PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 821574-3094398

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF TIGECYCLINE IN FEMALE ATHYMIC NUDE MICE BEARING MAST39 RMSTUMORS AFTER A SINGLE IP DOSE

SHORT TITLE:	Tigecycline Screening Plasma Tumor PK (SPTPK)
TEST ARTICLE:	Tigecycline (Tygacil, GAR-936)
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) Study

The plasma and tumor pharmacokinetic (PK) profiles of tigecyline was evaluated in normal female Athymic nude mice (Charles River) bearing rhabdomyosarcoma MAST39 xenograft tumors, approximately 12 to 16 weeks in age. Tigecycline for injection, USP (SJ000562689, Sandoz, Lot # NE4445) was diluted with 0.9% sodium chloride at 1.5mg/mL for a 33 mL/kg intraperitoneal (IP) injection, yielding 50 mg/kg dose. One blood sample was collected from each mouse after dosing using an IACUC-approved terminal cardiac puncture, with KEDTA as the anticoagulant and immediately processed to plasma, following which tumor tissue was extracted after perfusion with PBS. Samples were obtained at various times up to 24 hours post-dose and stored at -80 °C until analysis.

1.2 Bioanalysis (BA)

Tumor samples were weighed in 4.5 mL (tumor weight below 0.5 gram) or 15 mL (tumor weight above 0.5 gram) 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). Tumor samples were subjected to 3 - 10 cycles of 1 min vibration at 6.5 M/S speed, with 5 min ice baths between each cycle to prevent over-heating. The homogenates were then stored at -80 °C until analysis.

Plasma and tumor homogenate samples were analyzed for tigecycline (MedChemExpress, CAT # HY-B0117, LOT # 119745, Purity 99.55%) using a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma calibrators and quality controls were spiked with solutions, corrected for salt content and purity as necessary, prepared in ultrapure water. Plasma and tumor homogenate samples, 25 µL each, were protein precipitated with 115 µL of 15 ng/mL tigecycline-d9 (Cayman Chemicals, CAT# 25414, LOT# 593738, Purity ≥ 99%) in acetonitrile as an internal standard (IS). A 5 µL aliquot of the extracted supernatant was injected onto a SCIEX ExionLC high performance liquid chromatography system via a SCIEX ExionLC autosampler. The LC separation was performed using a Waters XSelect Premier HSST3 (2.5 µm, 50 mm x 2.1 mm) column maintained at 48 °C with gradient elution at a flow rate of 0.45 mL/min. The binary mobile phase consisted of 10mM ammonium acetate in water (pH 5.6) in reservoir A and acetonitrile in reservoir B. The initial mobile phase consisted of 10% B for 0.5 min with a linear increase to 98% B in 0.5 min. The column was then rinsed for 1.5 min at 98% B and returned to initial mobile phase conditions in 0.5 mins, then equilibrated the column for another 1.5 min for a total run time of 4.5 min. Under these conditions, the analyte and IS eluted at 1.75 and 1.74 min, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX Triple Quad 3500 in the positive ESI mode and the following mass transitions were monitored: tigecycline 586.3 → 513.3, Tigecycline-d9 595.3 → 514.4. The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fits the calibrators across the 5 to 250 ng/mL range, with a correlation coefficient (R) of ≥ 0.9951. 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 in plasma and 30 ng/mL in tumor. Sample dilution integrity was confirmed. No significant matrix effects, ion enhancement or suppression, were detected in blank EDTA plasma and tumor homogenate harvested from untreated female Athymic nude mice. The intra-run precision and accuracy was ≤ 5.6% CV and 85.60% to 105.20%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Plasma and tumor concentration-time (Ct) data for tigecycline were grouped by matrix and nominal time point. 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. Summary statistics were calculated, and the arithmetic 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).

A clinically relevant dose (CRD) for mice was estimated from unbound plasma PK and exposure. The CRD was defined as the mouse dose achieving a predicted mean steady state unbound plasma AUC (AUCu) similar to humans at the single agent maximum tolerated dose (MTD), recommended Phase II dose (RP2D), or FDA-approved dose. Dose proportional, linear, and time-invariant PK across species was assumed. Human and mouse plasma protein binding were assumed similar when data were not available. This is similar to the clinical relevance approach proposed by Spilker [1] which uses unbound plasma average steady state concentrations. Some latitude in dose rounding was permitted in the CRD recommendation, and an unbound exposure within 2-fold of the clinical target was considered acceptable. 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

