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13. ABSTRACT (Maximum 200) Annemycin is a non-cross resistant doxorubicin analog that is formulated in a liposomal carrier. We have completed a phase I study and initiated a phase II study of liposomal-Annemycin in patients with metastatic breast carcinoma. The maximum tolerated dose was 210/mg/m ² . The limiting toxicity was myelosuppression. Other toxicities were limited to allergic reactions. No significant cardiotoxicity, alopecia, mucositis, nor gastrointestinal toxicity have been observed. No antitumor responses were observed in the Phase I study. Three patients have been accrued in the Phase II study. Tumor tissue is being stored for determination of MDR-1 and MRP status at baseline and after therapy.			
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FOREWORD

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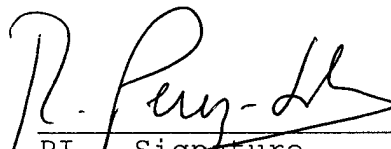
NA In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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Introduction

Doxorubicin is one of the most effective antitumor agents against hematological malignancies and certain human solid tumors such as breast carcinoma and osteosarcoma.¹ However, its use is limited by acute myelosuppression, chronic cardiotoxicity, and natural or acquired drug resistance. In the last few years, there have been important advances in understanding the mechanisms of acquired resistance to doxorubicin and other structurally unrelated antitumor agents. It is now widely accepted that the overexpression of a membrane glycoprotein, P-glycoprotein, that acts as a drug efflux pump, mediates acquired resistance to doxorubicin in some *in vitro* systems and *in vivo* animal tumor models.² It is also known that overexpression of P-glycoprotein occurs in a significant number of patients when their tumors progress or relapse after treatment with doxorubicin.³ The impact of the overexpression of P-glycoprotein in human tumors in mediating clinical drug resistance remains, nonetheless, unknown. Since the introduction of doxorubicin in the current anticancer armamentarium, extensive efforts have been devoted to the synthesis of analogs with improved properties. Initially, most efforts were directed towards the preparation of analogs with reduced cardiotoxic potential. Those efforts have been partially successful. More recently, and triggered by the discovery of the phenomenon of multidrug resistance and the identification of P-glycoprotein, the synthetic efforts have been more focused towards obtaining analogs with non-cross-resistance properties.⁴⁻⁷ Several non-cross resistant analogs have been identified. They all have in common a markedly increased lipophilicity. Some of them have a similar mechanism of cytotoxicity of that of doxorubicin, i.e., topoisomerase II inhibition. Others have a different mechanism of action, i.e., DNA alkylation.⁴

Liposomes have been used extensively as carriers of doxorubicin and daunorubicin.^{8,9} Liposomal-doxorubicin was found to be less cardiotoxic and more active than doxorubicin in models of liver metastases in mice by several investigators.^{10,11} Several clinical studies have been conducted with different liposomal formulations of doxorubicin. These trials have shown an MTD similar to that of free doxorubicin, a significant reduction of certain toxicities, such as gastrointestinal and vesicant effects, an unchanged dose-limiting toxicity, i.e., myelosuppression. These studies have also suggested, although have not proved, a reduced cardiotoxic potential.

Annamycin is an anthracycline antibiotic which was selected for its lack of cross-resistance properties and a very high affinity for lipid membranes.¹² Because of its latter property, Annamycin is an ideal compound for liposome entrapment. Because the drug is completely insoluble in water solutions, liposomes can be used as a carrier for its intravenous administration. The fundamental mechanism of action of Annamycin appears to be inhibition of topoisomerase-II. Liposomal-Annamycin was developed to combine the intrinsic favorable properties of the compound (lack of cross-resistance) with the potential advantages associated with liposome delivery (reduced cardiotoxicity and preferential distribution to certain organs). We have developed a liposomal formulation of Annamycin that has very high entrapment efficiency, physical stability, and chemical

stability. Liposomal-Annamycin has shown lack of cross-resistance *in vivo* in KB/-VI human xenografts and enhanced antitumor activity compared with doxorubicin several mouse tumor models *in vivo*.¹³ In mice, the dose-limiting toxicity of liposomal Annamycin is myelosuppression and its cardiotoxic potential less than that of doxorubicin. In dogs, the dose equivalent to the mouse LD10 was very well tolerated with no side effects, no blood chemical changes, and no pathological changes four weeks after drug administration.

Because MDR-1 and MRP overexpression have been observed in a high proportion of patients with breast cancer refractory to doxorubicin and paclitaxel, we performed a phase I study of liposomal-Annamycin in patients with breast carcinoma and other solid tumors and have started a phase II study in patients with breast carcinoma refractory to doxorubicin.

Study Design

1. Drug Information

Liposomal-Annamycin was prepared in Dr. Perez-Soler's laboratory at M. D. Anderson Cancer Center as a preliposomal lyophilized powder produced under sterile conditions. The preliposomal product was reconstituted on the same day of use.

For more information see copy of clinical protocol in Appendix. The study was performed under M. D. Anderson IND #46,869.

2. Eligibility Criteria

1. Patients with solid tumors with natural or acquired resistance to doxorubicin having received at least two prior chemotherapeutic regimens.
2. Life expectancy \geq 12 weeks.
3. Measurable or evaluable disease.
4. Prior chemotherapy given \geq 3 weeks prior to entry.
5. Adequate bone marrow, kidney, and liver function.
6. Consent form signed.

3. Treatment Plan

Liposomal-Annamycin was given as a 30-60 minute i.v. infusion using a peripheral vein via pump. Courses were repeated every three weeks or upon recovery of blood counts. The starting dose was 3 mg/m². Escalations were contemplated depending on the toxicity observed at the previous dose level.

Results

1. Patient Characteristics

Table 1 shows the characteristics of the patients entered in the study. Most patients had metastatic breast cancer refractory to doxorubicin and paclitaxel and had received ≥ 3 prior chemotherapeutic regimens. Therefore, the population was heavily pretreated but still most patients had a performance status of 1.

Table 1
Patient Characteristics

Patients	F	24
	M	<u>12</u>
		36
Age Median (Range)		56 (37-74)
Performance Status	0	6
	1	19
	2	11
Prior Chemotherapy Regimens	1	3
	2	11
	3	13
	>3	9
Tumor Type		
Breast	14	
NSCLC	6	
SCLC	1	
Colon	5	
Melanoma	2	
Others	7	

2. Dose Escalation

The starting dose was 3 mg/m². Subsequent dose levels studied were 6, 12, 24, 30, 60, 120, 180, 240, and 210 mg/m². The maximum tolerated dose was 210 mg/m² as discussed below.

3. Toxicity

a. Myelosuppression: The limiting toxicity was myelosuppression. Table 2 shows the myelosuppression observed at each dose level. Thrombocytopenia was the

dose limiting toxicity at 240 mg/m², with 2 of 4 developing grade 4 platelet counts. One additional patient with perfect hematologic tolerance through 5 courses at 120 mg/m² developed grade 3 thrombocytopenia after his first course increased to 180 mg/m², and recovered only to grade 2 as his overall condition continued to deteriorate.

Six patients have received 11 courses at 210/mg/m² liposomal-Annamycin. One had asymptomatic grade 4 granulocytopenia and two had grade 3 thrombocytopenia.

Thus for the phase II study, 210 mg/m² was designated as the dose of liposomal-Annamycin.

Table 2
Myelosuppression

Dose (mg/m ²)	Patients		Courses	Granulocytopenia	Thrombocytopenia
	New	Total		Number of Courses (Grade)	
3	3		5	0	0
6	3		5	0	0
12	3	6	11	0	3 (1)
24 ¹	3	7	13	0	2 (1), 1 (2)
30	3	6	7	0	0
60	2	7	14	1 (3)	1 (1)
120	3	4	12	4 (1), 1 (3)	1 (3)
180	6	9	19	7 (1)	4 (1)
				3 (2)	4 (2)
				5 (3)	1 ² (3)
				3 (4)	0 (4)
210	6	6	10	5 (3)	4 (3)
				1 (4)	0 (4)
240	3	5	7	1 (1)	4 (1)
				3 (2)	0 (2)
				1 (3)	0 (3)
				2 (4)	2 (4)

¹ One patient had congestive heart failure
(Cumulative Annamycin 60 mg/m², previous doxorubicin 250 mg/m² bolus)

² One patient had prolonged thrombocytopenia following cumulative dose 780 mg/m²

b. Non-Hematological Toxicities:

A few additional adverse reactions were noted on the phase I study. Five patients had apparent allergic reactions following the first dose. A subsequent grade 4 reaction after the third dose required stopping treatment. It does not appear that all patients require routine anti-allergic pre-medication, but patients who developed allergic reactions will be given routine dexamethasone and diphenhydramine for subsequent courses.

One patient with hypoxia secondary to extensive lung metastases and previous bolus adriamycin 250 mg/m² had heart failure after cumulative Annamycin 60 mg/m² over five courses. Subsequently, 4 patients had cardiac biopsy (at Ammamycin 360 mg/m²; 360 mg/m² after bolus adriamycin 300 mg/m² after adriamycin 300 mg/m² by 48 hour infusion; and 780 mg/m²). Only one of these (the one with previous adriamycin by 48 hour infusion) showed evidence of grade 0.5 anthracycline cardiotoxicity. Nineteen patients have now received 120-240 mg/m² Annamycin per dose without evidence of cardiac damage. Thus it is felt that routine cardiac surveillance will not require biopsy on the phase II study, but that it will be used only for changes in the cardiac function noted on echocardiographic surveillance.

On the phase I study there was minimal hair loss, nausea, and stomatitis. Skin toxicity was not a problem, and there was no evidence of cumulative myelotoxicity (except for the patient noted above) or any other organ toxicity. There have been no responses on the phase I study.

Table 3 shows a summary of the toxicity profile of liposomal-Annamycin.

Table 3
Summary of Toxicities with Liposomal-Annamycin

Bronchospasm	4 patients
Myelosuppression	Dose-limiting
Cardiac	Doubtful
Mucositis	Minimal or none (grade 2, 4 patients)
Hair Loss	Minimal or none
Skin	Minimal or none
Nausea/Vomiting	Minimal or none (5 patients nausea, 4 patients vomiting)

4. Pharmacokinetics

Figures 1-3 show the pharmacokinetic profile at three different doses of liposomal-Annamycin. The pharmacokinetics fit a 2-compartment model with a $T-1/2 \alpha$ of 7.7 - 27.9 minutes and a $T-1/2 \beta$ of 1.19 - 1.96 hours. Peak plasma levels at doses $> 120 \text{ mg/m}^2$ reached 3.8 - 5 $\mu\text{g/ml}$ which are in the range of the cytotoxic concentrations *in vitro*. Two metabolites peaking at 30 and 90 minutes were detected. Their identification is in progress.

Table 4 shows the pharmacokinetic parameters of liposomal-Annamycin.

