VII. MICROBIOLOGY SUMMARY

A Microbiology Summary is not presented in this Overall NDA Summary as DOXIL is not indicated for use as an anti-infective drug product.

However, microbiological data were developed in relation to validation of the sterile filtration and aseptic fill operations of the DOXIL production process. A summary of these data can be found in the Microbiology subsection of the Clinical Data Section of this NDA.

VIII. CLINICAL DATA SUMMARY

A. Introduction

DOXIL® (Stealth® liposomal doxorubicin hydrochloride) Injection is being developed in the United States under IND 36,778, originally filed [redacted], 19[redacted] by Liposome Technology, Inc. (LTI) in the Division of Oncology and Pulmonary Drug Products. DOXIL is not marketed in any country outside the United States, however DOXIL is made available on a Named Patient Sale basis in Austria, Ireland, Israel, Italy, and the United Kingdom.

DOXIL is proposed for intravenous (i.v.) administration at an initial dose of 20 mg/m^2 every 2 to 3 weeks. The proposed indication, based on the information contained in this New Drug Application (NDA), is for use in patients with AIDS-related Kaposi's sarcoma (KS) which is refractory to systemic combination chemotherapy or for use in those patients who have experienced unacceptable toxicity. In the principal clinical trial supporting this indication, patients were considered "refractory" by an independent panel of three physicians expert in the treatment of KS if they met both of the following criteria:

1) Prior treatment with at least two systemic chemotherapy drugs for treatment of AIDS-related KS for at least two cycles of therapy. (One, but not both, of the drugs could have been a vinca alkaloid; alpha-interferon was not considered a systemic chemotherapeutic drug.)

2) Had progressive disease or could not tolerate continued standard therapy due to drug toxicities. (Patients with stable disease did not qualify unless they could not tolerate continued therapy with conventional systemic chemotherapy.)

Given the seriousness of the indication, LTI is filing this NDA on the basis of interim efficacy results from two studies: Study 30-12, the principal study supporting efficacy in treatment-refractory patients, and Study 30-03, a supportive study. This NDA also contains results of three clinical pharmacology studies: Study 30-02, Study 30-05, and Study 30-14. An overview of DOXIL clinical trials contained in this NDA is presented in Table 34 and further described in Section VIII.C.

This clinical data summary is organized as follows:

- This introduction;
- Clinical pharmacology;
- Overview of clinical studies;
- Efficacy studies supporting the indication;
- Safety summary - general safety conclusions.

TABLE 34
Overview of DOXIL Clinical Studies Contained in NDA

Study Number	Location	Principal Investigator(s)	Dates of Conduct[a]	IND	Formulation(s)
		Clinical Pharmacology Studies			
30-02	Israel	[redacted]	[redacted]	Non-IND	DOXIL 1
30-05	U.S.	[redacted]	[redacted]	36,778	DOXIL 1 DOXIL 3
30-14	U.S.	[redacted]	[redacted]	36,778	DOXIL 3
		Phase 2/3 Efficacy Studies – AIDS-Related KS			
30-03	Australia/ Europe	Multicenter (22)	[redacted]	Non-IND	DOXIL 1 DOXIL 2 DOXIL 3
30-12	U.S./Europe	Multicenter (18)	[redacted]	36,778	DOXIL 3

[a] Study start date is date of initial dosing in first patient; study end date is the end of dosing.

During the course of development, the DOXIL formulation has evolved. The initial formulation, DOXIL 1, was unbuffered and stored frozen. Subsequently, a liquid formulation was developed. The early liquid formulation, designated DOXIL 2, had limited use in the clinic. The final liquid formulation, DOXIL 3 differs from DOXIL 2 solely in the buffer used. The final formulation has been used in the primary AIDS-related KS clinical study, Study 30-12, in part of the supportive clinical study, Study 30-03, and in a pharmacokinetic study, Study 30-14. DOXIL 3 (hereafter referred to as DOXIL) has been used in a complete nonclinical pharmacology and toxicology program and is the formulation proposed for commercial marketing. A summary of the DOXIL formulations used in the clinical trials follows in Table 35.

