PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 144948 - 1522849

STUDY TITLE:

PLASMA AND TUMOR PHARMACOKINETICS OF IRINOTECAN (IRN) AND SN-38 IN FEMALE ATHYMIC NUDE MICE BEARING ES-8 EWINGS SARCOMA ORTHOTOPIC XENOGRAFTS AFTER A SINGLE INTRAVENOUS DOSE OF ONIVYDE (NAL-IRI, NANOLIPOSOMAL IRINOTECAN, USP)

SHORT TITLE:	**Nal-IRI IV (IRN, SN-38) Plasma and ES-8 Tumor PK**
TEST ARTICLE:	Onivyde (nal-IRI, nanoliposomal irinotecan, USP)
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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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) Studies

The plasma pharmacokinetic (PK) profile of total (liposome encapsulated and non-encapsulated) irinotecan (IRN) and its active metabolite SN-38 were evaluated in female Athymic nude mice (Charles River), approximately 12 weeks in age, in a mix of non-tumor bearing mice and mice bearing ES-8 Ewing Sarcoma orthotopic xenografts (OTXs) across three separate studies. Onivyde (nal-IRI, nanoliposomal irinotecan, USP) was diluted with 5% dextrose in normal saline, to yield a dose of 5 mg/kg. A small study of tumor-bearing mice at a lower dose of nal-IRI 2.5 mg/kg IV was also conducted. In select mice across the studies, survival retro-orbital bleeds using Sarstedt 50 uL KEDTA POCT devices were obtained under isoflurane anesthesia, whereas terminal blood samples were obtained under IP Avertin (tribromoethanol) anesthesia. Blood samples were collected upon KEDTA, obtained at various times up to 168 hours post-dose, immediately processed to plasma, and stored on dry ice until transfer to -80°C where they remained 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 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 (KEDTA) and tumor homogenate samples were analyzed for total (liposome encapsulated and non-encapsulated free) 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 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

The irinotecan and SN-38 concentration-time (Ct) data were grouped by matrix and nominal time point, and 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, ELSE the entire time point's data were treated as missing. Ct summary statistics including the arithmetic mean and standard deviation were then generated. The mean (+SD) Ct profiles were then plotted by analyte and matrix, and presented as figures.

Mean Ct values were subjected to noncompartmental analysis (NCA) using Phoenix WinNonlin 8.1 (Certara USA, Inc., Princeton, NJ). The IV bolus 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 all presented mean Ct points past 32 hours until the end of the 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 the back-extrapolated concentration at time 0 (C0), observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), average concentration over 168 hours (Cavg,168hr = AUClast / 168 hr), clearance (CL = Dose/AUCinf), terminal volume of distribution (Vz), and volume of distribution at steady state (Vss). All these parameters are apparent, as the rates and fractions of liposomal liberation and SN-38 formation are unknown. 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.

An approximate clinically relevant dose (CRD) for mice was estimated from plasma PK and exposure of total IRN. The approximate CRD was estimated as the mouse dose achieving a predicted mean plasma average concentration (Cavg) of IRN similar to humans with the adult nal-IRI FDA-approved dose. Dose proportional, linear, and time-invariant PK across species was assumed; however, evidence suggests this may not the case with nal-IRI [1,2]. Human and mouse plasma protein binding were assumed to be similar. Additional considerations influenced the final recommended mouse dose, including mouse dosing regimens prevalent in the literature and the tolerability of the compound in mice.

2.0 RESULTS

Inconsistencies between data collection forms (DCFs) and sample labels were noted, with apparent time points between groups of mice switched during study conduct for logistical convenience by the In Vivo Scientists. The data found on sample labels took precedence over DCFs and was used in the analyses.

