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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.

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PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 155122-1630745

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF CX-5461 IN FEMALE ATHYMIC NUDE MICE AFTER A SINGLE INTRAPERITONEAL DOSE

SHORT TITLE:	**CX-5461 Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	CX-5461 (as dihydrochloride salt)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Geeleher, Paul <Paul.Geeleher@STJUDE.ORG>
SJCRH SRM2 O/R:	155122-1630745 Preclinical Pharmacokinetic Shared Resource
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REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2019-05-31

FOR P-PKSR APPROVED USE AND DISTRIBUTION

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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.

1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The plasma and tumor pharmacokinetic (PK) profile of CX-5461 as the dihydrochloride salt was evaluate in female Athymic nude mice (Charles River), approximately 12 weeks in age, bearing MAST3 neuroblastoma orthotopic xenografts. CX-5461 2HCl (SJ000879909-4, MedChemExpress, HY-13323A, 31261) was dissolved in 10% Captisol (SBECD, Ligand Pharmaceuticals) in 25 mM NaH2PO4 (pH 5.95), for a 25 mg/kg free base equivalents dose as a 10 mL/kg intraperitoneal injection. Terminal blood samples, under IP Avertin (tribromoethanol) anesthesia, were obtained at various times up to 24 hours post-dose, immediately processed to plasma, and stored at -80 °C until analysis. Following terminal bleeds, animals were perfused with PBS to flush blood from the vasculature. Tumors were then extracted, rinsed with PBS as necessary, and then placed in appropriately labeled microcentrifuge tubes in a cooler on dry ice. Tissue samples were then transferred to a -80°C freezer as soon as possible. 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 TEENPREP 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) for seven 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. Plasma and tumor samples were analyzed for CX-5461(SJ000879909-4, MedChemExpress, HY-13323A, 31261) 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 100 µL of 0.1% formic acid in acetonitrile and 25 µL of 250 ng/mL alisertib (MedChemExpress, HY-10971, purity 99.43%) 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 Kinetex 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) in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 10% B for 0.5 min with a linear increase to 80% B in 2 min. The column was then rinsed for 1 min at 80% B and then equilibrated at the initial conditions for 1.5 min for a total run time of 5 min. Under these conditions, the analyte and IS eluted at 2.71 and 3.68 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4000 in the positive ESI mode and the following mass transitions were monitored: CX-5461 514.2 -> 391.2, alisertib 519.2 -> 311.2. The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A quadratic model ($1/X^2$ weighting) fit the calibrators across the 5 to 250 ng/mL range, with a correlation coefficient (R) of ≥ 0.9961. 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 5 ng/mL. Sample dilution integrity was confirmed. The intra-run precision and accuracy was ≤ 7.15% CV and 93.5% to 115%, respectively.

1.3 Pharmacokinetic (PK) Analysis

CX-5461 plasma and tumor Ct data were grouped by matrix and 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, 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). The apparent plasma-to-tumor partition coefficient (Kp,inf) was estimated as the ratio of the AUCinf in tissue to AUCinf plasma, whereas Kp,last was similarly estimated using AUClast values.

2.0 RESULTS

The plasma and MAST3 neuroblastoma PK of CX-5461 were highly variable, with no discernable terminal phase for plasma. As such, all reported terminal parameters should be interpreted with caution. The plasma PK was rather distinct from the previous plasma only PK study, in that current observations showed a very flat profile with high concentrations and no apparent elimination phase. The absorption from the IP space appeared to be slow and variable, and the possibility of a saturable effect on absorption or metabolism / elimination cannot be ruled out. The plasma concentrations generally rose from 16 to 24 hours, which may suggest enterohepatic recirculation. CX-5461 tumor concentrations were slightly higher than unity compared with plasma (Kp,last = 1.16), with earlier time points showing lower concentration ratios, suggesting a slow permeation to tumor. The remaining formulation was found to be in specification.