TABLE 35
Summary of DOXIL Formulations used in Clinical Trials

	Frozen Formulation	Liquid Formulations	
Component	**DOXIL 1**	**DOXIL 2**	**DOXIL 3**
Doxorubicin HCl	[redacted] mg/mL	[redacted] mg/mL	2.0 mg/mL
HSPC	[redacted] mg/mL	[redacted] mg/mL	9.58 mg/mL
MPEG-DSPE	[redacted] mg/mL	[redacted] mg/mL	3.19 mg/mL
Cholesterol	[redacted] mg/mL	[redacted] mg/mL	3.19 mg/mL
Sucrose[a]	[redacted] mg/mL	[redacted] mg/mL	94 mg/mL
[redacted]	[redacted]	[redacted] mg/mL	--
Histidine	[redacted]	[redacted]	1.55 mg/mL
Ammonium Sulfate[a]	[redacted] mg/mL	[redacted] mg/mL	2 mg/mL
[redacted]	[redacted] mg/mL	[redacted]	--
[redacted]	[redacted] mg/mL	[redacted]	--
Water for injection	qs [redacted] mL	qs [redacted] mL	qs 1 mL
Formulation pH	[redacted]	[redacted]	6.5

[a] Estimated based on theoretically calculated liposome encapsulated volume.

B. Clinical Pharmacology

Three clinical pharmacology/pharmacokinetic studies are available. One study, Study 30-14, was an evaluation of the pharmacokinetics and KS tumor levels of DOXIL, the proposed commercial formulation. The previous frozen formulation, DOXIL 1, has been studied in two earlier clinical pharmacology trials, Study 30-02 and Study 30-05. Comparisons to conventional doxorubicin HCl (Adriamycin) were included in Study 30-05 and Study 30-02. The clinical pharmacology studies are summarized below in Table 36.

TABLE 36
Overview of Open-label, Randomized, Crossover Clinical Pharmacology Studies

Study No.	Principal Investigator	N M/F[a]	Diagnosis	Mean Age (range)	Dose	Formulation Code
30-02	[redacted]	6/10	Solid tumor	56.3 years (38 to 73 yrs)	25 - 50 mg/m^2 crossover[b]	DOXIL 1 Adriamycin
30-05	[redacted]	18/0	Kaposi's sarcoma	38.6 years (29 to 48 yrs)	10 - 40 mg/m^2 crossover[b]	DOXIL 1 Adriamycin
30-14	[redacted]	43/0	Kaposi's sarcoma	39.0 years (28 to 50 yrs)	10 - 20 mg/m^2 crossover 2-period	DOXIL

[a] M=male, F=female
[b] Originally a two-period crossover study; indefinite extension treatment allowed by protocol amendments.

Synopses for each of these pharmacokinetic studies are provided below. Following these summarizations, there is an overall discussion of the clinical pharmacokinetics of liposomal doxorubicin. The reader is referred to the Human Bioavailability and Pharmacokinetics subsection in the Clinical Data Section of this NDA for a more complete discussion.

1. Clinical Pharmacology/Pharmacokinetics of DOXIL

The pharmacokinetics and safety of DOXIL, the proposed commercial formulation was studied in Study 30-14, a two-period, randomized, crossover study. Doxorubicin HCl was given as single doses of DOXIL 10 and 20 mg/m^2 administered i.v. over 30 minutes. The doses were separated by a 3-week washout period. Plasma samples were collected over 10 days after each dose; tissue samples were collected 48 and 96 hours after a single dose of either DOXIL 10 or 20 mg/m^2.

