PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study: 158821-1671159

STUDY TITLE:

Screening Plasma and Tumor Pharmacokinetics of Irinotecan and SN-38 in Female Athymic Nude Mice Bearing MAST3 NB OTXs after a Single Intraperitoneal Dose of Irinotecan HCl

SHORT TITLE:	**IRN HCl MAST3 Screening Plasma and Tissue PK (SPTPK)**
TEST ARTICLE:	Irinotecan HCl
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
PRINCIPAL INVESTIGATOR(S)	Stewart, Elizabeth <Elizabeth.Stewart@STJUDE.ORG>
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REFERENCE STUDY NUMBERS:	NA
IN VIVO SCIENTIST(S)	Blankenship, Kaley B <Kaley.Blankenship@STJUDE.ORG> Hoffmann, Lauren <Lauren.Hoffmann@STJUDE.ORG>
BIOANALYTICAL SCIENTIST:	Caufield, William <William.Caufield@STJUDE.ORG>
REPORT AUTHOR(S):	Freeman, Burgess <Burgess.Freeman@STJUDE.ORG> Caufield, William <William.Caufield@STJUDE.ORG> Wang, Lindsey <Lindsey.Wang@STJUDE.ORG>
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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.

1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The plasma pharmacokinetic (PK) profile of irinotecan and its active metabolite SN-38 were evaluated in normal female Athymic nude mice (Charles River), approximately 12 weeks in age, bearing MAST3 neuroblastoma orthotopic xenografts (OTXs). Irinotecan HCl, USP was diluted as necessary for a dosage of 3.125 mg/kg as a 10 mL/kg intraperitoneal injection. Terminal blood samples, under IP Avertin (tribromoethanol) anesthesia, were obtained at various times up to 168 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. Tissues 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.

1.2 Bioanalysis

Frozen tumor samples were weighed in tared 15 mL Lysing Matrix D (MP Biomedical, Santa Ana, CA) tubes and diluted with a 5:1 volume of ultra-pure water. The tumor samples were then homogenized with a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). The homogenization consisted of four 6.0 M/S vibratory cycles of 1 min each on the FastPrep-24 system. To prevent over-heating due to friction, samples were placed on wet ice for 5 min between each cycle. The homogenates were then stored at -80 °C until analysis.

Plasma and tumor homogenate samples were analyzed for irinotecan (SJ000312345-15, MCE) and SN-38 (SJ000311679-8, TCI America) with a qualified LC MS/MS assay. Plasma calibrators and quality controls were spiked with solutions prepared in DMSO. Plasma samples, 25 µL each, were stabilized against carboxylesterase activity by the addition of 5 µL of 200 mM zinc sulfate and then protein precipitated with 100 µL of 10 ng/mL camptothecin (Cayman Chemical Co., Batch 0515272-12) in methanol as an internal standard. A 5 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 Kinetex EVO C18 (2.6 µm, 50 mm x 2.1 mm) column maintained at 50 °C with gradient elution at a flow rate of 0.6 mL/min. The binary mobile phase consisted of water-acetonitrile-200 mM ammonium acetate pH 6.0 (90:10:10 v/v) in reservoir A and acetonitrile-water-200 mM ammonium acetate pH 6.0 (90:10:10 v/v) in reservoir B. The initial mobile phase consisted of 15% B with a linear increase to 60% B in 2.0 min. The column was then rinsed for 1.0 min at 100% B and then equilibrated at the initial conditions for 2.0 min for a total run time of 5 min. Under these conditions, irinotecan, IS and SN-38 eluted at 1.22, 1.43 and 1.48 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX 5500 QTRAP in the positive ESI mode and the following mass transitions were monitored: Irinotecan 587.30 → 167.30, camptothecin (349.10 → 305.20) and SN-38 (393.10 → 305.20). The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the SN-38 calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9980 and 0.9992 for plasma and tumor, respectively. A linear model ($1/X^2$ weighting) fit the irinotecan calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of ≥ 0.9978 and 0.9972 for plasma and tumor, respectively. 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 both matrices, with a functional LLOQ of 6 ng/mL for tumor considering dilution. Sample dilution integrity was confirmed. The intra-run precision and accuracy for SN-38 in plasma was ≤ 11.6% CV and 93.2% to 107%, respectively. The intra-run precision and accuracy for irinotecan in plasma was ≤ 10.2% CV and 90.5% to 107%, respectively. For the MAST-3 tumor homogenate matrix, the intra-run precision and accuracy for SN-38 was ≤ 8.94% CV and 92.3% to 105%, respectively. The intra-run precision and accuracy for irinotecan in tumor homogenate was ≤ 11.5% CV and 93.1% to 104%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Irinotecan and SN-38 plasma 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 ratio of analyte in tumor to plasma (Kp,last) was caluculated as AUClast,tumor / AUClast, plasma. The metabolite ratio of SN-38 to IRN (MR,last) in plasma and tumor was calculated using the ratios of the AUClast estimates.