FIGURE 1
Liposomal - Annamycin
Plasma Clearance Curves

DOSE(mg/Msq) = 180

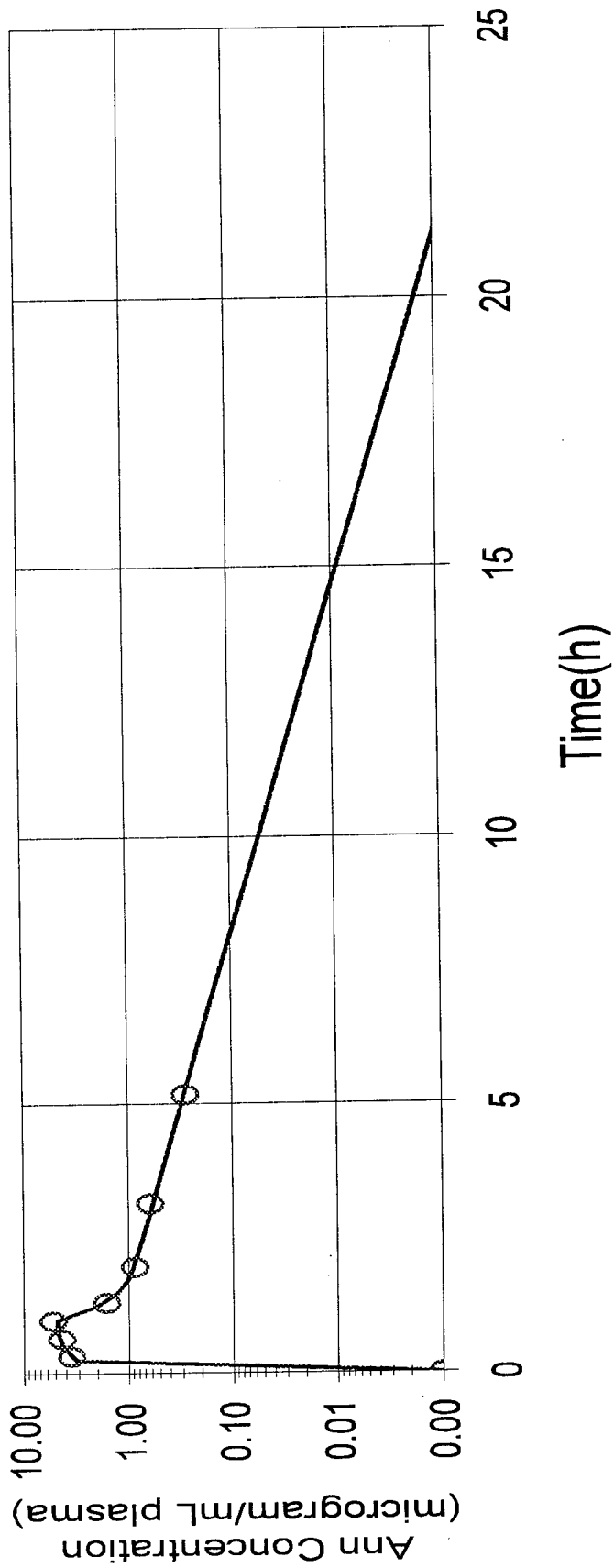


FIGURE 2
Liposomal - Annamycin
Plasma Clearance Curves

DOSE(mg/Msq) = 210

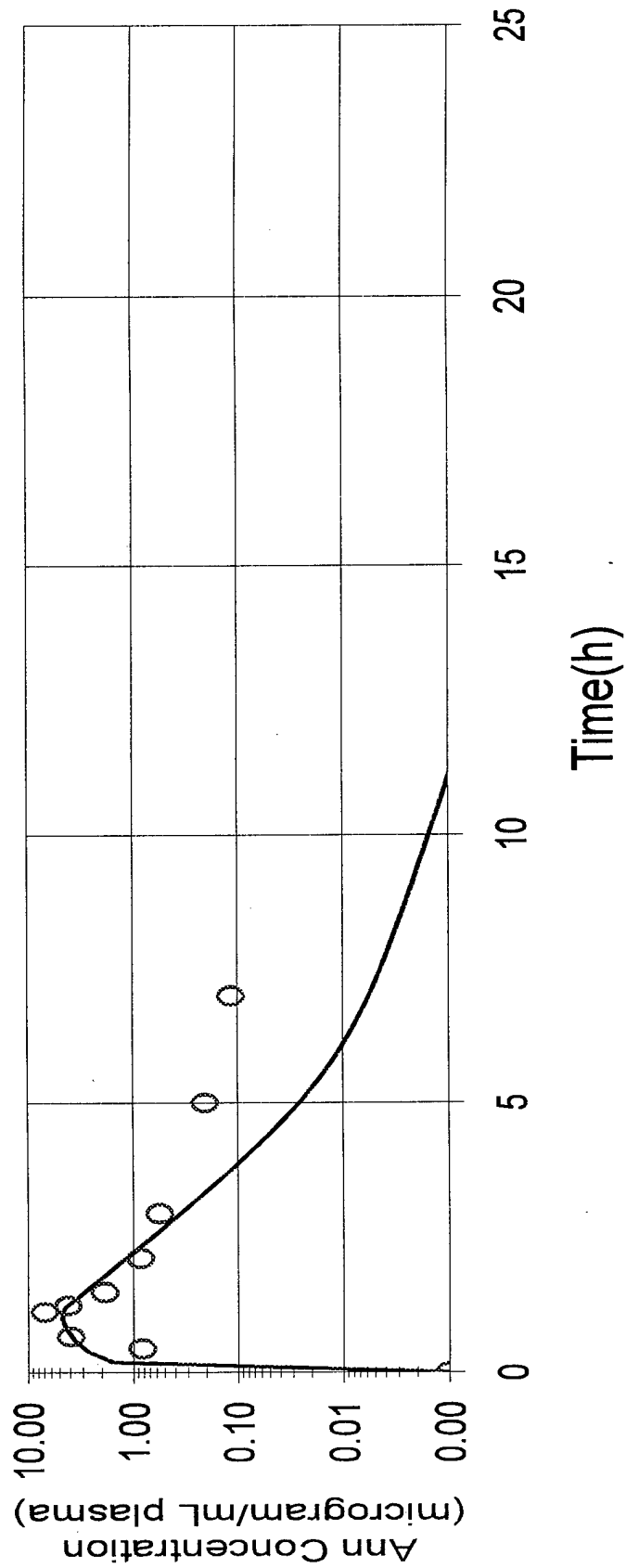


FIGURE 3
Liposomal - Annamycin
Plasma Clearance Curves

DOSE(mg/Msq) = 240

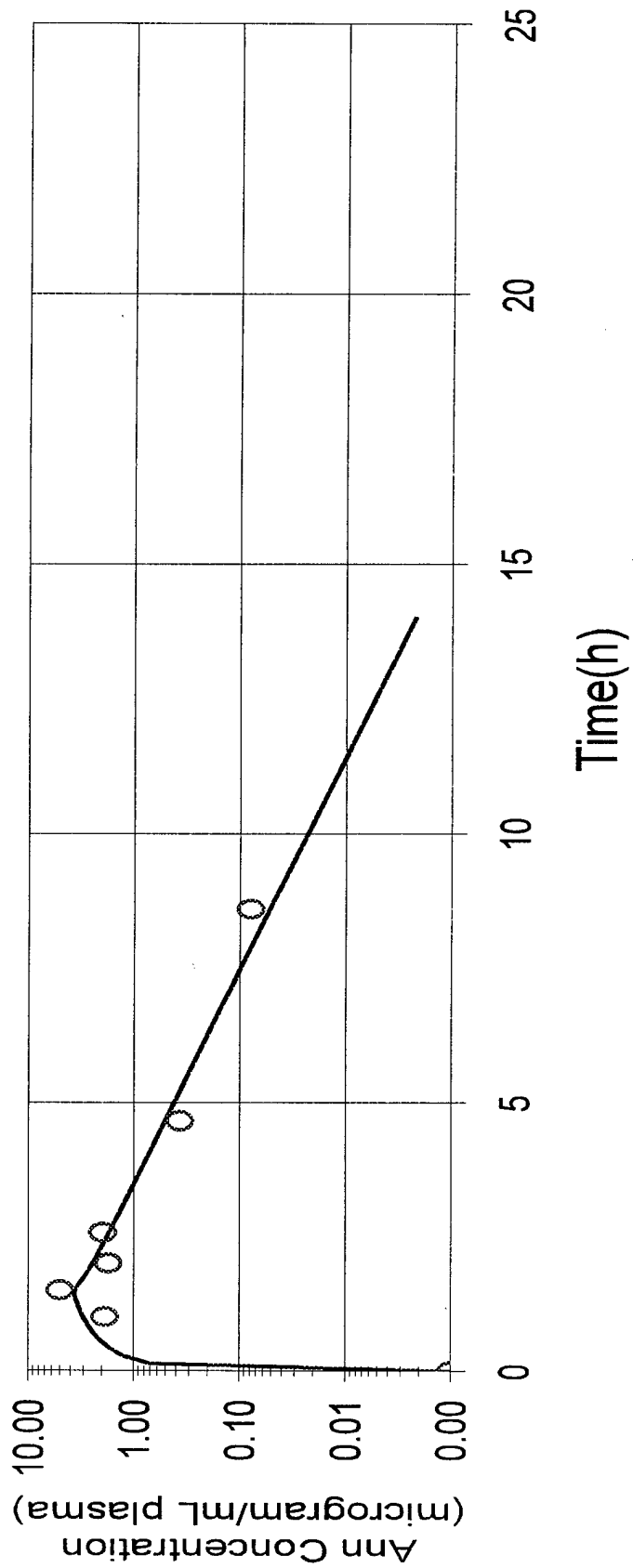


TABLE 4

L-Annamycin Pharmacokinetic Parameters

Dose (mg/M ²)	180	210	240
PARAMETER			
C _{MAX} (μg/mL)	5.99	5.00	3.79
CL (L/h)	38.9	51.2	46.6
V _{SS} (L)	61.5	36.4	73.8
t _{1/2} α (min)	7.72	27.9	8.70
t _{1/2} β (h)	1.96	1.77	1.19
AUC (μg. h/mL)	7.96	7.03	9.12

Discussion and Conclusions

The phase I study shows that liposomal-Annamycin is well tolerated, myelosuppression, mainly thrombocytopenia but also granulocytopenia, being dose-limiting. No episodes of life-threatening bleeding or neutropenic infection were observed. Non-hematological toxicity was limited to allergic reactions, probably to the Tween 20 present in the formulation. Definite cardiotoxicity was not observed. Alopecia, mucositis, and gastrointestinal toxicity were minimal. No tumor responses were observed in the phase I study. The phase II study is limited to patients with metastatic breast carcinoma refractory to doxorubicin and has already accrued 3 patients. Tumor biopsies are performed before therapy to correlate MDR-1 and MRP status with response.

The pharmacology studies demonstrate that Annamycin is rapidly cleared and metabolized. Metabolization into inactive species might result in loss of activity against MDR-1 and MRP cells. We are intensely studying the identity and biological activity of these metabolites.

In summary, liposomal-Annamycin is a well tolerated anthracycline with the interesting property of circumvention of MDR-1 and MRP. The current phase II study will definitely provide valuable information on the potential of this agent for the treatment of breast cancer resistant to standard chemotherapy.

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Appendix

1. Booser D, Esparza-Guerra L, Zou W, Priebe R, Perez-Soler R: Liposomal Annamycin. Phase I Clinical and Pharmacological Study. *ASCO* Abstract, p217a, 1997.
2. Phase I/II Clinical and Pharmacological Study of Liposomal Annamycin in Anthracycline-Resistant Breast Cancer. (Clinical Protocol).

Appendix 1

Liposomal annamycin. Phase I clinical and pharmacological study.
D. Booser, L. Esparza-Guerra, Y. Zou, W. Priebe, R. Perez-Soler, M.D.
Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX.