Forty-three male patients with AIDS-related KS were enrolled. Twenty-four patients enrolled in the crossover trial received both DOXIL 10 and 20 mg/m^2 in a randomized fashion; plasma pharmacokinetic data are summarized for 23 of these patients. The remaining 19 patients received a single dose; 8 patients received 10 mg/m^2 and 11 patients received 20 mg/m^2. Thirty-nine of the patients subsequently entered Study 30-12 for continued treatment, 5 of whom entered Study 30-12 prior to the data cut-off for this NDA.

DOXIL displayed linear pharmacokinetics best described by a two-compartment model. There were no carryover effects, although there were very low residual doxorubicin levels at period 2 in some patients. 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 in plasma concentration after the end of infusion. The cause of this second peak is not known, but its occurence did not affect the overall primary pharmacokinetic parameters of DOXIL in this patient population. 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. 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 $t_{½\lambda1}$ was 5.8 and 5.6 hours in the 10 and 20 mg/m^2 dose groups, respectively. Mean $t_{½\lambda2}$ was 50.1 and 56.6 hours in the 10 and 20 mg/m^2 dose groups, respectively. CLt was low (0.06 L/h/m^2 for the 10 mg/m^2 dose group and 0.04 L/h/m^2 for the 20 mg/m^2 dose group) and 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.

Biopsies of KS lesion tissue and adjacent normal skin were obtained in 22 patients. Data could not be analyzed statistically because of variability in the measured doxorubicin concentrations. 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 (15 ng/sample or 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 were 3-fold higher in the 10 mg/m^2 group and 16-fold higher in the 20 mg/m^2 group 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.

2. Clinical Pharmacology/Pharmacokinetics of DOXIL 1

The pharmacokinetics and safety of DOXIL 1 have been studied in two clinical trials. These are Study 30-05 and Study 30-02. These studies are summarized below.

a. Study 30-05

Study 30-05 was a two-period, crossover comparison of three dose levels of DOXIL 1 and Adriamycin. Eighteen male patients with AIDS-related KS were enrolled and participated in only one dose level group: 10, 20 or 40 mg/m^2 , given as a 15 minute i.v. infusion. Within each dose level group, patients were prospectively randomized (1:1) to receive a first dose of either DOXIL 1 or Adriamycin. There was a 3-week washout between periods. Six patients received both DOXIL 1 and Adriamycin 10 mg/m^2 and 6 received both DOXIL 1 and Adriamycin 20 mg/m^2. Three patients in the 40 mg/m^2 dose group received only the first dose. Subsequently, 12 patients continued to receive open-label DOXIL (administered initially as DOXIL 1 and subsequently as DOXIL when it became available). Doses in the open-label extension phase ranged from 10 to 20 mg/m^2; 3 patients received 12 cycles or more and 1 of these received 27 cycles. In addition, 4 of the patients entered Study 30-12 when Study 30-05 closed; 1 of these patients entered Study 30-12 prior to the NDA data cut-off.

Plasma samples were to be collected before dosing and repeatedly over 4 days (96 hours) following the end of the infusion. Lesions were to be biopsied 72 hours after the first dose. Following extraction from plasma and tissues, doxorubicin and doxorubicinol (plasma only) concentrations were determined using reverse-phase HPLC with fluorescence detection.

Dose-proportional increases were seen in doxorubicin C_{max} and in AUC following administration of DOXIL 1. Mean C_{max} was 4.3, 10.1, and 20.1 μg/mL in the 10, 20 and 40 mg/m^2 dose groups, respectively. Mean $AUC_{0\rightarrow\infty}$ was 184, 341, and 642 μg/mL·h, respectively. Doxorubicin disposition pharmacokinetics were independent of dose when administered as DOXIL 1. Mean $t_{½\lambda 1}$ was 4.3, 3.5, and 3.5 hours in the 10, 20 and 40 mg/m^2 dose groups, respectively. Mean $t_{½\lambda 2}$ was 43.7, 35.8, and 38.0 hours in the 10, 20, and 40 mg/m^2 dose groups, respectively. Doxorubicin CLt after DOXIL 1 ranged from 0.06 to 0.07 L/hr/m^2, and V_{ss} was small (3.2 to 3.5 L/m^2).

