VI. HUMAN PHARMACOKINETIC SUMMARY

A. Introduction

The plasma and tissue pharmacokinetics of DOXIL in humans are summarized in this section of the Overall NDA Summary. A comparison to conventional doxorubicin HCl, Adriamycin, and the previous formulation, DOXIL 1, are also summarized. For a more complete discussion of the pharmacokinetic results, the reader is referred to the Bioavailability and Pharmacokinetics subsection of the Clinical Data Section of this NDA. Table 25 lists the human pharmacokinetics studies included in this NDA.

TABLE 25
Pharmacokinetic Studies of DOXIL Formulations

Study	Design and Test Article	Patients	Dose Range
30-02	Single dose crossover, DOXIL 1 to Adriamycin	Solid Tumor	25 and 50 mg/m^2
30-05	Randomized, single dose, DOXIL 1 or Adriamycin	Kaposi's sarcoma	10, 20 and 40 mg/m^2
30-14	Randomized, crossover of single doses of DOXIL separated by 3-week washout period	Kaposi's sarcoma	10 and 20 mg/m^2

B. Pharmacokinetics of DOXIL (Study 30-14)

The pharmacokinetics of doxorubicin were investigated in Study 30-14, a two-period, randomized, crossover study of single doses of DOXIL 10 or 20 mg/m^2 administered intravenously (i.v.) over 30 minutes (LTI-30-14). The doses were separated by a 3-week washout period. Twenty-six male patients with AIDS-related KS enrolled into the study; plasma was sampled over 10 days following dosing and KS lesion and normal skin tissue samples were collected 96 hours after the first dose. Seventeen additional patients were subsequently enrolled for tissue sampling 48 or 96 hours post-dose because of tissue mishandling in the original cohort.

Twenty-four of 26 patients received both doses of the crossover; plasma pharmacokinetic data were generated for 23 of the patients. Plasma levels were extremely low and were considered statistical outliers in the remaining 1 patient. Results are summarized in Table 26 and discussed below.

TABLE 26
DOXIL Pharmacokinetic Parameter Estimates[a]

Parameter	10 mg/m^2	20 mg/m^2
Vss (L/m^2)	2.84 (0.124)	2.77 (0.129)
CLt (L/h/m^2)	0.0572 (0.0103)	0.0419 (0.00401)
$t_{½}\lambda_1$	5.79 (1.07)	5.61 (1.21)
$t_{½}\lambda_2$	50.1 (5.71)	56.6 (5.74)
AUC_{0-240} (μg/mL·h)	232 (22.7)	532 (45.6)
$AUC_{0-\infty}$ (μg/mL·h)	252 (28.5)	577 (57.2)

[a] Mean values (S.E.) for 23 patients. Includes only those patients in 'full' design group, i.e., patients from whom a total of 9 to 10 plasma samples were collected after DOXIL administration at both DOXIL dose levels.

DOXIL displayed linear pharmacokinetics best described by a two-compartment model. There were no carryover effects, although a few patients had low baseline doxorubicin plasma levels at Period 2.