2.0 RESULTS

The total irinotecan (IRN) and SN-38 plasma and tumor concentrations from IRN HCl were highly variable. Concentrations averaged 74.2% CV, which was similar to previously observed in Athymic nude mice with and without Ewing's Sarcoma OTXs. The apparent plasma half-life for both IRN and SN-38 was moderate at ca. 3.5 hours, and similar to our previous studies. The plasma ratio of SN-38 to IRN was approximately 12% and in line with prior studies. The plasma exposure of IRN and SN-38 appeared similar to previous studies, as quantified by AUCinf. However, the plasma AUC of IRN was slightly higher than previously observed in the EW8 PK studies – 1470 vs 1180 hr-ng/mL.

The tumor concentrations of IRN and SN-38 were higher than those previously observed in the EW8 orthotopic model. The intra-abdominal MAST3 OTX IRN concentrations were 4.33-fold higher than plasma, whereas EW8's were 79% of the plasma. The tumor SN-38 concentrations were also high, at 5.48-fold greater than plasma by AUClast. In comparison, the EW8 tumors had SN-38 concentrations 1.56-fold higher than plasma. It also appeared that SN-38 continued to accumulate into MAST3 tumors or was formed intra-tumorally by the IRN present. The 8 hour tumor SN-38 concentration was much higher than the previous time points.

Penetration of IRN and SN-38 resulting from an intraperitoneal dose of IRN HCl was relatively high compared with EW8 tumors. There is concern that the high tumor accumulation of IRN and SN-38 in the intra-abdominal MAST3 tumors could be due to direct diffusion of drug to tumor after IP dosing. One should carefully evaluate whether IP injection, a clinically irrelevant route of systemic administration, is appropriate in mouse models with a significant abdominal presence, such as neuroblastoma OTXs.

3.0 REFERENCES

None

4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profile of Analytes by Group

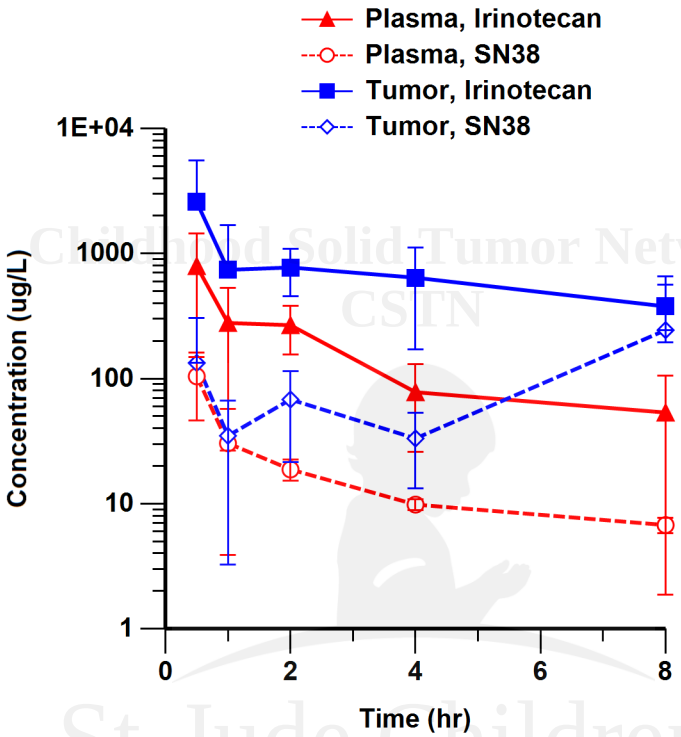

Table 4.1: NCA PK Parameter Estimates of Analytes by Group

		Analyte			
		Irinotecan		SN38	
		Group		Group	
		Plasma	Tumor	Plasma	Tumor
Parameter	Units	Estimate			
Cmax	ug/L	793	2580	104	244
Tmax	hr	0.500	0.500	0.500	8.00
AUClast	hr*ug/L	1280	5540	141	773
AUCinf	hr*ug/L	1470	8680	180	
Kel	1/hr	0.243	0.121	0.160	
T1/2	hr	2.85	5.71	4.34	
CL/F	L/hr/kg	2.12	0.360	17.4	
Vz/F	L/kg	8.73	2.96	109	

