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13. ABSTRACT *(Maximum 200 words)*

We are continuing study of the therapeutic and biological implications of the over-expression of MDR1-Pglycoprotein in breast cancer. Two major thrusts have been initiated: 1) organizing a clinical trial of paclitaxel without and with reversal of MDR1-Pglycoprotein action with the cyclosporin analog PSC-833; and 2) study of Pglycoprotein immunostaining in patients with primary and metastatic breast cancer available through our tumor bank. In addition, we are pursuing technical development to confirm these studies can be done from fixed section. The clinical trial that is to start will be a definitive test of the hypothesis that MDR1 is an important factor in determining response to paclitaxel in advanced breast cancer, and that a potent reversal agent will have therapeutic efficacy, at least in MDR1 overexpressing tumors. In addition, pharmacodynamic information in relation to paclitaxel without or with PSC-833 will be forthcoming.

14. SUBJECT TERMS
MDR1; Paclitaxel; Reversal of resistance; P-glycoprotein; PSC 833, breast cancer

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FOREWORD

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____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

10/27/95

PI - Signature Date

Table of Contents

Introduction

During year 1 the underpinnings for a randomized clinical trial to explore the role of MDR1 resistant reversal strategies on results of paclitaxel in advanced breast cancer were developed, and methods for studying the distribution of MDR1P glycoprotein (Pgp) immunostaining in a breast cancer population were set up. We shall elaborate on each one of these areas:

Body

1) **Clinical trials evaluating paclitaxel in relation to MDR1 expression.** We have used paclitaxel by 96-h infusion together with megestrol acetate (study #1B-93-8) in patients with advanced breast cancer previously failing paclitaxel. This study is ongoing but we have already concluded that the 96-h infusion will prove unsatisfactory for logistical reasons to evaluate the role of MDR1 and resistance reversal strategies in improving the therapeutic results of paclitaxel in breast cancer. The 96-h infusion requires central venous catheters and is also fraught with a propensity for drug precipitation within the catheter, which we have noted may predispose to catheter infections. In fact, we have initiated a prospective and retrospective study of such paclitaxel-associated catheter infections within our institution with other sources of support. Moreover, our future plans will seek MDR1- reversal strategies utilizing only the 3-h paclitaxel infusion (see Future Plans).

   Independently from this grant support, we have completed two studies utilizing paclitaxel as 3-h infusions (1,2) in order to further gain appreciation of its pharmacokinetics by itself (study #OC-93-5) and with an agent possibly acting via MDR1 reversal: estramustine phosphate (EMP) (study #OC-93-4). These studies have indicated that pharmacokinetics parameters are quite reproducible in an individual patient, and that EMP (preliminary data) does not appear to interfere with the pharmacokinetics disposition for paclitaxel. Moreover, these studies will allow us to propose and perform sophisticated pharmacodynamic analyses of similar data with paclitaxel alone or together with a resistance-reversal agent in patients with breast cancer (see Future Plans).

   We have also completed a study of paclitaxel, initially by 24-h infusion and subsequently by 3-h infusion, in patient with advanced breast cancer who have had biopsy evaluation for MDR1-Pgp immunostaining prior to therapy. Preliminary results of this study have been published (3). This study strongly suggests that MDR1 overexpression may occur in 20% of patients with breast cancer and that such overexpression confers resistance to paclitaxel. On the other hand, failure of doxorubicin therapy does not confer resistance to paclitaxel. Such trial is the direct forerunner of the currently proposed study, and will close upon activation of the proposed study.

2) **MDR1-Pglycoprotein (Pgp) immunostaining in relation to breast cancer patient characteristics.** We have been assembling a tumor bank of primary breast cancer specimens at a rate exceeding 100 specimens per year. We have also developed a computerized system to capture retrospectively and prospectively the key clinical information for subsequent analysis (Appendix

I). We have piloted this data collection on 86 patients with specimens in our breast cancer tumor bank.