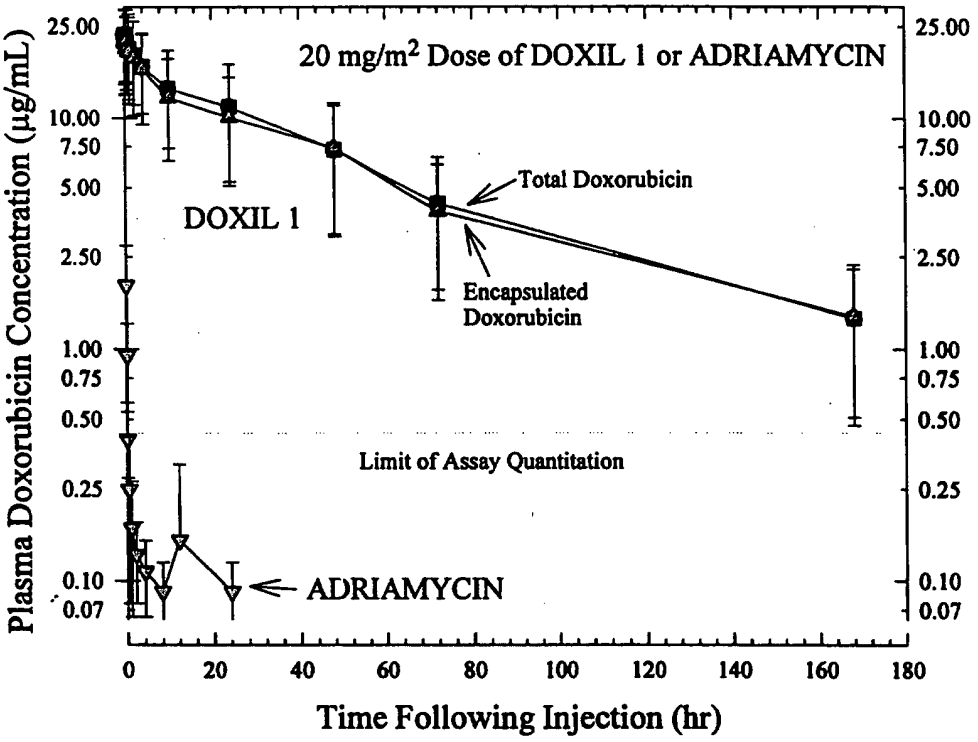
Peak plasma concentrations (C_{max}) and AUC were dose proportional. The mean C_{max} following 10 mg/m^2 was 4.1 μg/mL, and it was 8.9 μg/mL following 20 mg/m^2. Thirteen patients manifested a second peak plasma concentration after the end of infusion. The cause of this second peak is not known. Mean $AUC_{0\rightarrow240}$ was 232 μg/mL·h for the 10 mg/m^2 dose group and 532 μg/mL·h for the 20 mg/m^2 group. Mean $AUC_{0\rightarrow\infty}$ was 252 μg/mL·h for the 10 mg/m^2 group and 577 μg/mL·h for the 20 mg/m^2 group (Table 26).

DOXIL dispositional pharmacokinetics were independent of dose. Disposition of drug occurred in two phases after DOXIL administration, with a relatively short first phase and a prolonged second phase. Mean $t_{½}\lambda_1$ was 5.8 and 5.6 hours in the 10 and 20 mg/m^2 dose groups, respectively. Mean $t_{½}\lambda_2$ was 50.1 and 56.6 hours in the 10 and 20 mg/m^2 dose groups, respectively. Mean CLt was low (0.06 L/h/m^2 for the 10 mg/m^2 dose group and

0.04 for the 20 mg/m^2 dose group) and mean V_{ss} was small (2.8 L/m^2 for both dose groups).

Very low levels of doxorubicinol, the primary metabolite of doxorubicin, were detected in the plasma. Levels ranged from 0.8 to 11.9 ng/mL in patients who received DOXIL 10 or 20 mg/m^2 after both the first and second dose groups. This represented approximately 0.3% of the measured doxorubicin levels in plasma.

FIGURE 7
Plasma Clearance of Adriamycin and DOXIL 1



C. Amount of Non-liposomal Doxorubicin in Plasma

Several lines of evidence support the conclusion that the majority of the doxorubicin (between 93% and 99%) in plasma is encapsulated within the liposome after i.v. administration of DOXIL. In a study conducted with DOXIL 1, the fraction of the liposome-encapsulated and free, non-liposomal drug in circulation was quantitated

directly using a Dowex column separation method that is able to accurately and reproducibly quantitate ≥ 7% free drug in the plasma (LTI-30-02). Essentially all the doxorubicin measured in plasma was liposome-associated (Figure 7). Similar findings have been reported in nonclinical pharmacokinetic studies in rats (LTI Report No. 30-92-27). These findings suggest that at least 90 to 95% of the doxorubicin measured in plasma, and possibly more, is liposome-encapsulated.

Doxorubicinol levels have been reported to range from 40 to 60% of the doxorubicin levels after administration of Adriamycin due to its rapid and extensive metabolism.[81,116] In the study of DOXIL pharmacokinetics described in Section VIII (Study 30-14), plasma doxorubicinol concentrations ranged from 0.1 to 0.5% of the doxorubicin concentrations in plasma. Based on the conservative doxorubicinol:doxorubicin concentration ratio reported in the literature, free doxorubicin levels would be expected to be approximately 0.25 to 1.25% of the total measured drug concentration after DOXIL treatment. Since only non-liposomal doxorubicin can be metabolized, these findings suggest that 99% of the drug remains liposome-encapsulated after DOXIL treatment.

The results of a physiologically-based pharmacokinetic simulation model constructed using these data, support this prediction (LTI-30-94-16). The simulation, which included modeling components for the pharmacokinetic behavior of both liposomal and non-liposomal doxorubicin and accounted for the leakage rate of drug from the liposomes, also suggested that 1% or less of the doxorubicin in the plasma after DOXIL treatment was not liposome-encapsulated.