After nal-IRI 5 mg/kg IV, resultant irinotecan (IRN) and SN-38 Ct data demonstrated extreme variability between and within mice, with coefficients of variation of up to 191%. Most variability was observed with IRN and SN-38 in the plasma, with each matrix averaging 112% and 100% CV across Ct points, respectively. The tumor variability was moderate-to-high, averaging 66.2% and 69.5% CV for IRN and SN-38, respectively. The source of this variability is unclear, but it may result from varied pooling of liposomes into large, vascular, and heterogenous orthotopic tumors. The mean (SD) tumor weight was 3.19 ± 1.90 grams (N=30), averaging 11.6 ± 6.63 % of observed mouse body weight. Variability could have also been due to continuing plasma esterase activity in collected samples before freezing. Data from mice dosed with nal-IRI 2.5 mg/kg IV were not included in this analysis, as approximately 40% of the observations were below the limit of quantitation (BLOQ). This left the 2.5 mg/kg IV data uninterpretable with noncompartmental analysis, and therefore it is not presented.

At nal-IRI 5 mg/kg IV, the plasma back-extrapolated initial concentration (C0) of IRN was high at 74300 ug/L. This indicates a low initial volume of distribution for nal-IRI, as would be expected for a large liposome. The Cmax and C0 values appeared similar to those observed by Kang et al. after 5 mg/kg IV nal-IRI in mice [3]. The plasma IRN and SN-38 Ct profiles also appeared similar to Kang et al. over a 24-hour post-dose period. Plasma concentrations for IRN and SN-38 diminished in a bi-exponential manner, with a long apparent terminal phase starting at about 32-48 hours post-dose. The plasma terminal half-life was estimated at 52.6 hours and 211 hours respectively for IRN and SN-38. All derived terminal phase parameters should be interpreted with caution, given the high number of BLOQ observations and variability in this phase. It is hypothesized that this prolonged and variable terminal phase stems from redistribution of liposomal IRN, free IRN, and free SN-38 back into plasma from peripheral and tumor compartments.

As expected for a liposome, the apparent plasma clearance (CL) of IRN was very low at 0.405 mL/min/kg, or approximately 0.45% of murine hepatic blood flow (HBF). The plasma CL for the resultant SN-38 was low at 17 mL/min/kg or 18.9% of HBF, and lower than that reported for standard IV IRN in mice [4,5]. This is likely due to a "flip-flop" phenomenon, where the rate of IRN liposome liberation governs formation and the apparent clearance of resultant SN-38. The apparent volume of distribution at steady state was very low for IRN at 0.0810 L/kg. As with clearance, all volume of distribution parameters for IRN and SN-38 are apparent, as the rates and fractions of liberation and formation are unknown.

IRN penetrated into the ES-8 tumors to a modest degree, with an apparent Kp,inf value of 0.728. However, SN-38 concentrations in the tumor were much higher, showing a Kp,inf value of 3.51. Appearance of SN-38 in tumor, which occurs predominantly from intra-tumoral liposomal IRN release and esterase activation, was prolonged with the Tmax observed at 72 hours. SN-38 concentrations in tumor were quantifiable up to 168 hours post-dose, and showed an average tumor concentration of 67.9 ng/mL over this time period.

The PK of IRN and SN-38 arising from nal-IRI is complex in both murine tumor models and humans. Many factors and rates dictate the observed total IRN and SN-38 in both plasma and tumor tissues. Additionally, mice have higher plasma esterase activity vs. humans, and form larger amounts of SN-38 in plasma [6]. These challenges make deriving a PK-guided clinically relevant dose (CRD) for nal-IRI difficult for mice. Given these issues, there is uncertainty that plasma AUCs of either IRN or SN-38 are appropriate PK metrics to guide a CRD recommendation for nal-IRI.

However, in a population PK and exposure-response analysis, average plasma concentrations (Cavg) over a dosing interval were the PK metric of interest, and found to best correlate with clinical adverse events [2]. At the FDA-approved dose of nal-IRI 70 mg/m^2 IV every 2 weeks, the IRN Cavg was 1190 ug/L, which compares well with our observed Cavg,168hr of 1220 ug/L at 5 mg/kg IV in mice. At 70 mg/m^2, the corresponding plasma Cmax and Cavg values for SN-38 were very low in humans, at 2.64 ug/L and 0.721 ug/L, respectively. Assuming linear and proportional PK, which is unlikely in this situation, a mouse dose of nal-IRI ~0.2 mg/kg IV would yield a similar plasma SN-38 Cavg.