Future Plans/Conclusions:

The following three areas are about to be activated, in full development, or in the planning stage as noted:

1) **Clinical trials:** Protocol #1B-95-4 has completed internal review and is being submitted for approval by the DOD (Appendix II). We are the Principal Investigators and Central Laboratory for a Phase II randomized clinical trial to include 3 other institutions (each securing permission from respective IRB's and having their own sources of support for clinical trials: UC Davis and City of Hope are part of a consortium with us for Phase II studies under sponsorship by the NCI, and the University of Toronto's Bayside Hospital). This trial will randomize patients with advanced breast cancer and available pre-treatment biopsy material to paclitaxel or to paclitaxel + PSC-833, a multidrug resistance reversal agent that is a Pgp substrate, and has a superior therapeutic index. The drug PSC-833, a cyclosporin analogue being developed by Sandoz Pharmaceuticals, will be provided by this pharmaceutical company to the 4 institutions involved. The trial will provide definitive information on the role of MDR1-Pgp immunostaining in determining resistance to paclitaxel, and the ability of PSC-833 to reverse such resistance. The detailed pharmacokinetic study will, in addition, provide key information on pharmacological determinants of paclitaxel response as they apply to breast cancer pretreated with doxorubicin.

The previously approved study of 96-h paclitaxel and megestrol acetate will remain open for selected patients who fail short infusions of paclitaxel until its accrual goal is met, since no conflict with the above trial is present.

2) **MDR1-Pgp immunostaining: correlation with clinical features.** We anticipate steady accrual into this study now that methods for computerized clinical date retrieval and tissue banking have been established. The key personnel for this portion of the study (and in support of the clinical study) have been recruited. We, therefore, anticipate to have immediate accrual, initially from the existing banked tissue. As first planned, the study will be confined to the previously proposed correlates of MDR1-Pgp immunostaining with clinical characteristics: age, menopausal status, tumor size and stage, and hormone receptors. As has been suggested (4) other new important immunocytochemical correlates will be explored subsequently as these become suitable and relevant for future study. For example, experience with p53 immunostaining, and immunocytochemical identifiers of drug resistance other than MDR1 (5-10) such as MRP and LRP suggest there may also be important determinants of chemosensitivity for a wide range of drugs. Therefore, characterization in our banked tissue study may provide leads for our clinical trial where tissue specimens will be more limited in size, and therefore less suitable for exploratory study.

3) **Technical development:** Although MDR1-Pgp immunostaining has proved most reliable in identifying overexpression of MDR1 and presence of

multidrug resistance, it cannot provide a functional assessment of Pgp action. Rhodamine 123 exclusion assay has been used by others (11) to identify drugs which reverse rhodamine efflux. This functional study relies on flow cytometry and evaluating a single cell suspension. Confirmed MDR1 positive cell lines will be developed from patients, identified throughout studies, and subjected to this functional assay. Because of our initial experience with estramustine phosphate (2), in vitro studies with this drug will be expected to further investigate its interaction with Pgp (12-15) and with drugs such as paclitaxel (15).

We are also committed to explore development of MDR1-Pgp immunostaining from paraffin blocks. This may require special retrieval techniques and study with a wide range of antibodies. Even if this technique will be very time-consuming, achieving identification of MDR1-Pgp immunostaining paraffin tissues will greatly simplify future clinical studies and will expand the implications of any results that may be obtained in our initial study.

Budget Justification:

Franco Muggia, M.D. supervises the overall project, including the planning and execution of the clinical studies, and recruiting collaborators in biostatistics (Susan Groshen), in pathology (Michael Press), and in Pharmacology (Robert Koda). Cell line studies will be carried out by recruiting the collaboration of Peter Danenberg and Colin Hill.

Valerie Israel, M.D. (10%) and Christy Russell, M.D. (10 %) are medical oncologists who will carry out the clinical study of paclitaxel without or with PSC-833 in advanced breast cancer. Moreover, they will supervise the collections of specimens and clinical data on patients entered on studies as well as on those with tumor being banked.

Susan Groshen, Ph.D. (5%) is providing statistical input into the clinical study, data acquisition and laboratory determinants being studied. She is involved in the planning of all studies with Dr. Muggia.

Michael Press, M.D. (5%) supervises all work relating to MDR-Pgp expression in human tumor specimens. He also will supervise the development of new technical methods to detect Pgp, and other determinants of chemotherapy response. He is also responsible for the fresh tumor acquisition.

Xiao Wei Yang, Ph.D. (100%) has joined Dr. Press' lab to directly perform all studies dealing with immunostaining and to explore the development of new methods assessing the reliability of various monoclonal antibodies in paraffin-embedded tissues.