At the recommended Phase II dose (RP2D) of CX-5461 of 170 mg/m^2 IV as a 1 hour infusion every 3 weeks, adults with hematological malignancies exhibited a total plasma AUCinf of 17943 hr-ug/L [1], with a rapid distribution half-life of ~2 hr and terminal half-life of ~50 hr. The majority of the AUC (~90%) was represented by the terminal phase. The terminal half-life appeared to increase with dose, which the investigators attributed to suspected enterohepatic recirculation. The overall plasma Cavg, which included the infusion and distribution phases, over the first 168 hours post-dose was ~100 ug/L. Additional dose ranging of infusions on D1 and D8 of a 4-week schedule is ongoing in a separate Phase I study [2]; however, the RP2D has yet to be reported. The major toxicity to date has been photosensitivity. This study has also described “nonproportional” increases in Cmax and AUC with dose level suggestive of nonlinear PK. Previous data from CBT ATC has shown plasma protein binding of CX-5461 in mice and humans to be similar (RPT.159452-1677129).

Given the difficulties and variable PK CX-5461 has exhibited in our mouse studies, recommendation of a PK-based clinically relevant dose (CRD) is difficult. This is made more challenging since human PK of CX-5461 is so qualitatively different. CX-5461 has exhibited characteristics of nonlinear and non-dose proportional PK in our mouse studies as well as in human studies, which makes extrapolation of dose-exposure relationships unreliable.

With these caveats, and with the weak assumption of linear and proportional PK, a CX-5461 dosage of 2.5 mg/kg (range 1.25 – 5 mg/kg) IP QD x 5d would likely provide a plasma Cavg comparable to the standard RP2D dose. To recapitulate the D1,D8 schedule, a second QD x 5d course would be needed starting on D8. Of note, typical mouse CX-5461 doses in the literature have ranged from 12 – 50 mg/kg PO or IP, from once weekly to a continuous 5-on, 2-off schedule [3–7].

3.0 REFERENCES

1. Khot A, Brajanovski N, Cameron DP, Hein N, Maclachlan KH, Sanij E, Lim J, Soong J, Link E, Blombery P, Thompson ER, Fellowes A, Sheppard KE, McArthur GA, Pearson RB, Hannan RD, Poortinga G, Harrison SJ. First-in-Human RNA Polymerase I Transcription Inhibitor CX-5461 in Patients with Advanced Hematological Cancers: Results of a Phase I Dose Escalation Study. Cancer Discov. 2019 Jan 1;CD-18-1455.

2. Hilton J, Cescon DW, Bedard P, Ritter H, Tu D, Soong J, Gelmon K, Aparicio S, Seymour L. 44OCCTG IND.231: A phase 1 trial evaluating CX-5461 in patients with advanced solid tumors. Ann Oncol [Internet]. 2018 Mar 1 [cited 2019 May 30];29(suppl_3). Available from: https://academic.oup.com/annonc/article/29/suppl_3/mdy048.003/4917515

3. Drygin D, Lin A, Bliesath J, Ho CB, O'Brien SE, Proffitt C, Omori M, Haddach M, Schwaebe MK, Siddiqui-Jain A, Streiner N, Quin JE, Sanij E, Bywater MJ, Hannan RD, Ryckman D, Anderes K, Rice WG. Targeting RNA Polymerase I with an Oral Small Molecule CX-5461 Inhibits Ribosomal RNA Synthesis and Solid Tumor Growth. Cancer Res. 2011 Feb 15;71(4):1418–30.

4. Haddach M, Schwaebe MK, Michaux J, Nagasawa J, O'Brien SE, Whitten JP, Pierre F, Kerdoncuff P, Darjania L, Stansfield R, Drygin D, Anderes K, Proffitt C, Bliesath J, Siddiqui-Jain A, Omori M, Huser N, Rice WG, Ryckman DM. Discovery of CX-5461, the First Direct and Selective Inhibitor of RNA Polymerase I, for Cancer Therapeutics. ACS Med Chem Lett. 2012 Jul 12;3(7):602–6.

5. Li L, Li Y, Zhao J, Fan S, Wang L, Li X. CX-5461 induces autophagy and inhibits tumor growth via mammalian target of rapamycin-related signaling pathways in osteosarcoma. OncoTargets Ther. 2016 Sep 29;9:5985–97.