In conclusion, using total plasma IRN Cavg values, it can be asserted that doses of nal-IRI of up to 5 mg/kg IV in mice are likely to be clinically reasonable.

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (+SD) Ct Profile of Irinotecan (IRN) and SN-38 in Plasma (PLA) After Nal-IRI 5 mg/kg IV in Mice

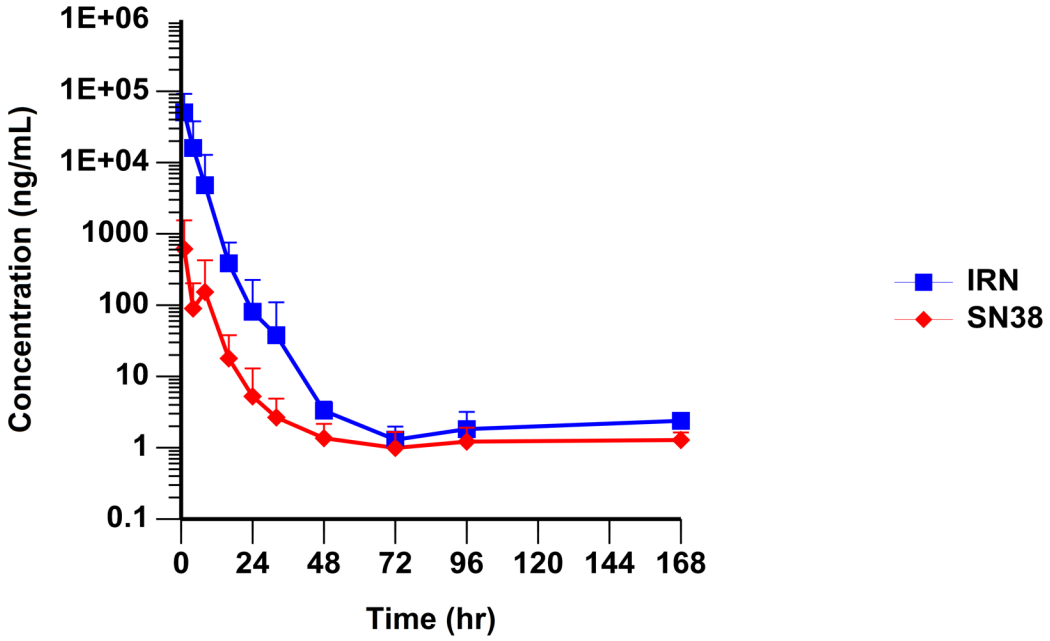

Figure 4.2: Mean (+SD) Ct Profile of Irinotecan (IRN) and SN-38 in ES-8 Tumor (TUM) After Nal-IRI 5 mg/kg IV in Mice