D. Comparison of DOXIL Pharmacokinetic Parameters (Study 30-14) to Literature Values for Adriamycin Pharmacokinetic Parameters

Plasma clearance kinetics of Adriamycin were measured in two studies; Study 30-02 and Study 30-05. However, plasma levels of doxorubicin fell rapidly below the quantitation limits of the assays employed in both studies (5.0 ng/mL and 500 ng/mL, respectively), preventing determination of pharmacokinetic parameters for Adriamycin. According to literature reports, an i.v. bolus injection of Adriamycin in humans produces high plasma concentrations of doxorubicin that decline quickly due to rapid and extensive distribution into tissues.[70] Apparent volumes of distribution range from 1400 to 3000 L, reflective of the drug's extensive tissue distribution. The Adriamycin plasma concentration-time curve in humans is biphasic, with a distribution half-life of 5 to 10 minutes and terminal phase elimination half-life of 30 hours.[71,72] A triphasic curve has also been described with a terminal plasma half-life of approximately 30 hours.[74] Clearance of doxorubicin

after Adriamycin administration ranges from 24 to 73 L/hour.[70] No accumulation in plasma occurs after repeated injections.[71,72]

The pharmacokinetics of DOXIL appear to be significantly different from those reported for Adriamycin. Administration of DOXIL results in a significantly higher doxorubicin AUC, lower rate of clearance (approximately 0.1 L/hour) and smaller volume of distribution (5 to 7 L) relative to administration of Adriamycin. The first phase of the biexponential plasma concentration-time curve after DOXIL administration is relatively short (approximately 5 hours), and the second phase, which represents the majority of the AUC, is prolonged (half-life 50 to 55 hours).

Doxorubicin C_{max} after DOXIL administration is 15- to 40-fold higher than after the same dose of Adriamycin, and the ratio quickly increases as doxorubicin is rapidly cleared from circulation. Importantly, the vast majority of the total plasma doxorubicin remains liposome-encapsulated after DOXIL treatment. Because of the high percentage of liposome encapsulation in DOXIL, the amount of free drug in the plasma appears to be significantly lower than that measured after administration of an equal dose of Adriamycin.

This conclusion is supported by the same type of calculations presented above, which derive the apparent concentration of free doxorubicin based on the reported relationship between doxorubicinol and doxorubicin concentrations in plasma. For example, five minutes after the end of the infusion, the mean doxorubicinol level following a 20 mg/m^2 dose of DOXIL was approximately 22 ng/mL. Using the doxorubicinol:doxorubicin concentration ratio reported in the literature, as described above, predicted free doxorubicin concentration at this time point would be 54 ng/mL in DOXIL-treated patients (the total plasma concentration measured at this time point was 8863 ng/mL). Compáratively, patients in Study 30-05 who received a dose of Adriamycin 20 mg/m^2, had initial plasma concentrations of doxorubicin of approximately 500 ng/mL.

E. Comparison of DOXIL and DOXIL 1 Pharmacokinetic Parameters (Studies 30-05 and 30-14)

No formal bioequivalence study of DOXIL, the proposed commercial formulation, and the previous formulation, DOXIL 1, was conducted in humans because of lack of supply of DOXIL 1. However, using the data from Study 30-14 and Study 30-05, the pharmacokinetics and KS lesion localization of DOXIL can be compared to those of DOXIL 1. These two studies were conducted in a population of patients with similar characteristics by the same investigator. Study 30-14 utilized a longer infusion time (30

minutes compared to 15 minutes in Study 30-05), a longer sampling interval (240 hours as compared to 96 hours in Study 30-05), and a more sensitive HPLC assay (lower limit of quantitation 5 ng/mL compared to 100 ng/mL in Study 30-05). The same modeling and parameter estimation techniques were used for both studies. The comparison of the pharmacokinetics included only the first dose pharmacokinetics of Study 30-14 and Study 30-05. For Study 30-14, the 10 and 20 mg/m^2 dose groups were combined for the determination of the median dose-independent parameters; dose-dependent parameters were normalized for dose.