Annamycin (2'-iodo, 3'-hydroxy, 4'-epi, 4-demethoxy-doxorubicin) is an anthracycline antibiotic with high affinity for lipid membranes, formulated in liposomes measuring 100-150 nm in diameter. Preclinical studies showed activity against MDRI-positive xenografts, and less cardiotoxicity than doxorubicin. Since May 1995, 27 patients have been treated at 9 dose levels ranging between 3 and 240 mg/m². A total of 83 courses have been given, administered intravenously over 30-60 minutes. Grade 4 thrombocytopenia is the dose limiting toxicity. Four patients had allergic reactions with the initial treatment (presumably related to the presence of 0.16%, Tween 20 in the liposomal annamycin suspension) at doses from 60 to 240 mg/m². Although retreatment was possible with dexamethasone premedication, one patient was subsequently taken off study because of a Grade 4 allergic reaction. Although one patient with extensive lung metastases developed heart failure at a cumulative dose of annamycin 60 mg/m² over five courses (after previous cumulative bolus doxorubicin 250 mg/m²), three patients had no electron microscopic changes on cardiac biopsy between cumulative annamycin doses 360 mg/m² (previous bolus doxorubicin 300 mg/m²) and 780 mg/m². Nausea was minimal. Other than occasional mild thinning of the hair, alopecia has not occurred. The pharmacokinetics fit a 2-compartmental model with a t 1/2 α of 12 min and a t 1/2 β of 1.68 hours. Dose-dependent peak plasma levels at doses higher than 120 mg/m² reached 3-4 μ g/ml, a cytotoxic concentration in vitro. Two metabolites peaking at 30 and 90 minutes were detected. Identification of metabolites is in progress. The recommended Phase II dose has not been determined but will be between 180 and 240 mg/m². A Phase II study in patients with breast carcinoma refractory to doxorubicin is planned to investigate the potential antitumor activity of liposomal-annamycin in tumors expressing P-glycoprotein.

THE UNIVERSITY OF TEXAS
M. D. ANDERSON CANCER CENTER
DIVISION OF MEDICINE

PHASE I/II CLINICAL AND PHARMACOLOGICAL STUDY OF LIPOSOMAL ANNAMYCIN
IN ANTHRACYCLINE - RESISTANT BREAST CANCER

- 1.0 OBJECTIVES
- 2.0 BACKGROUND
- 3.0 BACKGROUND DRUG INFORMATION
- 4.0 PATIENT ELIGIBILITY
- 5.0 TREATMENT PLAN
- 6.0 PRETREATMENT EVALUATION (See Appendix)
- 7.0 EVALUATION DURING STUDY (See Appendix)
- 8.0 EVALUATION OF TOXICITY
- 9.0 CRITERIA FOR RESPONSE
- 10.0 CRITERIA FOR REMOVAL FROM STUDY
- 11.0 NUMBER OF PATIENTS
- 12.0 REPORTING REQUIREMENTS
- 13.0 CLINICAL PHARMACOLOGY
- 14.0 BIOPSY PROCEDURES
- 15.0 REFERENCES

Appendix A: Evaluations Before and During Therapy
Appendix B: Toxicity Criteria
Appendix C: Reporting of Adverse Reactions
Appendix D: Zubrod Scale of Performance Status
Appendix E: Chemical Structure of Doxorubicin and Annamycin
Informed Consent

PROTOCOL ABSTRACT**Protocol:** (Give number and abbreviated title)

Phase I/II Clinical and Pharmacological Study of Liposomal Annamycin in Anthracycline - Resistant Breast Cancer

**Study
Chairman:**

Daniel J. Booser, M.D.

Patient Eligibility:

1. Metastatic breast cancer.
2. Anthracycline resistant (See 4.1), no more than 3 metastatic regimens.
(a) limit doxorubicin 350/m² bolus or 450/m² CI.
3. Measurable disease
4. Normal heart function.

(See Section 4.0 for additional details).

Treatment Plan: (Include dose adjustment)

1. Liposomal Annamycin 210 mg/m² over 1 hour in 250 cc NS. Catheter not required.
2. Repeat treatment q 3 weeks. Delay if AGC < 1500 or PLAT < 100,000.

(See Section 5.0 for additional details).

PATIENT EVALUATION:

1. H+P, PS, document measurable disease.
2. Document prior anthracycline treatment and response.
3. Use blue flow sheet and PDMS.
4. CBC/Diff/Plat; repeat weekly, q 2-3 days if AGC < 500 or Plat < 50,000.
5. Electrolytes, creatinine, calcium, total bilirubin, albumin, alkaline phosphatase, LDH, SGPT, glucose; then before every third course.
6. CEA or CA-27-29 if abnormal; repeat every third course.
7. CXR, CT abdomen, Bone scan + x-ray hot spots, clinical photos with measurement; repeat abnormal studies q 3 courses to PR, then after 1 mo. to confirm; then q 3-4 courses (bone scan only q 4 mo).
8. EKG, ECHO. Repeat ECHO q 3 courses (or more frequently if needed).
9. Pharmacology + biopsy: (See below).

(See sections 6 and 7 for additional details).

Miscellaneous Information:(Include any other information that you feel is pertinent to the study)

Pharmacology is outlined in 13.0.
Surgical biopsy, or perhaps needle biopsy, of superficial tumor for drug resistance mechanism studies and pharmacology.
Discuss with study chairman.

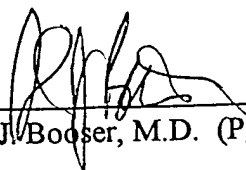
Statistical Considerations:

Phase II study will require 14 patients (7-14 months); if there is one response an additional 16 patients (8-16 additional months) will be added in order to detect a 20% response rate in this anthracycline-resistant population.

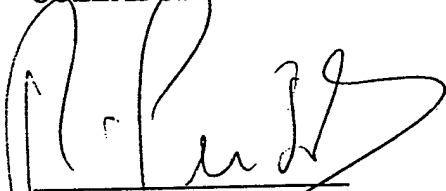
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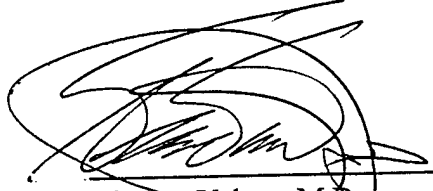
1. To document observed antitumor activity of liposomal Annamycin and frequency of response in anthracycline resistant breast cancer.
2. To refine the maximum tolerated dose of liposomal Annamycin administered by a 60 minute i.v. infusion every 21 days.
3. To study further the qualitative and quantitative toxicity and reversibility of toxicity of liposomal Annamycin administered in this fashion.
4. To investigate the clinical pharmacology of liposomal Annamycin and the rationale for dose and schedule chosen.

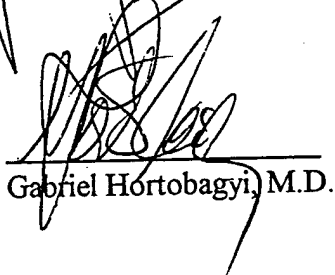
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Daniel J. Booser, M.D. (Pgr. 404-3338)

COLLABORATORS:


Roman Perez-Soler, M.D.


Vicente Valero, M.D.

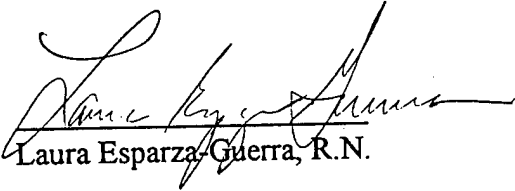

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1.0 OBJECTIVES

- 1.1 To document observed antitumor activity of liposomal Annamycin in anthracycline - resistant breast cancer.
- 1.2 To refine the maximum tolerated dose of liposomal Annamycin administered by a 60 min. i.v. infusion every 21 days.
- 1.3 To study further the qualitative and quantitative toxicity and reversibility of toxicity of liposomal Annamycin administered in this fashion.
- 1.4 To investigate the clinical pharmacology of liposomal Annamycin and the rationale for dose and schedule chosen.

2.0 BACKGROUND

Doxorubicin is one of the most effective antitumor agents against hematological malignancies and certain human solid tumors such as breast carcinoma and osteosarcoma.¹ However, its use is limited by acute myelosuppression, chronic cardiotoxicity, and natural or acquired drug resistance. In the last few years, there have been important advances in understanding the mechanisms of acquired resistance to doxorubicin and other structurally unrelated antitumor agents. It is now widely accepted that the overexpression of a membrane glycoprotein, P-glycoprotein, that acts as a drug efflux pump, mediates acquired resistance to doxorubicin in some *in vitro* systems and *in vivo* animal tumor models.² It is also known that overexpression of P-glycoprotein occurs in a significant number of patients when their tumors progress or relapse after treatment with doxorubicin.³ The impact of the overexpression of P-glycoprotein in human tumors in mediating clinical drug resistance remains, nonetheless, unknown. Since the introduction of doxorubicin in the current anticancer armamentarium, extensive efforts have been devoted to the synthesis of analogs with improved properties. Initially, most efforts were directed towards the preparation of analogs with reduced cardiotoxic potential. Those efforts have only been partially successful. More recently, and triggered by the discovery of the phenomenon of multidrug resistance and the identification of P-glycoprotein, the synthetic efforts have been more focused towards obtaining analogs with non cross-resistance properties.⁴⁻⁷ Several non-cross resistant analogs have been identified. They all have in common a markedly increased lipophilicity. Some of them have a similar mechanism of cytotoxicity of that of doxorubicin i.e., topoisomerase II inhibition. Others have a different mechanism of action, i.e., DNA alkylation.⁴

Liposomes have been used extensively as carriers of doxorubicin and daunorubicin.^{8,9} Liposomal-doxorubicin was found to be less cardiotoxic and more active than doxorubicin in models of liver metastases in mice by several investigators.^{10,11} Several clinical studies have been conducted with different liposomal formulations of doxorubicin. These trials have shown an MTD similar to that of free doxorubicin, a significant reduction of certain toxicities, such as gastrointestinal and vesicant effects, and an unchanged dose-limiting toxicity, i.e., myelosuppression. These studies have also suggested, although have not proved, a reduced cardiotoxic potential.

Annamycin is an anthracycline antibiotic which was selected for its lack of cross-resistance properties and a very high affinity for lipid membranes.¹² Because of this latter property, Annamycin is an ideal compound for liposome entrapment. Because the drug is completely insoluble in water solutions, liposomes can be used as a carrier for its intravenous administration. The fundamental mechanism of action of Annamycin appears to be inhibition of topoisomerase-II. Liposomal Annamycin was developed to combine the intrinsic favorable properties of the compound (lack of cross-resistance) with the potential advantages associated with liposome delivery (reduced cardiotoxicity and preferential distribution to certain organs). We have developed a liposomal formulation of Annamycin that has very high entrapment efficiency, physical stability, and chemical stability. Liposomal Annamycin has shown lack of cross-resistance *in vivo* in KB/-VI human xenografts and enhanced antitumor activity compared with doxorubicin in several mouse tumor models *in vivo*.¹³ In mice, the dose-limiting toxicity of liposomal Annamycin is myelosuppression and its cardiotoxic potential less than that of doxorubicin. In dogs, the dose equivalent to the mouse LD10 was very well tolerated with no side effects, no blood chemical changes, and no pathological changes four weeks after drug administration.

Thirty six patients were treated on the Phase I Clinical Study at the M.D. Anderson Cancer Center. Thrombocytopenia was the dose limiting toxicity at 240 mg/m², with 2 of 4 patients developing grade 4 platelet counts. One additional patient with perfect hemotologic tolerance through 5 courses at 120 mg/m² developed grade 3 thrombocytopenia after his first course increased to 180 mg/m², and recovered only to grade 2 as his overall condition continued to deteriorate.