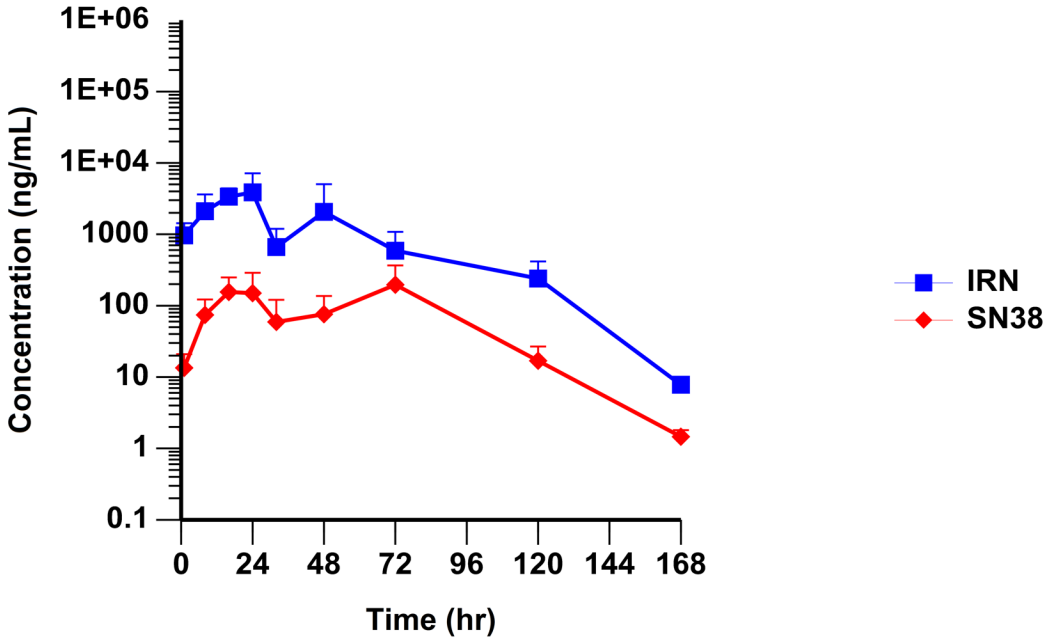

Table 4.0: Noncompartmental PK Parameter Estimates by Group and Analyte

		Group			
		PLA		TUM	
		Analyte		Analyte	
		IRN	SN38	IRN	SN38
Parameter	Units	Estimate			
Dosage	ug/kg	5000	3344*	5000	3344*
C0	ug/L	74200	1170	967	13.5
Cmax	ug/L	50500	614	3880	197
Tmax	hr	1.00	1.00	24.0	72.0
AUClast	hr*ug/L	205000	2960	149000	11400
Cavg,168hr	ug/L	1220	17.6	867	67.9
AUCinf	hr*ug/L	205000	3280	150000	11500
Kel	1/hr	0.0132	0.00329	0.0347	0.0294
T1/2	hr	52.6	211	20.0	23.6
CL	L/hr/kg	0.0243	1.02	0.0334	0.291
Vss	L/kg	0.0810	57.0	1.38	17.3
Vz	L/kg	1.85	310	0.963	9.87
Clast	ug/L	2.39	1.28	7.79	1.46
Tlast	hr	168	168	168	168
Kp,last	-	-	-	0.727	3.85
Kp,inf	-	-	-	0.732	3.51

*SN38 dosage in ug/kg adjusted for molar weight differences between IRN (MW 586.68) and SN38 (MW 392.40)

Table 4.1: Full Summary Statistics of Ct Data by Analyte and Group

		Analyte			
		IRN		SN38	
		Group		Group	
		PLA	TUM	PLA	TUM
Time (hr)		Concentration (ug/L)			
1.000	N	6	3	6	3
	Mean	50500	967	614	13.5
	SD	41300	464	927	7.50
	Min	992	449	8.44	6.97
	Median	61500	1100	97.1	11.7
	Max	88700	1350	2270	21.7
	CV%	81.7	48.0	151	55.7
	Geometric Mean	20600	874	131	12.1
	CV% Geometric Mean	705	63.9	1050	61.7