No statistical tests were utilized to compare the pharmacokinetics of the two formulations, since the studies were not prospectively intended to be compared. Instead, a direct visual comparison of median pharmacokinetic parameters was applied. Pharmacokinetic parameters are summarized in Table 27. By inspection, it appears that V_p, $t_{½\lambda_2}$, and $AUC_{0\rightarrow\infty}$ may be different between formulations. These observations are consistent with what is known about these formulations. The decreased peripheral volume of the DOXIL formulation may reflect the decreased leakage rate of doxorubicin. In contrast, the DOXIL 1 formulation, which has a higher leakage rate of free doxorubicin, will express a larger V_p, reflecting the large volume of distribution of free doxorubicin. The increase in terminal half-life and $AUC_{0\rightarrow\infty}$ of DOXIL are both the result of decreased release rate of doxorubicin from DOXIL liposomes. It appears that DOXIL may provide a larger and more sustained exposure profile to doxorubicin than does DOXIL 1.

TABLE 27
Summary of Median (range) DOXIL and DOXIL 1 Pharmacokinetic Parameters

Parameter	DOXIL (N = 42)	DOXIL 1 (N = 9)
$AUC_{0\rightarrow\infty}$[a] (μg/mL/mg·hr)	13.2 (1.50-28.3)	8.09 (4.87-14.7)
V_c (L/m^2)	2.34 (1.12 - 3.79)	2.27 (1.60-2.75)
V_p (L/m^2)	0.452 (0.206 - 1.29)	1.21 (0.463-1.65)
V_{ss} (L/m^2)	2.79 (1.44 - 4.51)	3.25 (2.16 - 4.39)
CLt (L/hr/m^2)	0.0413 (0.0171 - 0.358)	0.0670 (0.0340 - 0.108)
$t_{1/2\lambda 1}$ (hr)	3.13 (0.542 - 16.5)	3.76 (2.01 - 4.90)
$t_{1/2\lambda 2}$ (hr)	48.7 (6.00 - 98.9)	41.3 (19.8 - 54.0)

[a] $AUC_{0\rightarrow\infty}$ are normalized to dose.

A comparison of KS lesion levels of doxorubicin in patients treated with DOXIL and DOXIL 1 fails to show a difference between the two formulations. Results are

summarized below in Table 28. Direct comparison was limited by differences in the time period post-treatment at which biopsy samples were collected and the large variability in measured concentrations. In general, however, KS lesion concentrations appeared to be in the same range in both DOXIL-treated and DOXIL 1-treated patients, although some patients who received DOXIL had markedly higher concentrations of doxorubicin in their lesions, which is consistent with the pharmacokinetic results.

TABLE 28
Range of Doxorubicin Concentrations in KS Lesions from DOXIL- and DOXIL 1-treated Patients

Treatment	Dose (mg/m2)	Sampling Time	Concentration Range (μg/g tissue)
DOXIL[a]	10	48 hours	0.2 - 22.4
		96 hours	1.9 - 36.4
	20	48 hours	3.0 - 25.6
		96 hours	1.0 - 4.2
DOXIL 1[b]	10	72 hours	1.6 - 2.4
	20	"	0.8 - 2.4
	40	"	4.6 - 9.4

[a] Seven patients in each of the 48-hour sampling groups and 4 patients in each of the 96-hour sampling groups.
[b] Three patients in each dose group.

F. Doxorubicin Levels in KS Lesions (Studies 30-05 and 30-14)

In Study 30-14, biopsies of KS lesion tissue and adjacent normal skin were obtained in 22 patients (Table 29). Doxorubicin levels in KS lesions were higher than the levels in normal skin in 20 of the 22 patients; in 14 patients normal skin levels were below the lower limit of quantitation (0.4 μg/g tissue), whereas all KS lesion levels were quantifiable. Forty-eight hours after DOXIL administration, median doxorubicin levels in biopsies of KS lesions ranged from 3-fold to 16-fold higher than in normal skin from the same patients. The median doxorubicin concentration in KS lesions was 1.3 μg/g tissue in 7 patients receiving 10 mg/m^2 and 15.2 μg/g tissue in 7 patients receiving 20 mg/m^2; normal skin concentrations were 0.4 and 0.9 μg/g tissue in the 10 and 20 mg/m^2 dose groups, respectively. Ninety-six hours after drug treatment, KS lesion doxorubicin levels were 3-fold and 5-fold greater than in normal skin from the same patients in the 10 and 20 mg/m^2 groups, respectively. Median doxorubicin concentration in KS lesions was 4.3 and 3.3 μg/g tissue in 4 patients receiving 10 mg/m^2 and 4 patients

receiving 20 mg/m^2 dose, respectively; median concentration in the normal skin was 1.4 μg/g tissue for the 10 mg/m^2 dose group, and 0.7 μg/g tissue in the 20 mg/m^2 group.