Robert Koda, Ph.D. (5%) will supervise the pharmacology studies of paclitaxel and PSC-833 performed in the clinical trials and the pharmacodynamic and pharmacokinetic analyses.

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Appendix 1

USC-KNJCCC BREAST CANCER RESEARCH DATABASE

L.A. County Hospital (LACH) or Norris Hospital (NH)

----------DEMOGRAPHIC INFORMATION----------

Date First Seen at USC-LACH or NH for BREAST CANCER: __ __ / __ __ / 19 __ __

LACH Hospital Number: __ __ __ - __ __ - __ __
NH Hospital Number: __ __ - __ __ - __ __

Pathology Number:_____
__LACH __Norris Hospital

Tumor Procurement Number:_____

Name:(L)_____ (F)_____ (M)_____

Date of Birth: __ __ / __ __ / __ __ __ __

Ethnicity:

__Mexican
__Central American
__South American
__Cuban
__Puerto Rican
__Hispanic (but not sure of origen)

__African-American
__Black-Other

__Asian
__East Indian
__Pacific Islander
__Caucasian (non-Hispanic)
__Native Hawaiian
__Native Alaskan
__American Indian
__Other_____
_____

L.A. Address:_____
_____
_____
_____

Telephone: (__ __ __) __ __ __ - __ __ __ __

Foreign Address:_____
_____
_____
_____

Telephone: 011 - __ __ __ - __ __ __ - __ __ __ __ __ __ __
Country City tel. number

Person to Contact:_____
Relationship:_____

Telephone: (__ __ __) __ __ __ - __ __ __ __

Family History for Breast Cancer: __Yes __No
if yes, indicate which relatives, age at diagnosis, and if bilateral:_____
_____
_____
_____
_____
(If age at diagnosis is unknown indicate <50, >50, if this is known.)

History of Birth Control Use: __Yes __No __Don't Know

If yes, use of birth control pills: __Yes __No
If yes, indicate type if knonwn:_____

History Hormone Replacement Therapy: __Yes __No __Don't Know

If yes, use of estrogen replacement: __Yes __No
If yes, indicate type if knonwn:_____

If yes, use of estrogen + progesterone replacement: __Yes __No
If yes, indicate type if knonwn:_____

_____

Disease Status At First USC-KNJCCC Visit:

__ Newly Diagnosed (no prior treatment) When First Seen at LACH or NH
  __ loco-regional disease
  __ metastatic disease

__ Previously Treated When First Seen at LACH or NH
  __ loco-regional disease (at first USC-KNJCCC visit)
  __ metastatic disease (at first USC-KNJCCC visit)

_____

DATE THAT FORM IS COMPLETED: __ __ / __ __ / 199__

ABSTRACTOR'S INITIAL: __ __

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----------HISTORY OF PRIOR MALIGNANCIES ----------
(complete for all PRIOR cancers add pages if necessary)

Prior Malignancy? __No __Yes (if yes, complete below)

1. Site of Malignancy_____

Date of Diagnosis: __ __ / __ __ / __ __ __ __
Treatments Received for this Cancer (check all that apply)
__ Surgery
__ Chemotherapy
__ Radiation
__ Hormone/Endocrine Therapy
__ Immunotherapy
__ Bone Marrow Transplantation

Current Status __No Evidence of Disease (NED)
__With Active Disease

2. Site of Malignancy_____

Date of Diagnosis: __ __ / __ __ / __ __ __ __
Treatments Received for this Cancer (check all that apply)
__ Surgery
__ Chemotherapy
__ Radiation
__ Hormone/Endocrine Therapy
__ Immunotherapy
__ Bone Marrow Transplantation

Current Status __No Evidence of Disease (NED)
__With Active Disease

3. Site of Malignancy_____

Date of Diagnosis: __ __ / __ __ / __ __ __ __
Treatments Received for this Cancer (check all that apply)
__ Surgery
__ Chemotherapy
__ Radiation
__ Hormone/Endocrine Therapy
__ Immunotherapy
__ Bone Marrow Transplantation

Current Status __No Evidence of Disease (NED)
__With Active Disease

__ Check here if patient has more than 3 prior malignancies and another page is appended.

----------DIAGNOSIS INFORMATION, STAGING AND PATHOLOGY----------

Date of Initial Awareness of Mass or Symptoms: __ __ / __ __ / 19 __ __

Menopausal Status (at time diagnosis of breast cancer)
__Pre-menopausal
__Peri-Menopausal (1 year or less since LMP),
__Post-Menopausal (over 1 year since LMP)
__unknown