NaI-IRI IV (IRN, SN-38) Plasma and ES-8 Tumor PK

		Analyte			
		IRN		SN38	
		Group		Group	
		PLA	TUM	PLA	TUM
Time (hr)		Concentration (ug/L)			
4.000	N	3		3	
	Mean	16000		89.7	
	SD	21900		113	
	Min	992		1.63	
	Median	5760		50.1	
	Max	41100		217	
	CV%	137		126	
	Geometric Mean	6170		26.1	
	CV% Geometric Mean	558		2340	
8.000	N	9	6	9	6
	Mean	4820	2100	153	74.2
	SD	7980	1530	273	48.1
	Min	20.8	385	2.41	8.87
	Median	1400	2030	16.6	78.9
	Max	24500	4140	710	141
	CV%	166	72.7	179	64.8
	Geometric Mean	795	1530	22.6	54.8
	CV% Geometric Mean	2560	122	1020	133
16.000	N	6	3	6	3
	Mean	386	3360	17.9	156
	SD	370	1050	20.0	93.3
	Min	18.0	2710	2.54	63.3
	Median	317	2800	11.6	154
	Max	963	4570	57.6	250
	CV%	95.9	31.2	112	59.9
	Geometric Mean	190	3260	11.6	134
	CV% Geometric Mean	330	29.9	135	78.9
24.000	N	6	3	6	3
	Mean	80.8	3880	5.25	150
	SD	145	3280	7.72	139
	Min	9.59	122	1.24	10.1
	Median	21.6	5340	2.26	152
	Max	375	6170	21.0	288
	CV%	179	84.6	147	92.6

NaI-IRI IV (IRN, SN-38) Plasma and ES-8 Tumor PK

		Analyte			
		IRN		SN38	
		Group		Group	
		PLA	TUM	PLA	TUM
Time (hr)		Concentration (ug/L)			
	Geometric Mean	31.6	1590	3.00	76.2
	CV% Geometric Mean	214	1180	130	474
32.000	N	6	3	6	3
	Mean	37.8	662	2.66	59.2
	SD	71.9	533	2.22	61.4
	Min	4.78	51.4	0.500	8.04
	Median	9.21	893	2.04	42.3
	Max	184	1040	6.36	127
	CV%	190	80.6	83.4	104
	Geometric Mean	13.1	363	1.90	35.1
	CV% Geometric Mean	231	408	120	243
48.000	N	6	3	6	3
	Mean	3.31	2060	1.36	76.0
	SD	1.12	2970	0.798	60.5
	Min	1.76	36.1	0.500	8.11
	Median	3.53	679	1.37	95.7
	Max	4.57	5470	2.37	124
	CV%	33.8	144	58.6	79.6
	Geometric Mean	3.13	512	1.14	45.8
	CV% Geometric Mean	39.4	2400	78.5	294
72.000	N	6	3	6	3
	Mean	1.30	590	0.994	197
	SD	0.682	493	0.663	170
	Min	0.500	27.4	0.500	3.00
	Median	1.45	795	0.755	263
	Max	2.05	948	2.17	323
	CV%	52.6	83.6	66.7	86.6
	Geometric Mean	1.11	274	0.840	63.5
	CV% Geometric Mean	72.2	728	68.5	3300
96.000	N	3		3	
	Mean	1.83		1.22	
	SD	1.36		0.687	
	Min	0.500		0.500	
	Median	1.75		1.29	

		Analyte			
		IRN		SN38	
		Group		Group	
		PLA	TUM	PLA	TUM
Time (hr)		Concentration (ug/L)			
	Max	3.22		1.87	
	CV%	74.7		56.4	
	Geometric Mean	1.41		1.06	
	CV% Geometric Mean	121		76.6	
120.000	N	0	3	0	3
	Mean		241		16.9
	SD		176		9.90
	Min		109		6.37
	Median		172		18.4
	Max		440		26.0
	CV%		73.1		58.5
	Geometric Mean		202		14.5
	CV% Geometric Mean		81.1		84.4
168.000	N	3	2	3	2
	Mean	2.39	7.79	1.28	1.46
	SD	0.400	1.29	0.350	0.345
	Min	2.03	6.88	1.08	1.22
	Median	2.32	7.79	1.08	1.46
	Max	2.82	8.70	1.69	1.71
	CV%	16.8	16.6	27.3	23.6
	Geometric Mean	2.36	7.74	1.25	1.44
	CV% Geometric Mean	16.7	16.8	26.1	24.2