Tigecycline Ct data showed moderate-to-high variability in our mice, with coefficients of variation (CVs) ranging from 18.2% to 113% in plasma, and moderate variability ranging from 10.1% to 75.9% in tumor. The absorption rate of tigecycline was rapid, with the Tmax occurring at 0.5 hours post-dose for plasma, whereas the tumor appearance rate was moderate with the Tmax occurring at 1 hour. After Cmax, both plasma diminished in a bi-exponential manner, while the tumor declined in a mono-exponential fashion. The apparent plasma and tumor terminal half-life of tigecycline was 6.24 and 3.62 hours, respectively. The plasma and tumor Ct profiles generally mirrored each other after equilibration, and the T1/2 difference is most likely due to variance in the plasma Ct data at 24 hours. Tigicycline penetration into tumor was appreciable, with a Kp_inf value of 1.38. The apparent plasma clearance (CL/F) of tigecycline was low at 5.6 mL/min/kg, or approximately 6.22% of murine hepatic blood flow. The apparent plasma terminal volume of distribution (Vz/F) for tigecycline was moderate at 3.02 L/kg, in excess of total body water.

The PK profile of tigecycline in the current study differs modestly from previous findings in mice, with our exposures trending higher. Jitkova et al. reported tigecycline total plasma AUC value of 31870 hr-ug/L with a CL/F of 21.6 mL/min/kg after a 50 mg/kg in 0.9% NS IP x1 in NOD/SCID mice [2].The plasma AUC in the current study was 44700 hr-ug/L, approximately 1.4 fold higher than the reported plasma exposure, with a lower apparent clearance at 5.6 mL/min/kg. Another group reported a serum AUC 0-24h value of 49190 hr-ug/L with a terminal half-life of 7.36 hours in neutropenic ICR mice infected with S. aureus after 50 mg/kg subcutaneous injection – comparable with the plasma AUC of 44700 hr-ug/L and the T1/2 of 6.24 hours in our PK study [3]. Van Ogtrop et al. reported a serum AUC of 36500 hr-ug/L and the Cmax of 11100 ug/L in their study for tigecycline pharmacodynamic activity against S. pneumoniae bacterial infection after a 48 mg/kg single subcutaneous dose [4].

In a Phase 1 clinical study, the MTD of tigecycline was 300 mg IV as a 1 hour infusion weekly for 2 of 3 weeks (QD x 5) in patients with AML [5]. The MTD tigecycline plasma Ct profiles were digitized from this report (Plot Digitizer 2.6.8, plotdigitizer.sourceforge.net), and subjected to NCA. The resultant plasma PK estimates were: Cmax 5940 ug/L; AUC 29145 hr-ug/L and the T1/2 10.59 hours. Tigecycline displays atypical plasma protein binding behavior in both mice and humans. It was reported that the percentage of protein binding was increased with increasing concentrations in both mice and humans for tigecycline [3] [6]. Therefore, assuming similar PPB across the species, total plasma AUCs will be used for calculating

the CRDs. The estimated precise CRD for tigecycline, based on plasma AUCs, is 32.6 mg/kg IP QD. However, 50 mg/kg IP is within a reasonable range to be considered clinically relevant.

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profiles by Analyte and Group

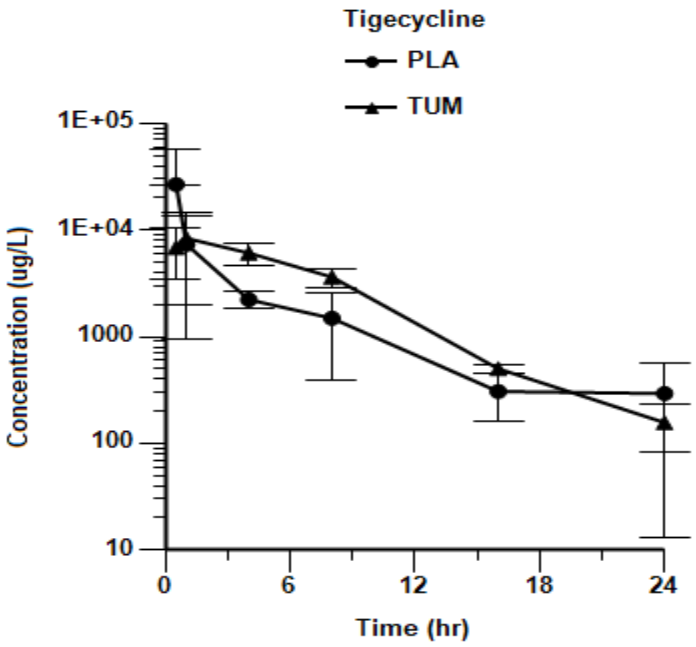

Table 4.1: NCA Parameter Estimates by Analyte and Group

		Analyte	
		Tigecycline	
		Group	
		PLA	TUM
Parameter	Unit	Value	
Cmax	ug/L	26700	8360
Tmax	hr	0.500	1.00
AUClast	hr*ug/L	42700	61000
AUCinf	hr*ug/L	44700	61700
Kel	1/hr	0.111	0.191
T1/2	hr	6.24	3.62
CL/F	L/hr/kg	0.336	0.243
Vz/F	L/kg	3.02	1.27
Clast	ug/L	293	157
Tlast	hr	24.0	24.0
Kp_last			1.43
Kp_inf			1.38