TABLE 29
Concentration of Doxorubicin in KS Lesions and Normal Skin After DOXIL Administration

Time after Infusion	No. of Patients	Dose (mg/m^2)	Doxorubicin Concentration (μg/g tissue) Median (range)		KS/Normal Skin
			KS Lesion	Normal Skin	
48 hr	7	10	1.32 (0.17-22.43)	0.40 (0.26-1.55)	2.43
	7	20	15.21 (2.98-25.56)	0.92 (0.38-1.74)	20.89
96 hr	4	10	4.26 (1.91-36.44)	1.42 (0.70-2.78)	3.20
	4	20	3.28 (1.03-4.17)	0.73 (0.55-1.14)	4.94

Although too few time points were studied to allow determination of an AUC for doxorubicin in KS lesions or skin, these data suggest that doxorubicin accumulates in KS lesions after DOXIL treatment.

The increased doxorubicin in KS lesions compared to normal skin is not simply due to increased vascularization and/or blood content of the lesions. All samples were blotted after collection to remove excess blood, which would minimize differences due to blood content. However, even the most conservative comparison of doxorubicin levels in KS lesions and normal skin suggests that doxorubicin selectively accumulates in KS lesions. A comparison based only on blood content is made when the entire weight of the lesion or skin sample is assumed to be due to blood (i.e., there is no weight contribution from tissue). Because plasma constitutes 60% of blood, lesion doxorubicin levels due to blood content will be a maximum of 0.6-fold the plasma level of doxorubicin at the time of collection. At 48 hours after DOXIL treatment, median ratios of the doxorubicin concentration in KS lesions to predicted levels if all drug was due to blood were 2.8 and 5.5 in the 10 and 20 mg/m^2 treatment groups, respectively, compared to 0.6 and 3.4 in normal skin (Table 30). At 96 hours after treatment, the median ratio of KS lesions to blood was 5.5 and 1.9 in the 10 and 20 mg/m^2 treatment groups, respectively, compared to 0.4 and 1.6 in the normal skin biopsies.

Table 30
Predicted Accumulation of Doxorubicin in KS Lesions and Skin Samples: (Median Values)

Time Point (hours)	DOXIL Dose (mg/m^2)	KS Lesion Ratio[a]	Skin Ratio[a]	KS Ratio / Skin Ratio
48	10	2.8	0.6	4.7
	20	5.5	3.4	1.6
96	10	5.5	0.4	13.8
	20	1.9	1.6	1.2

[a] Ratio of measured doxorubicin concentration to calculated concentration due to blood content. Lesions and skin sample weight assumed to be due to blood content only.

A more accurate estimate of the contribution of blood to the doxorubicin content measured in KS lesions and normal skin can be obtained using the published blood content of skin. Normal skin has 1.0 mL of blood per 40 grams of tissue (0.025 mL/g).[117] Plasma constitutes 60% of blood, and, thus, the blood content of skin can be calculated as 0.6 x sample weight in grams x 0.025. The ratio of actual sample concentration to predicted concentration based on blood content only can be determined on a patient-by-patient basis, providing an estimate of accumulation of drug in skin (Table 31). However, although definitive data are not available, it is possible that KS lesions have a higher blood content than normal skin, accounting for the greater accumulation of doxorubicin in the lesions. Thus, the data in Table 31 were calculated assuming the blood content of KS lesions was twice that of skin. Even with this assumption, the accumulation ratios calculated for the lesions are 2- to 7-fold higher than those for normal skin, with the exception of the 20 mg/m^2 dose group at the 96-hour time point, which was 0.6 that of normal skin.

TABLE 31
Accumulation Ratio of Doxorubicin in KS Lesions and Skin Samples[a] (Median Values)

Time Point (hours)	DOXIL Dose (mg/m^2)	KS Lesion Ratio[b]	Skin Ratio[b]	KS Ratio / Skin Ratio
48	10	55	26	2.1
	20	438	137	3.2
96	10	110	16	6.9
	20	37	63	0.6

[a] Ratio of measured doxorubicin concentration to calculated concentration due to blood content.
[b] Blood content of KS lesions assumed to be twice that of normal skin.

If the blood content of skin and KS lesions are assumed to be identical, even more strikingly increased ratios are calculated. Accumulation ratios range from 1.2 to nearly 14-fold higher in KS lesions compared to normal skin (Table 32).