Table 4.2: Ct Summary (Mean, SD, N) by Analyte and Group

Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
IRN	PLA	1.00	50500	41300	6
IRN	PLA	4.00	16000	21900	3
IRN	PLA	8.00	4820	7980	9
IRN	PLA	16.0	386	370	6
IRN	PLA	24.0	80.8	145	6
IRN	PLA	32.0	37.8	71.9	6
IRN	PLA	48.0	3.31	1.12	6
IRN	PLA	72.0	1.30	0.682	6
IRN	PLA	96.0	1.83	1.36	3

Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
IRN	PLA	120			0
IRN	PLA	168	2.39	0.400	3
IRN	TUM	1.00	967	464	3
IRN	TUM	8.00	2100	1530	6
IRN	TUM	16.0	3360	1050	3
IRN	TUM	24.0	3880	3280	3
IRN	TUM	32.0	662	533	3
IRN	TUM	48.0	2060	2970	3
IRN	TUM	72.0	590	493	3
IRN	TUM	120	241	176	3
IRN	TUM	168	7.79	1.29	2
SN38	PLA	1.00	614	927	6
SN38	PLA	4.00	89.7	113	3
SN38	PLA	8.00	153	273	9
SN38	PLA	16.0	17.9	20.0	6
SN38	PLA	24.0	5.25	7.72	6
SN38	PLA	32.0	2.66	2.22	6
SN38	PLA	48.0	1.36	0.798	6
SN38	PLA	72.0	0.994	0.663	6
SN38	PLA	96.0	1.22	0.687	3
SN38	PLA	120			0
SN38	PLA	168	1.28	0.350	3
SN38	TUM	1.00	13.5	7.50	3
SN38	TUM	8.00	74.2	48.1	6
SN38	TUM	16.0	156	93.3	3
SN38	TUM	24.0	150	139	3
SN38	TUM	32.0	59.2	61.4	3
SN38	TUM	48.0	76.0	60.5	3
SN38	TUM	72.0	197	170	3
SN38	TUM	120	16.9	9.90	3
SN38	TUM	168	1.46	0.345	2

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
S2_M1	IRN	PLA	1.00	42448.00
S2_M1	IRN	PLA	16.0	220.79
S2_M1	IRN	PLA	32.0	184.36
S2_M1	SN38	PLA	1.00	35.71
S2_M1	SN38	PLA	16.0	16.57
S2_M1	SN38	PLA	24.0	2.26
S2_M1	SN38	PLA	32.0	6.36

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
S2_M2	IRN	PLA	1.00	80524.00
S2_M2	IRN	PLA	16.0	962.74
S2_M2	IRN	PLA	32.0	12.33
S2_M2	SN38	PLA	1.00	154.16
S2_M2	SN38	PLA	16.0	12.21
S2_M2	SN38	PLA	24.0	2.88
S2_M2	SN38	PLA	32.0	2.81
S2_M3	IRN	PLA	1.00	992.28
S2_M3	IRN	PLA	16.0	18.04
S2_M3	IRN	PLA	32.0	13.08
S2_M3	SN38	PLA	1.00	8.44
S2_M3	SN38	PLA	16.0	2.54
S2_M3	SN38	PLA	24.0	2.26
S2_M4	IRN	PLA	4.00	41100.00
S2_M4	IRN	PLA	24.0	21.20
S2_M4	IRN	PLA	48.0	2.20
S2_M4	SN38	PLA	4.00	217.24
S2_M4	SN38	PLA	48.0	1.69
S2_M5	IRN	PLA	4.00	5759.90
S2_M5	IRN	PLA	24.0	42.81
S2_M5	IRN	PLA	48.0	4.57
S2_M5	SN38	PLA	4.00	50.11
S2_M5	SN38	PLA	48.0	2.07
S2_M6	IRN	PLA	4.00	992.11
S2_M6	IRN	PLA	24.0	22.01
S2_M6	IRN	PLA	48.0	4.26
S2_M6	SN38	PLA	4.00	1.63
S2_M6	SN38	PLA	32.0	4.00
S2_M6	SN38	PLA	48.0	2.37
S2_M7	IRN	PLA	8.00	21.08
S2_M7	IRN	PLA	72.0	1.17
S2_M7	IRN	PLA	96.0	3.22
S2_M7	SN38	PLA	8.00	4.03
S2_M7	SN38	PLA	72.0	2.17
S2_M7	SN38	PLA	96.0	1.87
S2_M8	IRN	PLA	8.00	2217.30
S2_M8	IRN	PLA	72.0	0.50
S2_M8	IRN	PLA	96.0	0.50
S2_M8	SN38	PLA	8.00	2.98
S2_M8	SN38	PLA	72.0	0.50
S2_M8	SN38	PLA	96.0	0.50
S2_M9	IRN	PLA	8.00	20.77
S2_M9	IRN	PLA	72.0	0.50