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

		Analyte	
		Tigecycline	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
0.500	N	3	3
	Mean	26700	6870
	SD	30300	3410
	Min	5460	3600
	Median	13300	6630
	Max	61400	10400
	CV%	113	49.5
	Geometric Mean	16400	6280
	CV% Geometric Mean	186	57.3
1.00	N	3	3
	Mean	7320	8360
	SD	6390	6350
	Min	764	1030
	Median	7670	12000
	Max	13500	12100
	CV%	87.3	75.9
	Geometric Mean	4300	5300
	CV% Geometric Mean	303	255
4.00	N	3	3
	Mean	2220	6080
	SD	405	1520
	Min	1760	4330
	Median	2420	6870
	Max	2490	7040
	CV%	18.2	25.0
	Geometric Mean	2200	5940
	CV% Geometric Mean	19.5	27.9
8.00	N	3	3
	Mean	1490	3620
	SD	1100	731
	Min	756	3160
	Median	960	3240
	Max	2750	4470
	CV%	73.7	20.2
	Geometric Mean	1260	3580
	CV% Geometric Mean	77.6	19.5
16.0	N	3	3
	Mean	306	501

		Analyte	
		Tigecycline	
		Group	
		PLA	TUM
Time (hr)		Concentration (ug/L)	
	SD	146	50.8
	Min	176	454
	Median	279	493
	Max	464	555
	CV%	47.7	10.1
	Geometric Mean	283	499
	CV% Geometric Mean	51.6	10.1
24.0	N	3	3
	Mean	293	157
	SD	280	74.3
	Min	33.6	70.7
	Median	256	199
	Max	590	200
	CV%	95.5	47.5
	Geometric Mean	172	141
	CV% Geometric Mean	279	65.7

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

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Tigecycline	PLA	0.500	61404.00
M1	Tigecycline	TUM	0.500	6628.70
M2	Tigecycline	PLA	0.500	13254.00
M2	Tigecycline	TUM	0.500	10396.00
M3	Tigecycline	PLA	0.500	5461.40
M3	Tigecycline	TUM	0.500	3598.60
M4	Tigecycline	PLA	1.00	13538.00
M4	Tigecycline	TUM	1.00	12089.00
M5	Tigecycline	PLA	1.00	7672.20
M5	Tigecycline	TUM	1.00	11968.00
M6	Tigecycline	PLA	1.00	764.16
M6	Tigecycline	TUM	1.00	1030.50
M7	Tigecycline	PLA	4.00	1757.80
M7	Tigecycline	TUM	4.00	4327.60
M8	Tigecycline	PLA	4.00	2420.90
M8	Tigecycline	TUM	4.00	6865.60
M9	Tigecycline	PLA	4.00	2491.80
M9	Tigecycline	TUM	4.00	7044.30

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M10	Tigecycline	PLA	8.00	755.74
M10	Tigecycline	TUM	8.00	3241.10
M11	Tigecycline	PLA	8.00	2748.50
M11	Tigecycline	TUM	8.00	3159.50
M12	Tigecycline	PLA	8.00	960.01
M12	Tigecycline	TUM	8.00	4465.00
M13	Tigecycline	PLA	16.0	175.55
M13	Tigecycline	TUM	16.0	493.44
M14	Tigecycline	PLA	16.0	279.04
M14	Tigecycline	TUM	16.0	454.35
M15	Tigecycline	PLA	16.0	463.82
M15	Tigecycline	TUM	16.0	555.06
M16	Tigecycline	PLA	24.0	33.58
M16	Tigecycline	TUM	24.0	70.72
M17	Tigecycline	PLA	24.0	256.10
M17	Tigecycline	TUM	24.0	199.44
M18	Tigecycline	PLA	24.0	590.20
M18	Tigecycline	TUM	24.0	199.50

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

Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Tigecycline	PLA	0.500	26700	30300	3
Tigecycline	PLA	1.00	7320	6390	3
Tigecycline	PLA	4.00	2220	405	3
Tigecycline	PLA	8.00	1490	1100	3
Tigecycline	PLA	16.0	306	146	3
Tigecycline	PLA	24.0	293	280	3
Tigecycline	TUM	0.500	6870	3410	3
Tigecycline	TUM	1.00	8360	6350	3
Tigecycline	TUM	4.00	6080	1520	3
Tigecycline	TUM	8.00	3620	731	3
Tigecycline	TUM	16.0	501	50.8	3
Tigecycline	TUM	24.0	157	74.3	3

5.0 ATTACHED FILES

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

Attached File 5.2 Tigecycline_SPTPK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*

Attached File 5.3 Tigecycline PK Sheet .pdf – completed *data collection form from client in vivo*