In contrast, doxorubicin was rapidly removed from the plasma following administration as Adriamycin. Five minutes after dosing, the mean plasma concentration of doxorubicin in Adriamycin-treated patients was 0.3, 0.5, and 1.5 μg/mL in the 10, 20, and 40 mg/m^2 dose groups, respectively. All remaining plasma concentrations were below the limit of quantitation of the HPLC assay (100 ng/mL). As a result, pharmacokinetic parameters could not be calculated for Adriamycin for this study.

Doxorubicinol was not detected in the plasma following administration of either DOXIL 1 or Adriamycin.

The mean doxorubicin concentration in KS lesions following DOXIL 1 was 2.1, 1.6, and 7.7 μg/g tissue in the 10, 20 and 40 mg/m^2 dose groups, respectively. The mean concentration following Adriamycin was 0.2, 0.3, and 0.8 μg/g tissue in the 10, 20 and 40 mg/m^2 dose groups, respectively. Consequently, concentrations were 11.4-fold, 5.2-fold, and 9.4-fold higher in lesions from patients given DOXIL 1 as compared to patients given Adriamycin, for the 10, 20 or 40 mg/m^2 dose groups, respectively.

b. Study 30-02

The pharmacokinetics, safety and efficacy of DOXIL 1 and Adriamycin were compared in Study 30-02, an open-label, randomized, two-period, crossover study. Sixteen patients with advanced malignancies, 6 male and 10 female, were randomized to receive either DOXIL 1 or Adriamycin.

The first 6 patients treated with DOXIL 1, and the first 3 patients treated with Adriamycin, received 25 mg/m^2 initial doses. After modification of the study design to allow a higher initial dose, three patients received DOXIL 1, 50 mg/m^2, and 4 received Adriamycin 50 mg/m^2, as the initial dose. In all cases, the second and subsequent doses were DOXIL 1, 50 mg/m^2, unless modified because of intolerance. There was a 3-week washout between doses. Initially, drug was infused over 5 minutes; subsequently dosing was modified such that drug was administered over 15 minutes. Patients were to be allowed to continue in open-label treatment with DOXIL 1 at 50 mg/m^2.

Plasma samples were collected prior to drug administration and at frequent intervals through 192 hours after DOXIL 1 administration and through 24 hours after Adriamycin injection. Plasma samples from DOXIL 1-treated patients were passed through a Dowex resin column to separate liposome-associated doxorubicin from free and protein-bound drug. Doxorubicin plasma concentrations were determined using a modified total

fluorescence assay that does not distinguish between doxorubicin and its fluorescent metabolites. The lower limit of quantitation was 0.4 μg/mL.

Pharmacokinetic modeling was performed and pharmacokinetic parameters for total doxorubicin were derived as described for Study 30-14.

DOXIL 1 pharmacokinetics were evaluated in 8 patients at a dose of 25 mg/m^2 and 14 patients at 50 mg/m^2. Adriamycin pharmacokinetics were evaluated in 3 patients at a dose of 25 mg/m^2 and 4 patients at 50 mg/m^2.

Following administration as DOXIL 1, approximately dose-proportional increases in doxorubicin levels were observed in C_{max}. The mean C_{max} was 13.7 and 17.2 μg/mL in the 25 and 50 mg/m^2 groups, respectively. The mean $AUC_{0\rightarrow\infty}$ was 595 and 760 mg·hr/L, respectively. The mean $t_{½\lambda 1}$ was 4 to 6 hours. The mean $t_{½\lambda 2}$ was 42 to 48 hours. Mean CLt after DOXIL 1 administration was 0.05 to 0.08 L/hr/m^2, and mean V_{ss} ranged from 2.5 to 4.5 L/m^2.

There was essentially no difference between the plasma concentration of total and liposomal doxorubicin.