Date of Diagnosis __ __ / __ __ / 19 __ __

Diagnosis at __LACH __NH
__Other (in U.S.)_____
__Other (outside U.S.)_____

Check one: __unilateral breast cancer __synchronous bilateral breast cancer (i.e. < 6 months apart)

Check one: __invasive cancer only __non-invasive only __both

If synchronous bilateral cancer, describe the breast with the largest lesion first. Then repeat for the second breast (use extra copy of this form).

Laterality: __Left __Right
Location: __Upper Outer __Lower Outer __Upper Inner __Lower Inner __Nipple

Histologic diagnosis (check primary diagnosis)
Ductal:
__ intraductal (in situ)
__ invasive with predominant intraductal component
__ invasive (infiltrating ductal)
__ comedo
__ inflammatory
__ medullary with lymphocyte infiltrate
__ mucinous (colloid)
__ papilllary
__ scirrhous
__ tubular
__ other_____
Lobular:
__ in situ
__ invasive with predominant in situ component
__ invasive (infiltrating)
Nipple:
__ Paget's disease (NOS?)
__ Paget's disease with intraductal carcinoma
__ Paget's disease with invasive ductal carcinoma
Other
__Yes:_____

DCIS Present in the Ipsilateral Breast: __Yes __No __Info. not Available
DCIS Present in the Contralateral Breast: __Yes __No __Info. not Available

__ Check here if contralateral breast has invasive disease and pages 2 & 3 are added to include diagnostic information regarding the second breast

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----------DIAGNOSIS INFORMATION, STAGING AND PATHOLOGY----------

Local/Regional Presentation of Tumor
Local: __single lesion
__multifocal
__diffuse
__none

Clinical Evidence of Involved Axillary Lymph Nodes: __yes __no

Other Sites of Disease: __no
__yes: (specify) _____
_____
_____

Local/Regional Signs/Symptoms (check all that apply)
__erythema
__discharge
__edema
__pain
__lump
__other local/regional symptoms_____
_____
__none

Systemic Signs/Symptoms
__no __yes (if yes, specify)_____
_____

Size of Primary Tumor
clinical assessment: __ __ __ mm X __ __ __ mm
radiographic/mammographic size: __ __ __ mm X __ __ __ mm
based on pathologic specimen: __ __ __ mm X __ __ __ mm

Number of lymph nodes involved: __ __ __ (or unknown__)
as determined at pathology after lymph node dissection
(if bilateral, only account for each lymph node once)

TNM Staging

T stage: primary tumor

Clinical:	Pathological:	
__TX:	__TX:	primary tumor cannot be assessed
__T0:	__T0:	no evidence of primary tumor
__Tis:	__Tis:	carcinoma in situ or Paget's disease of the nipple with no tumor
__T1:	__T1:	tumor 20 mm or less in greatest dimension
__T2:	__T2:	tumor more than 20 MM but less than or equal to 50 mm in greatest dimension
__T3:	__T3:	tumor greater than 50 mm in greatest dimension
__T4a:	__T4a:	extension to chest wall
__T4b:	__T4b:	edema (including peau d'orange) or ulceration of the skin of breast or satellite skin nodules confined to same breast
__T4c:	__T4c:	both T4a and T4b
__T4d:	__T4d:	inflammatory carcinoma

Was Pathologic Staging of Primary Following (Neoadjuvant) Chemotherapy?
__Yes __No __Information Not Available

N stage (lymph nodes)

Clinical:	Pathological:	
__NX:	__NX:	regional lymph nodes cannot be assessed
__N0:	__N0:	no regional lymph node metastasis
__N1:	__N1:	metastasis to movable ipsilateral axillary lymph node(s)
__N2:	__N2:	metastasis to ipsilateral axillary lymph node(s) fixed to one another or to other structures
__N3:	__N3:	metastasis to ipsilateral internal mammary lymph node(s)

M stage (distant metastasis)

Clinical:	Pathological:	
__MX:	__MX:	presence of distant metastasis cannot be assessed
__M0:	__M0:	no distant metastasis
__M1:	__M1:	distant metastasis (including metastasis to ipsilateral supraclavicular lymph nodes) List Sites:_____ _____