6 patients have received 11 courses at 210 mg/m² Annamycin. Of 10 evaluable courses to date, one had asymptomatic grade 4 granulocytopenia and two had grade 3 thrombocytopenia. Only one patient remains on study.

Thus, for this Phase 2 study, 210 mg/m² is designated as the dose of liposomal Annamycin.

A few additional adverse reactions were noted on the phase I study. Five patients had apparent allergic reactions following the first dose. A subsequent grade 4 reaction after the third dose required stopping treatment. It does not appear that all patients require routine anti-allergic pre-medication, but patients who develop allergic reactions will be given routine dexamethasone and diphenhydramine for subsequent courses.

One patient with hypoxia secondary to extensive lung metastases and previous bolus adriamycin 250mg/m² had heart failure after cumulative annamycin 60mg/m² over five courses. Subsequently 4 patients had cardiac biopsy (at annamycin 360 mg/m²; 360mg/m² after bolus adriamycin 300 mg/m²; 420 mg/m² after adriamycin 300 mg/m² by 48 hr infusion; and 780 mg/m²). Only one of these (the one with previous adriamycin by 48 hr infusion) showed evidence of grade 0.5 anthracycline cardiotoxicity. 19 patients have now received 120-240 mg/m² annamycin per dose without evidence of cardiac damage. Thus it is felt that routine cardiac surveillance will not require biopsy on the

Phase II study, but that it will be used only for changes in the cardiac function noted on ecocardiographic surveillance.

On the Phase I study there was minimal hair loss, nausea, and stomatitis. Skin toxicity was not a problem, and there was evidence of cumulative myelotoxicity (except for the patient noted above) or any other organ toxicity. There have been no responses on the Phase I study.

3.0 BACKGROUND DRUG INFORMATION

3.1 Liposomal Annamycin (2'-Iodo, 3'-hydroxy, 4'-epi, 4-demethoxy doxorubicin)

3.11 Chemistry (See Appendix F.)

Annamycin is a lipophilic anthracycline antibiotic that incorporates 4 structural modifications from doxorubicin: 2'-Iodo, 3'-hydroxy, 4'-epi, 4-demethoxy. The structure is shown in Appendix F. Annamycin is completely insoluble in water, but soluble in chloroform and DMSO. Annamycin will be synthesized and supplied by Argus Pharmaceuticals, Inc. The material supplied has two molecules of tetrahydrofuran per three molecules of Annamycin.

3.12 Liposomal Annamycin Pharmaceutical data

Liposomal Annamycin will be prepared in Dr. Perez-Soler's laboratory at M. D. Anderson in the form of a preliposomal lyophilized powder containing a mixture of phospholipids, Annamycin, and Tween 20. Each vial contains:

Annamycin	10 mg
DMPC	350 mg
DMPG	150 mg
Tween 20	17 mg

On the day of use, liposomes containing Annamycin are formed by reconstituting each vial with 10 ml of saline at 37°C and hand-shaking for 1 minute (final Annamycin concentration 1 mg/ml). The appropriate dose will be further diluted up to 100 ml of normal saline. The entrapment efficiency is > 95% and the mean liposome size: $0.4 \pm 0.30 \mu\text{m}$. All preliposomal powder batches are checked for sterility and pyrogenicity. The stability of the lyophilized preliposomal powder is > 60 days. Batches older than 60 days will not be used. The stability of the reconstituted liposome suspension is > 24 hours. Doses older than 24 hours will be discarded.

The study will be conducted under an Institutional IND.

3.13 Animal Toxicology.

Mice

1. LD10, LD50, LD90

Groups of 8-10 CD1 Swiss mice (6-8 weeks old, average weight 18-20 g) received one single i.v. bolus of different doses of Doxorubicin, free Annamycin, or liposomal Annamycin. Animals were observed and deaths recorded on day 14. Most deaths occurred between days 4 and 8. Results are shown below:

Drug	LD10	LD50 mg/kg	LD90
Doxorubicin	16.3 ± 1.7	19.9 ± 2.0	24.3 ± 2.5
Free Annamycin	6.8 ± 1.2	8.8 ± 0.9	11.6 ± 2.2
Liposomal Annamycin	10.4 ± 0.4	15.7 ± 1.5	23.7 ± 1.9

The LD10 and LD50 were 16.3 and 19.9 mg/kg for doxorubicin, 6.8 and 8.8 mg/kg for free Annamycin, and 10.4 and 15.7 mg/kg for liposomal Annamycin. The liposomal carrier, therefore, decreases the toxicity of the drug. However, free and liposomal Annamycin have similar antitumor activity and potency in the L1210 leukemia model in vivo.

2. Myelosuppression

CD1 mice were injected with the LD50 dose of Doxorubicin, free Annamycin or liposomal Annamycin. Blood was drawn at 96 hours and the white count, differential, and platelet count determined. Results are shown below.

Drug	WBC x10 ³ ml	Granulocytes /ml	Platelets x 10 ⁶ /ml
Doxorubicin	1.4±0.8	300±15	1.35±0.62
Free Annamycin	0.8±0.4	30±18	1.50±0.14
Liposomal Annamycin	0.6±0.4	10± 1	0.76±0.42

These results indicate that, at equitoxic doses, liposomal Annamycin is more myelosuppressive than doxorubicin and about as myelosuppressive as free Annamycin.

3. Skin toxicity

Groups of 6 CD1 mice were injected intradermally with 0.1 ml of a solution containing 1 mg/ml doxorubicin, free Annamycin or liposomal Annamycin.

Necrotic ulcers lasting 10-14 days until healing were observed in all animals receiving doxorubicin. Necrotic ulcers were also observed in all animals treated with free Annamycin but they were smaller and had resolved by day 8. No ulcers, only erythema, was observed in the animals treated with liposomal Annamycin.

Liposomal Annamycin had, therefore, a much reduced vesicant effect compared with doxorubicin when injected intradermally in the abdominal wall of CD1 mice.

4. Cardiotoxicity

CD1 mice were injected i.v. weekly for 5 consecutive weeks with doxorubicin, free Annamycin, or liposomal Annamycin at 50% the LD10. Animals were sacrificed on week 6. Cardiac lesions were evaluated using the Bertazzoli method. All animals treated with doxorubicin developed cardiac lesions; about 25% of animals treated with free Annamycin developed cardiac lesions. In contrast, none of the animals treated with liposomal Annamycin developed cardiac lesions. These results suggest that liposomal Annamycin may be less cardiotoxic than doxorubicin and confirm the well-described cardioprotective effect of liposomes.

5. Pathology

CD1 mice were injected i.v. with the LD50 of doxorubicin and liposomal Annamycin. Animals were sacrificed on day 4. Animals treated with doxorubicin showed histopathological changes in kidney (nephrosis), bone marrow (aplasia), and gastrointestinal tract (crypt cell necrosis). Animals treated with liposomal Annamycin showed more severe bone marrow aplasia, similar changes in the gastrointestinal tract, but no kidney lesions. Free Annamycin showed a toxicity pattern similar to that of liposomal Annamycin.

Dogs

Beagles were injected with 50% or 100% of the dose equivalent to the mouse LD10 (one male and one female per dose) over 15 minutes. No significant side effects, changes in blood chemistries, or autopsy gross or histologic changes were observed.

4.0 PATIENT ELIGIBILITY

- 4.1 Patient with metastatic breast cancer who must be anthracycline-resistant and have received no more than three prior regimens for metastatic disease. It is preferred, but not required, that Annamycin directly follow the failed anthracycline regimen. Resistance to anthraquinone (e.g. mitoxantrone) is not sufficient for the phase II part.

Resistance to anthracycline (usually doxorubicin) will be defined as no response (stable disease after 4 courses or progressive disease) to doxorubicin, or relapse within 6 months of discontinuing therapy with anthracycline as adjuvant therapy or 3 months as therapy for metastatic disease.

Cumulative prior anthracycline dose will be limited to doxorubicin-equivalent 350 mg/m² by bolus, or 450 mg/m² by prolonged (at least 48 hours) infusion.

- 4.2 All patients must have an estimated life expectancy of at least 12 weeks. Patients must have a performance status of ≤ 2 (Zubrod scale; see Appendix D).
- 4.3 Patients must sign an informed consent indicating that they are aware of the investigational nature of this study in keeping with the policies of the hospital. The only acceptable consent form is the one attached at the end of this protocol.
- 4.4 Patients may have measurable disease.
- 4.5 Patients must be at least 18 years old.
- 4.6 Patients must have been off all previous chemotherapy or radiotherapy for the 3 weeks prior to entering this study and recovered from toxic effects of that therapy. Six weeks will be required if the patient has received therapy which is known to have delayed toxicity (mitomycin or a nitrosourea).
- 4.7 Patients must have adequate bone marrow function (defined as a peripheral absolute granulocyte count $> 1,500$ and platelet count of $> 100,000$, adequate liver function (bilirubin of $\leq 1.5\%$), and adequate renal function (creatinine ≤ 1.5 mg%).
- 4.8 Patients with a history of heart failure are excluded. If the treating physician believes a history of cardiac (especially atrial) arrhythmia is clinically trivial, this may be discussed with the study chairman. Patients with 2D echocardiographic ejection fraction $< 55\%$ are ineligible.
- 4.9 Patients with brain metastases treated by surgery and/or radiotherapy are eligible if neurologic status is stable 2 weeks after stopping dexamethasone.
- 4.10 Pregnant or nursing women are ineligible. Women not using adequate contraception are ineligible.
- 4.11 Patients with prior malignant disease in addition to the one to be treated on this study will be eligible as long as treatment was given with curative intent and the diagnosis of the metastatic breast cancer requiring treatment is clear.

5.0 TREATMENT PLAN

- 5.1 All patients shall be registered with PDMS. A prestudy form shall be necessary on each patient.
- 5.2 All therapy will be given at the M. D. Anderson Cancer.
- 5.3 Dose Schedule: Liposomal Annamycin 210 mg/m² intravenously over 60 min. in a 250 ml volume of normal saline. Peripheral vein may be used. Treat at q 3 weeks if AGC > 1500, platelets > 100,000. If hematologic recovery is delayed, repeat blood counts q 2-3 days until recovered.
- 5.4 Dosage escalation shall follow the schema below. Discuss with study coordinator.
- 5.41 If the preceding course nadir AGC > 1000 and platelets > 100,000 increase dose by 20% to liposomal annamycin 250 mg/m², in absence of any other significant toxicity.

All dose escalations will be reviewed with the study coordinator.

- 5.42 Dose adjustments for individual patients based on toxicity.

DECREASE 1 LEVEL:

- a) Less than 500 granulocytes and/or less than 50,000 platelets for > 5 days;
- b) Grade II nonhematologic toxicity other than nausea, vomiting, and stomatitis.
- c) Neutropenic fever.

DECREASE 2 LEVELS:

Documented infection with AGC < 500.

DISCONTINUE TREATMENT:

2D Echocardiographic ejection fraction < 50%.

Filgrastim may be given to patients receiving antibiotic treatment for neutropenic fever, but may not be used prophylactically. Erythropoietin may be given for refractory anemia as clinically indicated. Blood products, antibiotics, antiemetics, and pain medications may be given as needed.