6. Maclachlan KH, Cuddihy A, Hein N, Cullinane C, Harrison SJ, Hannan R, Poortinga G. Novel Combination Therapies with the RNA Polymerase I Inhibitor CX-5461 Significantly Improve Efficacy in Multiple Myeloma. Blood. 2017 Dec 7;130(Suppl 1):1805–1805.

7. Xu H, Di Antonio M, McKinney S, Mathew V, Ho B, O'Neil NJ, Santos ND, Silvester J, Wei V, Garcia J, Kabeer F, Lai D, Soriano P, Banáth J, Chiu DS, Yap D, Le DD, Ye FB, Zhang A, Thu K, Soong J, Lin S, Tsai AHC, Osako T, Algara T, Saunders DN, Wong J, Xian J, Bally MB, Brenton JD, Brown GW, Shah SP, Cescon D, Mak TW, Caldas C, Stirling PC, Hieter P, Balasubramanian S, Aparicio S. CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. Nat Commun. 2017 Feb 17;8:14432.

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profile of CX-5461 by Group

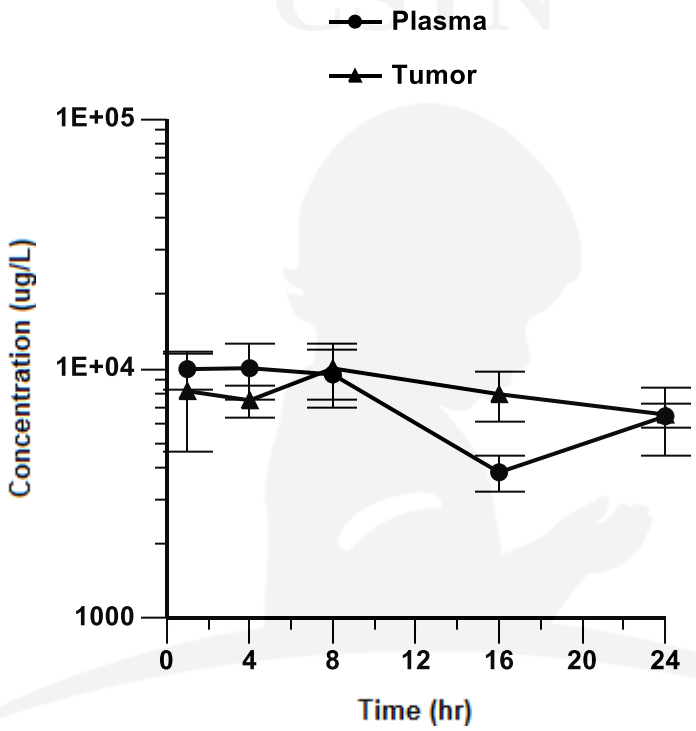

Table 4.1: NCA PK Parameter Estimates of CX-5461 by Group

		Analyte	
		CX-5461	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Cmax	ug/L	10000	10000
Tmax	hr	4.00	8.00
AUClast	hr*ug/L	165000	191000
AUCinf	hr*ug/L	376000	435000
Kel	1/hr	0.0241	0.0266
T1/2	hr	28.8	26.0

		Analyte	
		CX-5461	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
CL/F	L/hr/kg	0.0664	0.0575
Vz/F	L/kg	2.76	2.16
Clast	ug/L	6450	6550
Tlast	hr	24.0	24.0
Kp,inf	-	-	1.16
Kp,last	-	-	1.16

Table 4.2: Full Summary Statistics of CX-5461 Ct Data by Group

		Analyte	
		CX-5461	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
1.000	N	3	3
	Mean	9960	8130
	SD	1780	3440
	Min	8140	5960
	Median	10000	6330
	Max	11700	12100
	CV%	17.9	42.3
	Geometric Mean	9850	7700
	CV% Geometric Mean	18.3	40.8
4.000	N	3	3
	Mean	10000	7470
	SD	2510	1060
	Min	7180	6830
	Median	11100	6890
	Max	11900	8690
	CV%	25.0	14.2

CX-5461 Screening Plasma Tumor PK (SPTPK)