TABLE 32
Accumulation Ratio of Doxorubicin in KS Lesions and Skin Samples[a]
(Median Values)

Time Point (hours)	DOXIL Dose (mg/m^2)	KS Lesion Ratio[b]	Skin Ratio[b]	KS Ratio / Skin Ratio
48	10	110	26	4.2
	20	876	137	6.4
96	10	218	16	13.6
	20	75	63	1.2

[a] Ratio of measured doxorubicin concentration to calculated concentration due to blood content.
[b] Blood content of KS lesions and normal skin assumed to be equivalent.

In Study 30-05, doxorubicin levels in KS lesions 72 hours after treatment with DOXIL 1 were found to range from five- to 11-fold higher than after the same dose of Adriamycin (Table 33). These levels may not represent the peak tumor levels achieved. Considering the shorter half-life of the major disposition phase of doxorubicin after delivery as Adriamycin, it is possible that peak tumor levels occurred earlier than 72 hours in the Adriamycin-treated patients. However, doxorubicin remains in bone marrow, blood cells and other tissues for weeks after administration of Adriamycin.[74,88,118] Doxorubicin concentrations in blood cells, which always exceed plasma concentration, do not decline markedly in the week after treatment with Adriamycin.[87,119] Animal studies have shown that tumor levels of doxorubicin are higher after treatment with DOXIL even at 1 hour post-treatment.[49] These observations suggest that doxorubicin levels in the KS lesions of Adriamycin-treated patients probably never attained the levels measured after DOXIL treatment.

TABLE 33
Comparison of Doxorubicin Concentration in KS Lesions After DOXIL 1 or Adriamycin Administration

Dose Level mg/m^2	Doxorubicin Concentration (µg/gm tissue) Mean ± SD (n=3)		Selectivity[a] Index
	DOXIL	Adriamycin	
10	2.06 ± 0.42	0.18 ± 0.07	11.4
20	1.61 ± 0.80	0.31 ± 0.16	5.2
40	7.71 ± 2.72	0.82 ± 0.18	9.4

[a] Selectivity Index = Quotient of doxorubicin concentration measured in patients receiving DOXIL divided by the doxorubicin concentration in patients receiving Adriamycin.

The mechanism by which liposome-encapsulated drug accumulates within KS lesions is not fully understood. Stealth liposomes of the same size and lipid composition as DOXIL, but containing entrapped colloidal gold designed to serve as a marker to follow liposome distribution by light and electron microscopy, have been shown to enter solid colon tumors implanted in mice[45] and KS-like lesions in HIV-transgenic mice.[46] In the latter mouse model, transcytosis of liposomes from the lumen of blood vessels into the extravascular compartment of KS lesions and intracellular uptake of liposomes by spindle cells within lesions were observed.[46] Extravasation of liposomes may also occur by passage of the particles through endothelial cell gaps which have been reported to be present in certain solid tumors[8,9] and in KS-like lesions.[7,120] These processes may contribute to the selective uptake of DOXIL in KS lesions seen here.

Clinical Pharmacology Reports Referenced

Report Number: LTI-30-14
Report Title: Randomized, crossover, pharmacokintics and tumor localization study of Stealth® liposomal doxorubicin hydrochloride in patients with AIDS and Kaposi's sarcoma.
Study Dates: [redacted], 19[redacted] to [redacted], 19[redacted] (Report date [redacted], 19[redacted])
Principal Investigator: [redacted]
Study Site: [redacted]
DOXIL Lot No.: 0193

Report Number: LTI-30-05
Report Title: Randomized pharmacokinetics, dose-finding and tumor localization study of Stealth® liposomal doxorubicin hydrochloride compared to Adriamycin® in patients with AIDS and Kaposi's sarcoma.
Study Dates: [redacted], 19[redacted] to [redacted], 19[redacted] (Report date [redacted], 19[redacted])
Principal Investigator: [redacted]
Study Site: [redacted]
DOXIL 1 Lot No.: 146P, 0161, 0163, 0168, 0175, 0190, 0192, 0193

Report Number: LTI-30-02
Report Title: A pilot clinical study of doxorubicin USP encapsulated in Stealth® liposomes.
Study Dates: [redacted], 19[redacted] to [redacted], 19[redacted] (Report date [redacted], 19[redacted])
Principal Investigator: [redacted]
Study Site: [redacted]
DOXIL Lot No.: 0137, 140, 141, 146P, 152

Report Number: LTI-30-94-16
Report Title: A physiologically based pharmacokinetic simulation model of DOXIL™ (Stealth liposomal doxorubicin HCl) Injection plasma pharmacokinetics in humans.
Report Dates: [redacted], 19[redacted]
Author: [redacted]