- 5.5 If there is an allergic reaction, patients will be treated as appropriate with steroids, diphenhydramine, and/or epinephrine. If the allergic reaction is not life-threatening and it is felt to be in the patient's best interest, subsequent courses may be given with pre-medication including dexamethasone 20 mg and diphenhydramine 50 mg IV.
- 5.6 A minimum of 2 courses shall be required for a patient to be considered as having received an adequate trial to evaluate efficacy. All patients will be considered evaluable for toxicity.

6.0 PRETREATMENT EVALUATION (See Appendix A)

- 6.1 A complete history and physical, including documentation of all measurable disease as well as signs and symptoms and performance status shall be required. All information will be entered on blue flow sheets and Patient Data Management System. Measurable disease on physical exam should be documented by photographs with measurement. Cumulative anthracycline dose and previous response will be recorded.
- 6.2 Laboratory studies shall include CBC, differential, platelet count and electrolytes, creatinine, calcium, total bilirubin, albumin, alkaline phosphatase, LDH, SGPT, glucose. CEA or CA27-29 may be obtained. Baseline EKG and echocardiogram. Chest x-ray, CT abdomen, bone scan and x-ray of hot spots. Optional biopsy for MDR1, MRP, and other drug resistance studies.

All women of childbearing potential will have a serum pregnancy test within 48 hours prior to administration of each annamycin dose.

7.0 EVALUATION DURING STUDY (See Appendix A)

- 7.1 Patients shall be followed with weekly CBC, differential, and platelet count. These will be obtained every 2-3 days if AGC < 500 or platelets < 50,000.
- 7.2 Electrolytes, creatinine, calcium, total bilirubin, albumin, alkaline phosphatase, LDH, SGPT, and glucose shall be performed with every third course.
- 7.3 If any patient has prolonged myelosuppression, a bone marrow examination will be performed to rule out the possibility of tumor invasion of the marrow.
- 7.4 Tumor measurements on physical examination will be recorded every 3 weeks.
- 7.5 Abnormal radiologic studies and clinical photos should be repeated after 3 courses of therapy to partial response; one month later to confirm response; and then at least every 3-4 courses to evaluate duration of response. Bone scans should not be repeated more frequently than q 4 months unless clinically indicated. If a patient has evidence of a quick, dramatic response (or conversely if increasing disease is suspected), scans should be repeated at a shorter interval.
- 7.6 Echocardiogram will be repeated every 3 courses, and more frequently if there is a drop in ejection fraction and it is felt that continuing Annamycin would be in the patient's best interest. Cardiology consultation should be requested if any question arises about cardiac function. Annamycin will be discontinued if the 2D echocardiographic ejection fraction is < 50%. All cardiac abnormalities should be discussed with protocol chairman.
- 7.7 A cardiac biopsy should be done only if there is a clinical concern about cardiac toxicity based on clinical signs or symptoms or a drop in the echocardiographic ejection fraction. Patients with histologic grade >1 evidence of cardiac toxicity will be removed from the study.

8.0 EVALUATION OF TOXICITY

- 8.1 Toxicity shall be evaluated for each dosage level and each course of therapy.
- 8.2 Myelosuppressive toxicity shall be reported at lowest observed WBC, AGC, and platelet count. Anemia and red blood cell transfusion will be noted.
- 8.3 Other toxicities including cutaneous and mucosal will be described according to Appendix B. Because a small amount of Tween-20 is used in the formulation, patients should be watched for the possibility of allergic reactions. (See 5.5)
- 8.4 Patient data will be analyzed for evidence of cumulative toxicity with repeated courses of therapy.
- 8.5 Every effort will be made to obtain an autopsy on patients who die during the study. The pathologists will be informed of the nature of the study and the special concerns regarding the drug under study. Careful evaluation of potential target organs for toxicity will be made.

9.0 CRITERIA FOR RESPONSE

- 9.1 All tumor measurements must be recorded in centimeters and must have the longest diameter and its perpendicular applied at the widest portion of the tumor recorded.
- 9.2 Complete Remission: disappearance of all clinical evidence of active tumor for a minimum of 4 weeks. The patient must be free of all symptoms.
- 9.3 Partial Remission: 50% or greater decrease in the sum of the product of the diameters of measured lesions for 4 weeks. No simultaneous increase in the size of any lesion or the appearance of new lesions may occur. This improvement must continue for 4 weeks to be considered a partial remission.
- 9.4 No Change: No change or a change in tumor size $< 50\%$ decrease or $< 25\%$ increase for a minimum of 4 weeks.
- 9.5 Progressive Disease: Increase $> 25\%$ in the size of any measurable lesions. Appearance of new lesions.

Bone Criteria for Response

Complete Remission

- Lytic or mixed lesions recalcify completely.
- Blastic lesions unchanged or reduced in size.
- Bone scan normal or substantially improved: fewer and less intense lesions present.
- CEA, CA 27-29 normal.
- Patient asymptomatic.

Partial Remission

- Lytic or mixed lesions show some recalcification.
- No new lytic lesions appear.
- Bone scan stable or improved.
- Alkaline phosphatase normal or decrease $\geq 50\%$.
- CEA, CA 27-29 decreased by $> 50\%$.
- Symptomatic improvement.
- Analgesic requirement decreased (if present initially)

Stable Disease

- No evidence of recalcification.
- No change in the number or size of lesions.
- No new lesions.
- Bone scan stable.
- CEA or CA 27-29 decreased $< 50\%$ or increased $< 25\%$.
- Symptoms unchanged.

Progressive Disease

- New lytic lesions.
- New blastic lesions after 6 months of therapy.
- Increasing CEA or CA 27-29

A pathologic fracture is not necessarily a sign of progressive disease.

10.0 CRITERIA FOR REMOVAL FROM THE STUDY

- 10.1 Increasing disease (as defined above) after 2 courses of therapy.
- 10.2 The development of unacceptable toxicity.
- 10.3 Patients who become pregnant while enrolled in the study.

11.0 NUMBER OF PATIENTS

The phase II study will require 14 patients (estimated 7-14 months). If one response is observed, an additional 16 patients will be entered, an additional 8-16 months. This will permit estimation of the response rate with a standard error not greater than 10%. A 20% response rate in anthracycline-resistant patients is regarded as potentially useful.

12.0 REPORTING REQUIREMENTS
(See Appendix C)

- 12.1 An adverse drug reaction on a Phase II study is a previously unknown or a life-threatening reaction (Grade IV) which may be due to drug administration, or any death on study. The investigator is responsible for the prompt reporting of an Adverse Drug Reaction (ADR) to the Division of Medicine Clinical Trials Administration Office (x2-7770, Box 92). This office will in turn notify both the Surveillance Committee and the study sponsor, and the Human Use Review and Regulatory Affairs Division of USAMRMC.

The M.D. Anderson Cancer Center, the sponsor of this IND is responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the regulations promulgated thereunder. Those responsibilities include reporting any unexpected fatal or life-threatening experiences by telephone to this Agency no later than three working days after receipt of the information, reporting of any serious and unexpected adverse experiences in writing within 10 working days, and the submission of annual progress reports [21 CFR 312.32(a) and 312.33].

Dr. Gabriel N. Hortobagyi, Chairman of the Department of Breast Medical Oncology, is designated study monitor.

13.0 CLINICAL PHARMACOLOGY

13.1 Clinical Pharmacology Objectives

13.11 To study the pharmacokinetics of liposomal Annamycin.

13.12 To quantify the drug's urinary excretion.

13.13 To study *in vivo* drug metabolism.

13.2 Pharmacology Background

Mouse pharmacology studies have shown that the plasma peak levels, plasma half-life, and plasma AUC of liposomal Annamycin are 2-3 times higher than those achieved with an equimolar dose of doxorubicin.¹⁴ Compared with doxorubicin, the organ AUC's of liposomal Annamycin were 3-fold higher in brain, 2-fold higher in liver and kidney, 6-fold higher in lung, and 10-fold higher in spleen and s.c. B16 melanoma tumors. The heart AUC was similar for both drugs.

13.3 Patient Eligibility

All patients eligible for treatment on this study will be eligible for pharmacologic studies provided: (1) that they consent to such studies; (2) that they have adequate veins for repeated blood sampling; and, (3) that they can be hospitalized or return multiple times to the Clinic for drug sampling after treatment. Patients may receive liposomal Annamycin if they decline to participate in the pharmacology study.

13.4 Pharmacology Treatment Plan

13.41 All pharmacologic studies will be initiated in patient receiving a single intravenous dose of chemotherapy. The dose will be adjusted according to the total dose per course of drug utilized on the therapeutic schedule in this particular protocol.

13.42 Plasma Collection: 5 ml of heparinized blood (green stopper vacutainer tube) will be collected for each sample. Samples will be centrifuged immediately and the plasma separated from cells and frozen immediately. A baseline sample is to be collected before the drug is administered. Samples will be collected every 15 minutes for the first hour, and then at 1.5, 2, 4, 8, 12, 24, 36, 48 and 72 hours, and 7 days after drug administration. To facilitate collection of multiple samples, a heparin lock may be used. Care must be taken to clear the tubing of heparin before obtaining the sample, as excess heparin would dilute the plasma. This can be accomplished by withdrawing 3 ml of blood prior to sample collection. It is imperative that the drug be given through a separate IV so as not to contaminate the tubing of the heparin lock with the drug.

- 13.43 Urine samples are to be collected in dark bottles as voided, and stored (with inhibitor, if necessary) in a refrigerator for the first 24 hours following drug administration. 24-hour urine collection in a single container is then continued daily for 5 days (or a shorter or longer period of time, depending on the excretion characteristics of the individual drug). A baseline urine sample for use as a blank for interfering substances is to be collected prior to drug administration.

13.5 Drug Determination

Annamycin and metabolites will be determined in total blood, plasma, plasma proteins and plasma lipoproteins by an HPLC method previously described.¹⁵

13.6 Clinical Pharmacology Data Evaluation

13.61 Pharmacokinetics: Upon completion of a study, results will be subjected to multiple linear regression analysis. Most results can be described by an open 2 compartment model. The various pharmacokinetic parameters are computed as follows:

- A Intercept of the initial distribution slope, α , with ordinate usually in $\mu\text{g/ml}$ or ng/ml .
 P Intercept (in 3 compartment model) of back-extrapolated, second mono-exponential slope, π , describing distribution into second tissue compartment. Ordinate is usually given in $\mu\text{g/ml}$ or ng/ml .
- B Intercept of back-extrapolated monoexponential elimination slope, β , with ordinate usually given in $\mu\text{g/ml}$ or ng/ml .
- a, π ,b Slopes of these linear segments, with the dimension of t^{-1}
- C Drug concentration in the central (plasma) compartment, in $\mu\text{g/ml}$ or ng/ml .
- C₀ Drug concentration in the central compartment at time zero.
- D Dose (quantity) administered, in mg/kg or mg/m^2 .
 t Time in min or hr

$t_{1/2}$ Half-life

$t_{1/2\alpha}$ Half-life during the shallow tissue distribution (α) Phase

$t_{1/2\pi}$ Half-life during the deep tissue distribution (π) Phase

$t_{1/2\beta}$ Half-life during the terminal or elimination (β) Phase

V_c Volume of distribution of the drug in the open 1-compartment model, but volume of the central compartment in the 2- or 3-compartment models, in ml/kg or ml/m^2 .

V_{area} Volume of distribution by the area method.