		Analyte	
		CX-5461	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	Geometric Mean	9810	7420
	CV% Geometric Mean	27.8	13.7
8.000	N	3	3
	Mean	9480	10000
	SD	2520	2500
	Min	7570	7280
	Median	8520	10600
	Max	12300	12200
	CV%	26.6	24.9
	Geometric Mean	9270	9800
	CV% Geometric Mean	25.9	27.1
16.000	N	3	3
	Mean	3840	7900
	SD	656	1750
	Min	3100	5990
	Median	4100	8300
	Max	4330	9410
	CV%	17.1	22.1
	Geometric Mean	3800	7760
	CV% Geometric Mean	18.1	23.7
24.000	N	3	3
	Mean	6450	6550
	SD	1940	756
	Min	4240	6010
	Median	7250	6210
	Max	7850	7410
	CV%	30.1	11.5
	Geometric Mean	6230	6520
	CV% Geometric Mean	34.5	11.3

Table 4.3: CX-5461 Ct Data Listings by Subject, Analyte, Group, and Time

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	CX-5461	Plasma	1.00	8138.10
M1	CX-5461	Tumor	1.00	6331.10
M2	CX-5461	Plasma	1.00	11690.00
M2	CX-5461	Tumor	1.00	5961.30
M3	CX-5461	Plasma	1.00	10040.00
M3	CX-5461	Tumor	1.00	12090.00
M4	CX-5461	Plasma	4.00	11103.00
M4	CX-5461	Tumor	4.00	8686.60
M5	CX-5461	Plasma	4.00	11851.00
M5	CX-5461	Tumor	4.00	6825.70
M6	CX-5461	Plasma	4.00	7178.30
M6	CX-5461	Tumor	4.00	6885.10
M7	CX-5461	Plasma	8.00	8518.50
M7	CX-5461	Tumor	8.00	7279.30
M8	CX-5461	Plasma	8.00	12335.00
M8	CX-5461	Tumor	8.00	10624.00
M9	CX-5461	Plasma	8.00	7574.30
M9	CX-5461	Tumor	8.00	12165.00
M10	CX-5461	Plasma	16.00	4099.10
M10	CX-5461	Tumor	16.00	9413.70
M11	CX-5461	Plasma	16.00	3095.60
M11	CX-5461	Tumor	16.00	5988.80
M12	CX-5461	Plasma	16.00	4329.00
M12	CX-5461	Tumor	16.00	8297.00
M13	CX-5461	Plasma	24.00	4237.00
M13	CX-5461	Tumor	24.00	7409.90
M14	CX-5461	Plasma	24.00	7249.40
M14	CX-5461	Tumor	24.00	6212.30
M15	CX-5461	Plasma	24.00	7854.90
M15	CX-5461	Tumor	24.00	6013.00

Table 4.4: CX-5461 Ct Summary (Mean, SD, N) by Group

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	CX-5461	Plasma	1.00	9956.03	1777.44	3.00
Concentration	ug/L	CX-5461	Plasma	4.00	10044.10	2509.88	3.00
Concentration	ug/L	CX-5461	Plasma	8.00	9475.93	2520.63	3.00
Concentration	ug/L	CX-5461	Plasma	16.00	3841.23	655.89	3.00
Concentration	ug/L	CX-5461	Plasma	24.00	6447.10	1937.80	3.00
Concentration	ug/L	CX-5461	Tumor	1.00	8127.47	3436.63	3.00
Concentration	ug/L	CX-5461	Tumor	4.00	7465.80	1057.66	3.00
Concentration	ug/L	CX-5461	Tumor	8.00	10022.77	2497.72	3.00
Concentration	ug/L	CX-5461	Tumor	16.00	7899.83	1746.65	3.00
Concentration	ug/L	CX-5461	Tumor	24.00	6545.07	755.57	3.00

5.0 ATTACHED FILES

Attached File 5.1 CX-5461 Screening Plasma and Tumor PK V1.0.docx– *Final in vivo study plan as executed*

Attached File 5.2 Copy of 155122-1630745_CX5_SPTPK_2019-05-08.xlsx– *Submitted in vivo study digital data collection form (DCF)*

Attached File 5.3 CX-5461 study sheet.jpg– *Submitted in vivo study worksheet #1*

Attached File 5.4 CX-5461 Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*