		Analyte			
		Irinotecan		SN38	
		Group		Group	
		Plasma	Tumor	Plasma	Tumor
Parameter	Units	Estimate			
Clast	ug/L	53.5	378	6.74	244
Tlast	hr	8.00	8.00	8.00	8.00
MR,last				0.110	0.140
Kp,last			4.33		5.48

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

		Analyte			
		Irinotecan		SN38	
		Group		Group	
		Plasma	Tumor	Plasma	Tumor
Time (hr)		Concentration (ug/L)			
0.500	N	3	3	3	3
	Mean	793	2580	104	133
	SD	645	2960	57.8	170
	Min	361	418	58.5	32.0
	Median	485	1370	85.1	38.1
	Max	1530	5950	169	330
	CV%	81.3	115	55.4	128
	Geometric Mean	645	1510	94.4	73.8
	CV% Geometric Mean	89.2	221	58.1	210
1.000	N	3	3	3	3
	Mean	278	740	30.4	34.9
	SD	252	954	26.5	31.6
	Min	3.54	6.37	1.43	0.856
	Median	333	395	36.3	40.4
	Max	499	1820	53.5	63.3
	CV%	90.5	129	87.2	90.7
	Geometric Mean	83.8	166	14.1	13.0
	CV% Geometric Mean	4350	7210	715	1640
2.000	N	3	3	3	3
	Mean	267	774	18.8	68.0
	SD	112	319	3.60	46.7
	Min	198	450	16.2	25.9

		Analyte			
		Irinotecan		SN38	
		Group		Group	
		Plasma	Tumor	Plasma	Tumor
Time (hr)		Concentration (ug/L)			
	Median	207	784	17.2	60.0
	Max	397	1090	22.9	118
	CV%	42.0	41.3	19.2	68.6
	Geometric Mean	253	726	18.6	56.8
	CV% Geometric Mean	40.4	47.0	18.6	88.6
4.000	N	3	3	3	3
	Mean	77.8	641	9.83	33.2
	SD	51.8	470	0.987	19.9
	Min	34.9	233	9.23	17.7
	Median	63.2	533	9.28	26.2
	Max	135	1160	11.0	55.7
	CV%	66.6	73.4	10.0	60.0
	Geometric Mean	66.8	524	9.80	29.6
	CV% Geometric Mean	76.7	94.7	9.80	63.6
8.000	N	3	3	3	3
	Mean	53.5	378	6.74	244
	SD	51.6	183	0.953	410
	Min	19.7	269	6.03	7.30
	Median	27.9	275	6.37	7.90
	Max	113	589	7.82	717
	CV%	96.5	48.4	14.1	168
	Geometric Mean	39.6	352	6.70	34.6
	CV% Geometric Mean	116	46.9	13.8	3140

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Irinotecan	Plasma	0.50	360.50
M1	Irinotecan	Tumor	0.50	5951.70
M1	SN38	Plasma	0.50	85.09
M1	SN38	Tumor	0.50	329.70
M2	Irinotecan	Plasma	0.50	484.69
M2	Irinotecan	Tumor	0.50	418.30