V_{dss} Volume of distribution at steady state.

k_{12} Specific first-order rate constant (in t^{-1}) for drug transfer from the central compartment to the peripheral compartment (2-compartment model), or to the first ("shallow") peripheral compartment (3-compartment model).

k_{21} Rate constant of the reverse process.

k_e Elimination constant (2-compartment model).

k_{13} Rate constant for drug transfer from the central compartment to second ("deep") peripheral compartment.

k_{31} Rate constant of the reverse process.

k_{10} Rate constant (in t^{-1}) for elimination of drug from central compartment (3-compartment model).

One-Compartment Model

	Drug	Central Compartment	α	in which $k_e = \alpha$
C	=	$C_0 e^{-\alpha t}$		
$t_{1/2}$	=	$\ln 2 / \alpha$		
V_c	=	D / C_0		
AUC or $C \times t$	=	$C_0 (1 - e^{-\alpha t}) / \alpha$		
AUC_{∞} or $(C \times t)_{\infty}$	=	C_0 / α		

$C \times t$ is the area under the plasma drug disappearance curve, $(C \times t)_{\infty}$ denotes the $C \times t$ estimated to the complete disappearance of the drug.

Two-Compartment Model

By back-extrapolation, the biphasic drug disappearance curve is resolved into 2 linear segments (44), therefore

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

Drug	Central Compartment	k_{12}	Peripheral Compartment
	k_e	k_{21}	
$(t_{1/2})\alpha$	=	$\ln 2 / \alpha$	
$(t_{1/2})\beta$	=	$\ln 2 / \beta$	
AUC or $C \times t$	=	$A(1 - e^{-\alpha t}) / \alpha + B(1 - e^{-\beta t}) / \beta$	
AUC_{∞} or $(C \times t)_{\infty}$	=	$A / \alpha + B / \beta$	
V_c	=	$D / (A + B)$	
V_{area}	=	$D / \beta (C \times t)_{\infty} = D\alpha / (A\beta + B\alpha)$	

$$V_{dss} = D(A\beta^2 + B\alpha^2)/(A\beta + B\alpha)^2$$

$$k_{12} = \frac{AB(\beta - \alpha)^2}{(A+B)(A\beta + B\alpha)}$$

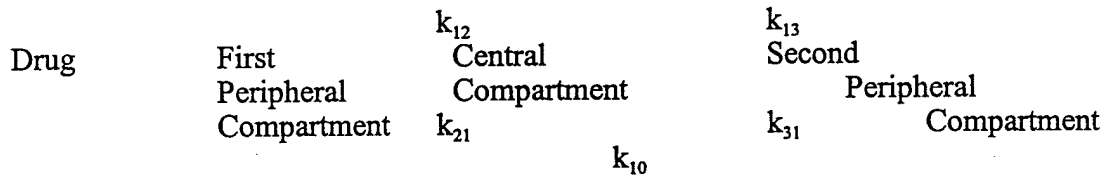
$$k_{21} = (A\beta + B\alpha)/(A+B), \text{ and}$$

$$k_e = \frac{A + B}{A/\alpha + B/\beta}$$

Three-Compartment Model

By back extrapolation, the triphasic drug disappearance curve is resolved into 3 linear segments (44), therefore

$$C = Ae^{-\alpha t} + Pe^{-\pi t} + Be^{-\beta t}$$



$$t_{1/2\alpha} = \ln 2/\alpha$$

$$t_{1/2\pi} = \ln 2/\pi$$

$$t_{1/2\beta} = \ln 2/\beta$$

$$\text{AUC or } C \times t = A(1 - e^{-\alpha t})/\alpha + P(1 - e^{-\pi t})/\pi + B(1 - e^{-\beta t})/\beta$$

$$\text{AUC}_{\infty} \text{ or } C \times t_{\infty} = A/\alpha + P/\pi + B/\beta$$

$$V_c = D/(A + P + B)$$

$$V_{\text{area}} = D/[\beta^{\circ} \times t]$$

$$V_{dss} = \frac{A}{D \alpha^2 + \frac{P}{\pi^2} + \frac{B}{\beta^2}} \times t^2$$

$$k_{21} = \frac{1}{2}(-b - b^2 - 4c)$$

and

$$k_{31} = \frac{1}{2}(-b + b^2 - 4c)$$

where

$$b = \frac{\pi\beta + \pi A + \beta P + \beta A + \alpha P + \beta B}{P + A + B}$$

$$\text{and } c = \frac{\alpha\pi B + \pi\beta A + \alpha\beta P}{P + A + B}$$

$$k_{10} = \alpha\pi\beta/k_{21}k_{31}$$

For all models, Cl_{renal} (renal clearance, in ml/min), is calculated as follows:

$$Cl_{\text{renal}} = \frac{C_u \times V}{C_p} \quad \text{where } C_u = \text{drug concentration in urine,}$$

$V =$ volume of urine collected during time interval in ml/min), and

$C_p =$ drug concentration in plasma at mid-point of collection interval.

13.62 Number of Patients and Patient Groups: Data will be analyzed first in at least 5 patients with entirely normal renal and hepatic function. Patients with borderline abnormalities of renal function (serum creatinine > 1.2 mg/dl or creatinine clearance < 50 ml/min) or hepatic excretory function (serum bilirubin > 1.2 mg/dl) will be analyzed separately. Patients with lesser abnormalities of liver function (elevated transaminases, alkaline phosphatase, or markedly abnormal hepatic scan) will be analyzed as still a separate group. Patients with abnormalities of both hepatic and renal function comprise another group. In addition, patients whose renal and hepatic function were insufficient for eligibility for the phase I study may undergo evaluation after sufficient data have been accumulated to define the relative roles of hepatic and renal clearance on the clinical pharmacology of the particular drug in question.

13.63 Clinical Correlation: In cases where sufficient patients can be studied pharmacologically, attempts to correlate pharmacokinetic parameters with drug toxicity will be undertaken.

14.0 BIOPSY PROCEDURES

Patients with superficial tumors may be requested to undergo biopsy to obtain tumor to be sent to Dr. Perez-Soler's laboratory for investigational studies. This may include, but is not limited to, MDR-1 and MRP (Multidrug Resistance Protein)¹⁶. These will be obtained prior to treatment, and may be repeated as indicated by response to treatment. Tumor also may be requested after treatment for pharmacology studies. Surgical biopsy will be needed for some of these studies, but needle biopsy may suffice for others. This will depend on the cellularity of the tumor and also the evaluation of technical factors in the assays.

Surgical biopsy may be arranged with Dr. Merrick Ross (Pager 404-3192; Office 2-7217).

15.0 REFERENCES

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**APPENDIX A
 EVALUATIONS BEFORE AND DURING THERAPY**

	PRE- STUDY	WEEKLY	Q 3 WEEKS	ABNORMAL STUDIES AFTER 3 COURSES TO PR; THEN 1 MO. TO CONFIRM PR; & Q 3-4 COURSES*****
History and Physical Exam	X		X	
Tumor Measurements	X		X	
CBC/Diff/Platelets	X	***X	X	
Electrolytes, creatinine, calcium, total bilirubin, albumin, alkaline phosphatase, LDH, SGPT, glucose	X			X
CEA or CA 27-29 (if abnormal)	X			X
EKG	X			
2D Echocardiogram**	X			
Chest x-ray, CT abdomen, Bone scan**** with x-ray "hot spots", clinical photos	X			X

- ** To be repeated q 3 courses and when clinically indicated. Discontinue treatment if EF < 50%.
- *** Repeat q 2-3 days if AGC < 500 and/or platelets < 50,000
- **** Repeating bone scan more frequently than every four months is not indicated unless increasing disease is suspected.
- ***** Sooner if a patient has evidence of a quick, dramatic improvement; or conversely if increasing disease is suspected.

APPENDIX B

NCI COMMON TOXICITY CRITERIA

TOXICITY (Adverse Event)	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	GRADE 4 (or Life- Threatening)
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BLOOD/BONE MARROW

WBC	≥ 4.0	3.0 - 3.9	2.0 - 2.9	1.0 - 1.9	<1.0
PLT	WNL	75.0 - normal	50.0 - 74.9	25.0 - 49.9	<25.0
Hgb	WNL	10.0 - normal	8.0 - 10.0	6.5 - 7.9	<6.5
Granulocytes/ Bands	≥ 2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	<.05
Lymphocytes	≥ 2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	<.05

LIVER

Bilirubin	WNL	-	<1.5 x N	1.5 - 3.0 x N	>2.0 x N
Transaminase (SGOT, SGPT)	WNL	≤ x 2.5 x N	2.6 - 5.0 x N	5.1 - 20.0 x N	>20.0 x N
Alk Phos or 5' nucleotidase	WNL	≤ x 2.5 x N	2.6 - 5.0 x N	5.1 - 20.0 x N	>20.0 x N

GASTROINTESTINAL

Nausea	none	able to eat reasonable intake	intake significantly decreased, but can eat	no significant intake	-
Vomiting	none	1 episode in 24 hours	2-5 episodes in 24 hours	6-10 episodes in 24 hours	> 10 episodes in 24 hours or requiring parental support
Diarrhea	none	increase of 2-3 stools/day over pre Rx	increase 4-6 stools/day, or nocturnal stools, or moderate cramping	increase of 7-9 stools/day, or incontinence, or severe cramping	increase of ≥ 10 stools/day, or grossly bloody diarrhea, or need for parenteral support
Stomatitis	none	painless ulcers, erythema, or mild soreness	painful erythema, edema, or ulcers, but can eat	painful erythema, edema, or ulcers, but can eat	requires parenteral or enteral support

KIDNEY/BLADDER

Creatinine	WNL	<1.5 x N	1.5 - 3.0 x N	3.1 - 6.0 x N	>6.0 x N
Proteinuria	no change	1+ or <0.3 g% or <3 g/l	2-3+ r 0.3 - 1.0 g% or 3 - 10 g/l	4+ or ? 1.0 g% or > 10 g/l	nephrotic syndrome
Hematuria	neg	micr only	gross, no clots	gross + clots	requires transfusion

APPENDIX E (Continued)

TOXICITY (Adverse Event)	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	GRADE 4 (or Life-Threatening)
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BLOOD/BONE MARROW

Cardiac dysrhythmias	none	asymptomatic, transient, requiring no therapy	recurrent or persistent, no therapy required	requires treatment	requires monitoring; or hypotension, or ventricular tachycardia, or fibrillation
Cardiac function	none	asymptomatic, decline of resting ejection fraction by less than 20% of baseline value	asymptomatic, decline of resting ejection fraction by more than 20% of baseline value	mild CHF, responsive to therapy	severe or refractory CHF
Cardiac-ischemia	none	non-specific T-wave flattening	asymptomatic, ST and T wave changes suggesting ischemia	angina without evidence for infraction	acute myocardial infraction
Cardiac-pericardial	none	asymptomatic effusion, no intervention required	pericarditis (rub, chest pain, ECG changes)	symptomatic effusion; drainage required	tamponade; drainage urgently required
Pulmonary	none or no change	asymptomatic, with abnormality in PFT's	dyspnea on significant exertion	dyspnea at normal level of activity	dyspnea at rest
Weight gain/loss	<5.0%	5.0 - 9.9%	10.0 - 19.9%	≥ 20.0%	-

BLOOD PRESSURE

Hypertension	none or no change	asymptomatic, transient increase by greater than 20 mm HG(D) or to > 150/100 if previously WNL; no treatment required	recurrent or persistent, increase by greater than 20 mm HG(D) or to > 150/100 if previously WNL; no treatment required	requires therapy	hypertensive crisis
Hypotension	none or no change	changes requiring no therapy (including transient orthostatic hypotension)	requires fluid replacement or other therapy	requires therapy and resolves within 48 hours of stopping the agent	requires therapy for > 48 hours after stopping the agent