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
S2_M9	IRN	PLA	96.0	1.75
S2_M9	SN38	PLA	8.00	2.41
S2_M9	SN38	PLA	72.0	1.01
S2_M9	SN38	PLA	96.0	1.29
S4_M1	IRN	PLA	1.00	88686.00
S4_M1	IRN	TUM	1.00	1346.90
S4_M1	SN38	PLA	1.00	1174.40
S4_M1	SN38	TUM	1.00	21.66
S4_M2	IRN	PLA	1.00	87711.00
S4_M2	IRN	TUM	1.00	449.18
S4_M2	SN38	PLA	1.00	2270.50
S4_M2	SN38	TUM	1.00	6.97
S4_M3	IRN	PLA	1.00	2918.10
S4_M3	IRN	TUM	1.00	1104.40
S4_M3	SN38	PLA	1.00	40.00
S4_M3	SN38	TUM	1.00	11.74
S4_M4	IRN	PLA	8.00	24502.00
S4_M4	IRN	TUM	8.00	3371.70
S4_M4	SN38	PLA	8.00	710.08
S4_M4	SN38	TUM	8.00	63.50
S4_M5	IRN	PLA	8.00	183.83
S4_M5	IRN	TUM	8.00	651.67
S4_M5	SN38	PLA	8.00	67.21
S4_M5	SN38	TUM	8.00	35.35
S4_M6	IRN	PLA	8.00	5777.30
S4_M6	IRN	TUM	8.00	4143.10
S4_M6	SN38	PLA	8.00	544.79
S4_M6	SN38	TUM	8.00	101.96
S4_M7	IRN	PLA	16.0	652.39
S4_M7	IRN	TUM	16.0	2797.20
S4_M7	SN38	PLA	16.0	57.63
S4_M7	SN38	TUM	16.0	153.68
S4_M8	IRN	PLA	16.0	45.21
S4_M8	IRN	TUM	16.0	4570.00
S4_M8	SN38	PLA	16.0	7.56
S4_M8	SN38	TUM	16.0	249.80
S4_M9	IRN	PLA	16.0	414.15
S4_M9	IRN	TUM	16.0	2711.00
S4_M9	SN38	PLA	16.0	10.90
S4_M9	SN38	TUM	16.0	63.32
S4_M10	IRN	PLA	24.0	375.48
S4_M10	IRN	TUM	24.0	6172.30
S4_M10	SN38	PLA	24.0	20.98