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M2	SN38	Plasma	0.50	58.46
M2	SN38	Tumor	0.50	31.98
M3	Irinotecan	Plasma	0.50	1534.90
M3	Irinotecan	Tumor	0.50	1372.40
M3	SN38	Plasma	0.50	169.21
M3	SN38	Tumor	0.50	38.13
M4	Irinotecan	Plasma	1.00	3.54
M4	Irinotecan	Tumor	1.00	6.37
M4	SN38	Plasma	1.00	1.43
M4	SN38	Tumor	1.00	0.86
M5	Irinotecan	Plasma	1.00	333.06
M5	Irinotecan	Tumor	1.00	395.24
M5	SN38	Plasma	1.00	36.28
M5	SN38	Tumor	1.00	40.39
M6	Irinotecan	Plasma	1.00	498.61
M6	Irinotecan	Tumor	1.00	1819.30
M6	SN38	Plasma	1.00	53.46
M6	SN38	Tumor	1.00	63.33
M7	Irinotecan	Plasma	2.00	396.76
M7	Irinotecan	Tumor	2.00	783.63
M7	SN38	Plasma	2.00	22.89
M7	SN38	Tumor	2.00	25.87
M8	Irinotecan	Plasma	2.00	198.31
M8	Irinotecan	Tumor	2.00	449.51
M8	SN38	Plasma	2.00	16.21
M8	SN38	Tumor	2.00	118.25
M9	Irinotecan	Plasma	2.00	206.50
M9	Irinotecan	Tumor	2.00	1088.00
M9	SN38	Plasma	2.00	17.22
M9	SN38	Tumor	2.00	60.03
M10	Irinotecan	Plasma	4.00	34.87
M10	Irinotecan	Tumor	4.00	233.30
M10	SN38	Plasma	4.00	9.23
M10	SN38	Tumor	4.00	26.25
M11	Irinotecan	Plasma	4.00	135.37
M11	Irinotecan	Tumor	4.00	1155.20
M11	SN38	Plasma	4.00	9.28

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M11	SN38	Tumor	4.00	55.67
M12	Irinotecan	Plasma	4.00	63.20
M12	Irinotecan	Tumor	4.00	533.37
M12	SN38	Plasma	4.00	10.97
M12	SN38	Tumor	4.00	17.68
M13	Irinotecan	Plasma	8.00	27.91
M13	Irinotecan	Tumor	8.00	588.80
M13	SN38	Plasma	8.00	6.03
M13	SN38	Tumor	8.00	717.22
M14	Irinotecan	Plasma	8.00	112.93
M14	Irinotecan	Tumor	8.00	268.73
M14	SN38	Plasma	8.00	7.82
M14	SN38	Tumor	8.00	7.30
M15	Irinotecan	Plasma	8.00	19.65
M15	Irinotecan	Tumor	8.00	275.24
M15	SN38	Plasma	8.00	6.37
M15	SN38	Tumor	8.00	7.90

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

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Irinotecan	Plasma	0.50	793.36	645.18	3.00
Concentration	ug/L	Irinotecan	Plasma	1.00	278.40	252.02	3.00
Concentration	ug/L	Irinotecan	Plasma	2.00	267.19	112.29	3.00
Concentration	ug/L	Irinotecan	Plasma	4.00	77.81	51.82	3.00
Concentration	ug/L	Irinotecan	Plasma	8.00	53.50	51.64	3.00
Concentration	ug/L	Irinotecan	Tumor	0.50	2580.80	2958.01	3.00
Concentration	ug/L	Irinotecan	Tumor	1.00	740.30	954.45	3.00
Concentration	ug/L	Irinotecan	Tumor	2.00	773.71	319.36	3.00
Concentration	ug/L	Irinotecan	Tumor	4.00	640.62	470.22	3.00
Concentration	ug/L	Irinotecan	Tumor	8.00	377.59	182.94	3.00
Concentration	ug/L	SN38	Plasma	0.50	104.25	57.81	3.00
Concentration	ug/L	SN38	Plasma	1.00	30.39	26.51	3.00
Concentration	ug/L	SN38	Plasma	2.00	18.77	3.60	3.00
Concentration	ug/L	SN38	Plasma	4.00	9.83	0.99	3.00
Concentration	ug/L	SN38	Plasma	8.00	6.74	0.95	3.00
Concentration	ug/L	SN38	Tumor	0.50	133.27	170.14	3.00

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	SN38	Tumor	1.00	34.86	31.60	3.00
Concentration	ug/L	SN38	Tumor	2.00	68.05	46.71	3.00
Concentration	ug/L	SN38	Tumor	4.00	33.20	19.93	3.00
Concentration	ug/L	SN38	Tumor	8.00	244.14	409.70	3.00

5.0 ATTACHED FILES

Attached File 5.1 158821_IRN SN-38 Screening Plasma Tumor PK.docx – *Final in vivo study plan as executed*

Attached File 5.2 2019-09-16 Irinotecan_SPTPK.xlsx – *Submitted in vivo study digital data collection form (DCF)*

Attached File 5.3 Irinotecan Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*