After administration of Adriamycin 25 and 50 mg/m^2, mean C_{max} was 1.6 and 1.9 μg/mL, respectively. Plasma levels fell below the assay limit of quantitation by 30 minutes after the infusion, and pharmacokinetic parameters could not be determined.

3. Overall Summary of the Clinical Pharmacokinetics of DOXIL

The plasma and tissue pharmacokinetics of DOXIL are summarized in section VI of this Overall NDA Summary (Human Pharmacokinetic Summary). A detailed summary of the amount of nonliposomal doxorubicin in plasma, doxorubicin levels in KS lesions and a comparison of DOXIL and Adriamycin pharmacokinetics has also been presented in Section VI of this Overall NDA Summary. In the following section, highlights of these studies are reviewed and critical pharmacologic findings summarized.

The pharmacokinetics of DOXIL are characterized by a biexponential plasma concentration-time curve, with a short first phase and a prolonged second phase that accounts for the majority of the AUC. Plasma concentration and AUC are dose-dependent, given as 10 or 20 mg/m^2, but disposition kinetics are independent of dose. Volume of distribution is relatively small, just several-fold the plasma volume and clearance is low.

Several lines of evidence support the conclusion that the majority of the doxorubicin remains encapsulated within the liposome after i.v. administration of DOXIL. In Study 30-02, the fraction of the liposome-encapsulated and total drug in circulation after DOXIL treatment was quantitated. Essentially all the doxorubicin measured in plasma was liposome-associated (see, for example, Figure 7, Section VI of this Summary). Similar results have also been reported in nonclinical pharmacokinetic studies in rats, i.e., at least 90 - 95% of the doxorubicin measured in plasma, and possibly more, is liposome-encapsulated (see LTI Study Report LTI-30-93-27). The low blood levels of doxorubicinol suggest that 99% of the drug remains liposome-encapsulated after DOXIL treatment. A physiologically-based pharmacokinetic simulation model (LTI-30-94-16) constructed using plasma data from Study 30-14 supported these findings and suggested that as little as 1% or less of the doxorubicin in the plasma after DOXIL treatment was not liposome-encapsulated.

In Study 30-14, the doxorubicin concentration of KS lesions ranged from approximately 3- to 16-fold higher than doxorubicin levels in normal skin biopsies collected from the same patients at the same time points. In Study 30-05, doxorubicin levels in KS lesions 72 hours after treatment with DOXIL 1 were found to range from 5- to 11-fold higher than after the same dose of Adriamycin. Animal studies have shown that tumor levels of doxorubicin are higher after treatment with DOXIL even at 1 hour post-treatment.[49]

The pharmacokinetics of DOXIL appear to be significantly different from those reported for Adriamycin. DOXIL has a significantly higher AUC, lower rate of clearance (approximately 0.1 L/hour) and smaller volume of distribution (5 to 7 L) than those parameters reported for Adriamycin. The first phase of the biexponential plasma concentration-time curve after DOXIL administration is relatively short (half-life 1 to 3 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 Adriamycin is rapidly cleared from circulation; however, the majority of plasma doxorubicin remains liposome-encapsulated after DOXIL treatment. Because of the high percentage of liposome encapsulation in DOXIL, initial free drug levels in the plasma appear to be significantly lower than those measured after administration of an equal dose of Adriamycin.

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 patients with similar characteristics by the same investigator. 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 37. By inspection, it appears that only 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 its decreased leakage rate of doxorubicin. In contrast, the DOXIL 1 formulation, which has a higher leakage rate of free doxorubicin, expresses 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 to doxorubicin than does DOXIL 1.

TABLE 37
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] $AUC_{0-\infty}$ is normalized to dose.

Clinical Pharmacology Reports Referenced

Report Number:	LTI-30-14
Report Title:	Randomized, crossover, pharmacokinetics and tumor localization study of Stealth® liposomal doxorubicin HCl 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]