APPENDIX E (Continued)

TOXICITY (Adverse Event)	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	GRADE 4 (or Life- Threatening)
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NEUROLOGIC

Neuro-sensory	none or no change	mild paresthasias, loss of deep tension reflexes	mild or moderate objective sensory loss; moderate paresthasias	severe objective sensory loss or paresthasias that interfere with function	-
Neuro-motor	none or no change	subjective weakness; no objective findings	mild objective weakness without significant impairment of function	objective weakness with impairment of function	paralysis
Neuro-cortical	none	mild somnolence or agitation	mild somnolence or agitation	severe sommolence, agitation, confusion, disorientation, or hallucinations	coma, seizures, toxic, psychosis
Neuro-cerebellar	none	slight incoordination dysdiakokinesis	intention tremor, dysmetria, slurred speech, nystagmus	locomotor ataxia	cerebellar necrosis
Neuro-mood	no change	mild anxiety or depression	moderate anxiety or depression	severe anxiety or depression	suicidal ideation
Neuro-headache	none	mild	moderate or severe but transient	unrelenting and severe	ileus > 96 hours
Neuro- constipation	none or no change	mild	moderate	severe	deafness not correctable
Neuro-hearing	none or no change	asymptomatic, hearing loss on audiometry only	tinnitus	hearing loss interfering with function but correctable with hearing aid	blindness
Neuro-vision	none or no change	-	-	symptomatic subtotal loss of vision	

APPENDIX E (Continued)

TOXICITY (Adverse Event)	GRADE 0	GRADE 1 (Mild)	GRADE 2 (Moderate)	GRADE 3 (Severe)	GRADE 4 (or Life-Threatening)
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DERMATOLOGIC

Skin	none or no change	scatter macular or papular eruption or erythema that is asymptomatic	scatter macular or papular eruption or erythema with pruritus or other associated symptoms	generalized symptomatic	exfoliative dermatitis or ulcerative dermatitis
Palmar-Plantar Erythro-dysesthesia	no symptoms	Mild erythema, swelling, or desquamation not interfering with daily activities.	Erythema, desquamation, or swelling interfering with, but not precluding, normal physical activities small blisters or ulcerations less than 2 cm in diam.	Blistering, ulceration, or swelling interfering with walking or normal daily activities; cannot wear regular clothing.	Diffuse or local process causing infectious complications, or a bed ridden state or hospitalization.
Allergy	none	transient rash, drug fever <38°C, 100.4°F	urticaria, drug fever = 38° C, 100.4°F, mild bronchospasm		anaphylaxis
Alopecia	no loss	mild hair loss	pronounced or total hair loss	-	-

METABOLIC

Hyperglycemia	<116	116 - 160	161 - 250	251 - 500	> 500 or ketoacidosis
Hypoglycemia	>64	55 - 64	40 - 54	30 - 39	< 30
Amylase	WNL	< 1.5 x N	1.5 - 2.0 x N	2.1 - 5.0 x N	> 5.1 x N
Hypercalcemia	<10.6	10.6 - 11.5	11.6 - 12.5	12.6 - 13.5	> 13.5
Hypocalcemia	>8.4	8.4 - 7.8	7.7 - 7.0	6.9 - 6.1	≤ 6.0
Hypomagnesemia	>1.4	1.4 - 1.2	1.1 - 0.9	0.8 - 0.6	≤ 0.5

COAGULATION

Fibrinogen	WNL	0.99 - 0.75 X n	0.74 - 0.50 X N	0.49 - 0.25 x N	≤ 0.24 x N
Prothrombin time	WNL	1.01 - 1.25 x N	1.26 - 1.50 x N	1.51 - 2.00 x N	> 2.00 x N
Partial thromboplastin time	WNL	1.01 - 1.66 x N	1.67 - 2.33 x N	2.34 - 3.00 x N	>3.00 x N
Hemorrhage (Clinical)	none	mild, no transfusion	gross, 1-2 units transfusion per episode	gross, 3-4 units transfusion per episode	massive, >4 units transfusion per episode

OTHER

Chills (rigors)	none	any rigor, mild	rigors requiring medication	rigors not controlled by medication	-
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Source (modified from): National Institute of Health, National Cancer Institute, Cancer Therapy Evaluation Program, Bethesda, Maryland 20892

THIS APPENDIX APPLIES TO NCI PROTOCOLS ONLY

APPENDIX C

NCI
DIVISION OF CANCER TREATMENT

ADVERSE DRUG REACTIONS
WITH INVESTIGATIONAL AGENTS

RESPONSIBILITIES OF INVESTIGATORS

The prompt reporting of adverse drug reactions (ADRs) to DCT is the responsibility of each investigator engaged in clinical research with investigational drugs supplied by DCT. Detailed definitions and reporting requirements are provided in this Attachment. In general, ADRs are defined as: 1) PREVIOUSLY UNKNOWN TOXICITIES (not included in the list of known toxicities provided by DCT); and 2) LIFE-THREATENING OR FATAL TOXICITIES (regardless of whether or not previously unknown). It is the policy of DCT that the investigators are encouraged to submit such reports even if there is only a suspicion of a drug effect. Procedures for reporting ADRs are outlined in the attached chart. In all instances investigators should be prepared to provide additional information to DCT if requested.

Any investigator who is dubious about whether a particular adverse reaction needs to be reported should call the IDB at 301-496-7957. After normal working hours, a recorder will be available.

Failure to report adverse reactions in a timely manner may result in discontinuation of the study, and, in some cases, revocation or suspension of the investigator's permission to perform clinical research using investigational agents under the Division of Cancer Treatment, NCI, sponsored INDs.

This reporting of adverse reactions is in addition to and does not supplant the reporting of toxicities as part of the report of the results of the research protocol, e.g., study summary forms or cooperative group data reporting. All adverse reactions should also be reported to your local Institutional Review Board.

ADVERSE DRUG REACTIONS OCCURRING ON PHASE I TRIALS

Life-threatening reactions which may be due to drug administration and all fatal reactions occurring on Phase I studies should be reported immediately by phone. A written report should be submitted within ten (10) working days. The first occurrence of any toxicity, regardless of grade, should be reported by phone with 24 hours to the appropriate Senior Investigator in IDB. A written report is required.

ADVERSE DRUG REACTIONS FOR COOPERATIVE GROUP STUDIES

Investigators on Cooperative Group protocols should read the protocol and/or check with the Operations Office and/or the Chairman of the New Agents Committee about their Group-specific practices for handling these reports. Some Groups route them through the Operations Office (if a mechanism is in place to do that on an emergency basis) while other Groups report directly to the DCT and copy the Operations Office.

ADVERSE DRUG REACTIONS OCCURRING WITH COMMERCIAL DRUGS

These toxicities should be reported promptly in writing (FDA Form 1639) according to the guidelines in Attachment 5. The report should be sent to the IDB within ten (10) working days of its occurrence.

THIS CHART APPLIES TO NCI PROTOCOLS ONLY

INVESTIGATIONAL AGENT ADVERSE DRUG REACTION REPORTING CHART
NATIONAL CANCER INSTITUTE, DIVISION OF CANCER TREATMENT

PHASE I STUDIES

- | | | |
|----|---|--|
| a. | ALL-LIFE THREATENING EVENTS(GRADE 4) ²
WHICH MAY BE DUE TO DRUG ADMINISTRATION. | REPORT BY PHONE TO IDB
WITHIN 24 HOURS ¹ . A
WRITTEN REPORT TO FOLLOW
WITHIN 10 WORKING DAYS ³ .
ALL FATAL EVENTS (GRADE 5)
WHILE ON STUDY. |
| b. | FIRST OCCURRENCE OF ANY TOXICITY
(REGARDLESS OF GRADE) | REPORT BY PHONE TO IDB
SENIOR INVESTIGATOR WITHIN
24 HOURS ¹ .

A WRITTEN REPORT IS
REQUIRED. |

PHASE II AND III STUDIES

UNKNOWN REACTION^{4,5}

KNOWN REACTION^{4,5}

GRADES 2-3²

GRADES 4 AND 5

GRADES 1-3

GRADES 4 AND 5

WRITTEN REPORT
TO IDB WITHIN

REPORT BY PHONE

NOT TO BE REPORT-
TO IDB WITHIN

WRITTEN REPORT
ED AS ADRs. THESE
TO IDB WITHIN

10 WORKING
DAYS.

24 HRS¹.
WRITTEN REPORT
TO FOLLOW WITHIN

TOXICITIES SHOULD
BE SUBMITTED AS
PART OF STUDY
RESULTS.

10 WORKING DAYS.

GRADE 4 MYELO-
SUPPRESSION NOT TO BE
REPORTED, BUT SHOULD
BE SUBMITTED AS PART
OF STUDY RESULTS.

GRADE 5 APLASIA IN
LEUKEMIA PATIENTS--
WRITTEN REPORT WITHIN
10 WORKING DAYS

USE ATTACHMENT 4 TO REPORT ADRs WITH DCT SPONSORED INVESTIGATIONAL AGENTS.

¹Telephone number available 24 hours daily: 301-496-7959 (Recorder after hours)

²See enclosed Toxicity Grading Chart

³Report to: Investigational Drug Branch

 P.O. Box 30012

 Bethesda, Maryland 20824-9998

⁴A list of all known toxicities can be found in the protocol document or consent form.

⁵Reactions judged definitely not treatment related should not be reported. However, a report should be submitted if there is only a reasonable suspicion of drug effect.

APPENDIX D

ZUBROD SCALE OF PERFORMANCE STATUS

Zubrod Status

- 0 = No symptoms
- 1 = Symptoms, fully ambulatory
- 2 = Bedridden < 50% of normal day
- 3 = Requires nursing assistance or equivalent,
bedridden > 50% of normal day
- 4 = Bedfast

Reference

Zubrod CG, Schneiderman M, Frei E, Brindley C, Gold GL, Shnider B, Oviedo R, Gorman J, Jones R, Jonsson V, Colsky J, Chalmers T, Ferguson W, Dederick M, Holland J, Selawry O, Regelson W, Lasagna L, and Owens AH: Appraisal of methods for the study of chemotherapy of cancer in man: Comparative therapeutic trial of nitrogen mustard and triethylene thiophosphoramide. *J Chron Dis* 11:7-33, 1960.

GUIDELINES FOR REPORTING OF ADVERSE DRUG REACTIONS (ADRs) TO THE SURVEILLANCE COMMITTEE (IRB)

In general, ADRs are defined as:

- 1) PREVIOUSLY UNKNOWN TOXICITIES (not included in the list of known toxicities provided by the Division of Cancer Treatment (DCT); and
- 2) LIFE-THREATENING OR FATAL TOXICITIES (regardless of whether or not previously unknown).

The timely reporting of adverse drug reactions is required by the Food and Drug Administration (FDA). The reporting of adverse reactions is in addition to and does not supplant the reporting of toxicities as part of the report of the results of the clinical trial. The Surveillance Committee (IRB) must be notified of any significant life-threatening and/or serious adverse reactions or experiences regardless of cause on a timely basis and must be appraised of all adverse experiences by written report on a periodic and timely basis, at least annually.

1. Reporting ADRs occurring with Investigational Agents

Phase I Studies

Life-threatening events (Grade 4) which may be due to drug administration

All fatal events (Grade 5) while on study (or within 30 days of treatment)

First occurrence of any previously unknown clinical event (Regardless of Grade).