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
S4_M10	SN38	TUM	24.0	287.67
S4_M11	IRN	PLA	24.0	13.82
S4_M11	IRN	TUM	24.0	122.22
S4_M11	SN38	PLA	24.0	1.24
S4_M11	SN38	TUM	24.0	10.14
S4_M12	IRN	PLA	24.0	9.59
S4_M12	IRN	TUM	24.0	5339.70
S4_M12	SN38	PLA	24.0	1.91
S4_M12	SN38	TUM	24.0	151.88
S4_M13	IRN	PLA	48.0	3.72
S4_M13	IRN	TUM	48.0	36.12
S4_M13	SN38	PLA	48.0	0.50
S4_M13	SN38	TUM	48.0	8.11
S4_M14	IRN	PLA	48.0	3.34
S4_M14	IRN	TUM	48.0	679.07
S4_M14	SN38	PLA	48.0	0.50
S4_M14	SN38	TUM	48.0	95.72
S4_M15	IRN	PLA	48.0	1.76
S4_M15	IRN	TUM	48.0	5471.30
S4_M15	SN38	PLA	48.0	1.06
S4_M15	SN38	TUM	48.0	124.22
S6_M1	IRN	PLA	8.00	1395.10
S6_M1	IRN	TUM	8.00	384.68
S6_M1	SN38	PLA	8.00	5.73
S6_M1	SN38	TUM	8.00	8.87
S6_M2	IRN	PLA	8.00	405.87
S6_M2	IRN	TUM	8.00	1390.90
S6_M2	SN38	PLA	8.00	16.59
S6_M2	SN38	TUM	8.00	141.22
S6_M3	IRN	PLA	8.00	8831.40
S6_M3	IRN	TUM	8.00	2661.90
S6_M3	SN38	PLA	8.00	21.65
S6_M3	SN38	TUM	8.00	94.38
S6_M4	IRN	PLA	32.0	6.10
S6_M4	IRN	TUM	32.0	51.42
S6_M4	SN38	PLA	32.0	1.27
S6_M4	SN38	TUM	32.0	8.04
S6_M5	IRN	PLA	32.0	5.87
S6_M5	IRN	TUM	32.0	893.44
S6_M5	SN38	PLA	32.0	1.04
S6_M5	SN38	TUM	32.0	127.33
S6_M6	IRN	PLA	32.0	4.78
S6_M6	IRN	TUM	32.0	1039.80

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
S6_M6	SN38	PLA	32.0	0.50
S6_M6	SN38	TUM	32.0	42.33
S6_M7	IRN	PLA	72.0	1.72
S6_M7	IRN	TUM	72.0	795.34
S6_M7	SN38	PLA	72.0	0.50
S6_M7	SN38	TUM	72.0	263.27
S6_M8	IRN	PLA	72.0	1.84
S6_M8	IRN	TUM	72.0	27.40
S6_M8	SN38	PLA	72.0	0.50
S6_M8	SN38	TUM	72.0	3.00
S6_M9	IRN	PLA	72.0	2.05
S6_M9	IRN	TUM	72.0	947.64
S6_M9	SN38	PLA	72.0	1.28
S6_M9	SN38	TUM	72.0	323.43
S6_M10	IRN	PLA	120	
S6_M10	IRN	TUM	120	440.39
S6_M10	SN38	PLA	120	
S6_M10	SN38	TUM	120	26.01
S6_M11	IRN	PLA	120	
S6_M11	IRN	TUM	120	109.19
S6_M11	SN38	PLA	120	
S6_M11	SN38	TUM	120	18.42
S6_M12	IRN	PLA	120	
S6_M12	IRN	TUM	120	172.05
S6_M12	SN38	PLA	120	
S6_M12	SN38	TUM	120	6.37
S6_M13	IRN	PLA	168	2.82
S6_M13	SN38	PLA	168	1.69
S6_M14	IRN	PLA	168	2.32
S6_M14	IRN	TUM	168	8.70
S6_M14	SN38	PLA	168	1.08
S6_M14	SN38	TUM	168	1.22
S6_M15	IRN	PLA	168	2.03
S6_M15	IRN	TUM	168	6.88
S6_M15	SN38	PLA	168	1.08
S6_M15	SN38	TUM	168	1.71

5.0 ATTACHED FILES

Attached File 5.1 Nal-IRI Study DCFs.zip – *Digital collection forms for nal-IRI in vivo PK studies*
Attached File 5.2 Nal-IRI IV (IRN, SN-38) Plasma and ES-8 Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*