Submit a written report within 10 working days to the Surveillance Committee.

Phase II and III Studies - Unknown Reaction

Grades 2-3

Grades 4 and 5

Submit a written report within 10 working days to the Surveillance Committee

Phase II and III Studies - Known Reactions

Grades 1-3

Grades 4 and 5

No report is required, except as part of study results.

Submit a written report within 10 working days to the Surveillance Committee.

Exception:

- o Grade 4 myelosuppression need only be submitted as part of the study results.

Guidelines for Reporting ADRs to the Surveillance Committee

2. Reporting ADRs Occurring with Commercial Drugs

Any increased incidence of a known ADR as reported in the package insert and/or the literature, any ADR which is both serious (life-threatening, fatal) and unexpected or any death on study if clearly related to commercial agent.

Submit a written report to the Surveillance Committee within 10 working days.

3. Devices in Clinical Research

Grade 4 and 5 toxicities

Submit a written report to the Surveillance Committee within 10 working days.

Note: Report event by telephone within 24 hours to study sponsor or FDA (if study is conducted under an institutional IND)

The University of Texas
M.D. ANDERSON CANCER CENTER

INFORMED CONSENT

PROTOCOL TITLE: *Phase I/II Clinical and Pharmacological Study of Liposomal Annamycin in Anthracycline-resistant breast cancer*

1. _____
Participant's Name I.D. Number

You have the right to know about the procedures that are to be used in your participation in clinical research so as to afford you an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm you; it is simply an effort to make you better informed so you may give or withhold your consent to participate in clinical research. This informed consent does not supersede other informed consents you may have signed.

This clinical trial is so designed that no person shall on the grounds of race, color, gender, or national origin be excluded from participation in or be denied the benefits, or be otherwise subjected to discrimination through or under this study.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY:

TREATMENT: The goal of this research study is to see if annamycin shrinks or prevents the growth of tumors in patients with advanced breast cancer. The study of annamycin and its effect on how long patients live will also be studied.

The investigational agent to be used in this study is not approved by the Food and Drug Administration (FDA) for commercial use; however, the FDA has permitted its use in this research study.

OPTIONAL PROCEDURES: In order to better understand the actions of Annamycin, Pharmacology and Biopsy tests will be done to study the metabolism and distribution of the drug.

IRB Approved Consent
Date Of Approval 8-18-97
Signature C. Sallinger

____ Patient's Initials _____ Witness ____/____/____/Date

3. DESCRIPTION OF RESEARCH:

Liposomal Annamycin will be given as an infusion into the vein over a 60-minute period. This will be repeated every twenty-one days. The drug will be administered at the M. D. Anderson Cancer Center in the outpatient chemotherapy area.

Therapy will continue as long as the tumor does not increase in size, and the side effects are not too severe. Blood tests will be performed at least weekly to determine the effects of this treatment. Other tests such as x-ray examination and scans will be performed frequently to evaluate the effects of the treatment with abnormal tests repeated at least every 3 courses. Up to 30 patients will be treated under the protocol study.

OPTIONAL PROCEDURES

Optional procedures without direct benefit will be offered to patients in this clinical research study. Patients do not have to consent to these optional studies in order to receive treatment.

PHARMACOLOGY: Patients in the pharmacology phase of the study will be asked to provide specimens of blood and urine. A baseline blood sample will be collected before the drug is administered. Samples then will be collected every 15 minutes for the first hour, and then at 1.5, 2, 4, 8, 12, 24, 36, 48, and 72 hours, and 7 days after drug administration. To make collection of multiple samples easier, a venous catheter called a heparin lock may be used. Between 70 and 100 cc blood (about 1/5 pint) will be obtained for each pharmacology study sequence. The amount of blood drawn for the study may increase the chances that the patient may require a blood transfusion. Urine samples for pharmacology will be collected in dark bottles, and stored in a refrigerator for the first 24 hours following drug administration. Twenty-four hour urine collections in a single container then will be continued daily for about 4 days (depending on the excretion characteristics of the drug).

BIOPSY: Patients who have a tumor that is easily accessible may be asked to have a biopsy performed before the start of treatment. Additional biopsies may be requested. Many of these will require surgical incision, although some studies may be performed on needle biopsy specimens. This will be done under local anesthetic.

4. RISKS, SIDE EFFECTS AND DISCOMFORTS TO PARTICIPANTS:

This clinical research study may involve unforeseeable risks to the participant.

TREATMENT: The side effects of this drug may include hair loss, nausea, vomiting, allergic reactions, and decrease in peripheral blood counts. Low white blood count may result in infection. Low platelet count may result in bleeding. There is a small risk of allergic reaction. Heart damage may occur with heart failure from weakening of the heart muscle. Laboratory studies suggest this may be less common than with other members of this drug class such as Adriamycin, and heart biopsy has been successful in frequently avoiding heart failure with Adriamycin. Low hemoglobin may result in loss of energy and weakness. Since this is a new drug, unexpected side effects may occur.

_____ Patient's Initials _____ Witness ____/____/____/Date

. IRB Approved Consent
. Date Of Approval 8-18-97
. Signature C. Salinas

- 4a. This clinical research may involve unforeseeable risks to unborn children; therefore, the participants should practice adequate methods of birth control throughout the period of their involvement in the clinical study if they are sexually active. To help prevent injury to children, the female participants should refrain from breast feeding during participation in the clinical research study.

OPTIONAL PROCEDURES - PHARMACOLOGY AND BIOPSY:

The amount of blood drawn for the study may increase the chances that the patient may require a blood transfusion.

With some biopsies underlying structures can be damaged. This complication is rare but in certain circumstances structural damage may require further diagnostic tests or even surgery to try to correct the injury.

5. POTENTIAL BENEFITS:

TREATMENT: The cancer may shrink or stop growing. However, patients might not benefit at all.

OPTIONAL PROCEDURES - PHARMACOLOGY AND BIOPSY:

There are no potential benefits for the individual patient undergoing the pharmacology and biopsy studies. Future patients may benefit from the information gathered from this clinical research study, especially about drug metabolism and mechanisms of drug resistance. This information may or may not be useful for the participants but will enable physicians to learn more about the use of this drug in cancer treatment.

6. ALTERNATE PROCEDURES OR TREATMENTS:

TREATMENT: Patients may receive standard chemotherapy drugs without taking part in this study. Other investigational studies may be available. Patients may also choose not to treat the cancer at all. In this case, only symptoms will be treated.

OPTIONAL PROCEDURES - PHARMACOLOGY AND BIOPSY:

Treatment with liposomal Annamycin may be given without participating in the optional studies.

UNDERSTANDING OF PARTICIPANTS

7. I have been given an opportunity to ask any questions concerning the investigational drug regimen and optional procedures involved and the investigator has been willing to reply to my inquiries. This investigational drug regimen will be administered under the above numbered, titled and described clinical research protocol at this institution. I hereby authorize Dr. _____, the attending physician/investigator and designated associates to administer the investigational drug regimen.

____ Patient's Initials

____ Witness

___/___/___/Date

. IRB Approved Consent
. Date Of Approval 8-18-97
. Signature C. Sallings

8. I have been told and understand that my participation is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution.

Should I decide not to participate or withdraw my consent from participation in this clinical research, I have been advised that I should discuss the consequences or effects of my decision with my physician.

In addition, I understand that the investigator may discontinue the clinical research study if, in the sole opinion and discretion of the investigator, the study or treatment offers me little or no future benefit, or the supply of medication ceases to be available or other causes prevent continuation of the clinical research study. The investigator will notify me should such circumstances arise and my physician will advise me about available treatments which may be of benefit at that time.

I will be informed of any new findings developed during the course of this clinical research study which may relate to my willingness to continue participation in the study.

9. I have been assured that confidentiality will be preserved, except that qualified monitors from the Food and Drug Administration (FDA) and of the U.S. Army Medical Research and Materiel Command may review my records where appropriate and necessary as a part of the responsibility to protect human subjects in research. Qualified monitors shall include assignees authorized by the Surveillance Committee of this institution provided that confidentiality is assured and preserved. My name will not be revealed in any reports or publications resulting from this study, without my expressed consent. In special circumstances the FDA might be required to reveal the names of participants.

10. You are authorized all necessary medical care for injury or disease which is the proximate result of your result of your participation in this research. The U.S. Army requires that this institution provide such medical care when conducting research with private citizens. Other than medical care that may be provided, you will not receive any compensation for your participation in this research study; however, you should understand that this is not a waiver or release of your legal rights.

11. I have been informed that I should inquire of the attending physician whether or not there are any services, investigational agents or devices, and/or medications being offered by the sponsor of this clinical research project at a reduced cost or without cost. Should the investigational agent become commercially available during the course of the study, I understand that I may be required to cover the cost of subsequent doses.

Costs related to my medical care including expensive drugs, tests or procedures that may be specifically required by this clinical research study shall be my responsibility unless the sponsor or other agencies contribute toward said costs. I have been given the opportunity to discuss with Daniel J. Booser, M.D., Study Chairman, and my insurance company the expenses or costs associated with my participation in this research activity.

12. It is possible that this research project will result in the development of beneficial treatments, devices, new drugs, or possible patentable procedures, in which event I understand that I cannot expect to receive any compensation or benefits from the subsequent use of information acquired and developed through my participation in this research project.

_____ Patient's Initials

_____ Witness

____/____/____/Date

. IRB Approved Consent
. Date Of Approval 8-18-97
. Signature C. Sallinger

13. I understand that refraining from breast feeding and practicing effective contraception are medically necessary and a prerequisite for my participation in this clinical research study. Should contraception be interrupted or if there is any suspicion of pregnancy, my participation in this clinical research study will be terminated at the sole discretion of the investigator.
14. It is a policy of the U.S. Army Medical Research and Materiel Command that data sheets are to be completed on all volunteers participating in research for entry into this commands volunteer registry data base. The information to be entered into this confidential data base includes your name, address, social security number, study name, and dates. The intent of the data base is twofold: First, to readily answer questions concerning an individual's participation in research sponsored by the USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years.
15. I may discuss questions or problems during or after this study with Daniel J. Booser, M.D. at (713) 792-2817. In addition, I may discuss any problems I may have or any questions regarding my rights during or after this study with the Chairman of the Surveillance Committee at (713) 792-2933 and may in the event any problem arises during this clinical research contact the parties named above.

_____ Patient's Initials

_____ Witness

____/____/____/Date

. IRB Approved Consent
. Date Of Approval 8-18-97
. Signature C. Sallings

CONSENT FOR TREATMENT AND OPTIONAL PROCEDURES (Mark choice with an "X").

1. I elect to _____ or not to _____ undergo pharmacology studies as an optional procedure. Patient's initials _____.
2. I elect to _____ or not to _____ undergo the tumor biopsy as an optional test for the study of possible drug resistance. Patient's initials _____.

CONSENT

Based upon the above, I consent to participate in the research and have received a copy of the consent form.

DATE

SIGNATURE OF PARTICIPANT

WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language which is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks, and I believe that the participant understood this explanation.

PHYSICIAN/INVESTIGATOR

I have translated the above informed consent into _____ for this patient.
(NAME OF THE LANGUAGE)

NAME OF TRANSLATOR

SIGNATURE OF TRANSLATOR AND DATE

Patient's Initials

Witness

___/___/___
/Date

IRB Approved Con
 Date Of Approval 8-18-97
 Signature C. Sallinger