Histologic grade
__ low
__ moderate
__ high
__ special type - not graded
__ cannot evaluate

ER status By Immunocytochemistry __positive __negative
By Biochemistry __ __ __ fmoles

PR status By Immunocytochemistry __positive __negative
By Biochemistry __ __ __ fmoles

Vascular Invasion: __yes __no __information not available
Lymphatic Invasion: __yes __no __information not available

Additional (other than surgical/pathological) Methods of Staging:
CHECK ALL THAT APPLY

__Clinical Assessment
__X-ray
__Bone Scan (isotopes)
__Liver Scan (isotopes)
__CT Scan (chest)
__CT Scan (abdomen)
__CT Scan (pelvis)
__MRI
__PET Scan
__IHC (micrometastasis in BM or LN)_____
__Other_____

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----------TREATMENT FOLLOWING INITIAL DIAGNOSIS OF BREAST CANCER----------

Surgical Procedure for Removal of Primary: __no __yes.
If yes, date __ __ / __ __ / 19 __ __

Surgery performed at __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Surgical Procedures for Initial Treatment of Primary (check all that apply):
__radical/extended radical mastectomy
__simple extended/modified radical mastectomy
__total mastectomy
__partial mastectomy/lumpectomy
__excisional biopsy
__needle-directed biopsy

Was Patient Free of All Gross Tumor After Surgery __yes __no __not sure
Negative Margins __yes __no __not sure/information not available

Axillary Lymph Node Dissection Performed: __yes __no

Radiation Therapy (RT) __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Date start __ __ / __ __ / 19 __ __
Date finish __ __ / __ __ / 19 __ __

Cumulative RT Dose Delivered: __ __ __ __ cGy

Was RT Completed? __yes __no __information not available
Number of Days of RT Planned: __ __
Number of Days Actually Given: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

---

Was patient considered free of disease at end of surgery and/or radiation:
__yes __no __information not available __not applicable

Chemotherapy (CX) __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Date start __ __ / __ __ / 19 __ __
Date finish __ __ / __ __ / 19 __ __

Purpose of CX: __neoadjuvant (Neo)
__adjuvant (Adj)
__front line (F-L)
__other (Oth)_____

CIC Protocol Number (if applicable): _____

Chemotherapy Agents Received (check all that apply):

Neo	Adj	F-L	Oth	
__	__	__	__	Doxorubicin (Adriamycin)
__	__	__	__	5-FU (5-Fluorouracil)
__	__	__	__	Ifosfamide
__	__	__	__	Cyclophosphamide (Cytoxan)
__	__	__	__	Methotrexate
__	__	__	__	Vinca alkaloids (Vinblastine, Vincristine, Navelbine)
__	__	__	__	Paclitaxel (Taxol)
__	__	__	__	other Taxanes
__	__	__	__	other alkylating agents (specify)_____
				_____
__	__	__	__	other drugs (specify)_____
				_____

Number of Kept Appointments for Chemotherapy: __ __
Number of Missed Appointments for Chemotherapy: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

Response to Chemotherapy
__ CR
__ PR
__ Stable
__ Progression
__ Not Evaluable
__ not applicable (e.g. adjuvant therapy)
__ information not available
__ too early to assess

Reason CX stopped:
__ completed course
__ disease progression
__ complications
__ other_____
__ too early to evaluate

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----------TREATMENT FOLLOWING INITIAL DIAGNOSIS OF BREAST CANCER----------

Endocrine Therapy (ET) __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Purpose of ET: __neoadjuvant (Neo) __adjuvant (Adj)
__front line (F-L) __other (Oth)_____

CIC Protocol Number (if applicable): _____

Endocrine Therapies Received (check all that apply and list the agent):

Neo	Adj	F-L	Oth		
__	__	__	__	Tamoxifen	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	__	Progestins (e.g. Megace) _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	__	Other Hormones _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	__	Aromatase Inhibitors _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	__	LH/RH agonists _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	__	Surgical ablation procedure:_____	Date __ __ / __ __ / 19 __ __

Number of Kept Appointments for Endocrine Therapy: __ __
Number of Missed Appointments for Endocrine Therapy: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

Response to Endocrine Therapy
__ CR
__ PR
__ Stable
__ Progression
__ Not Evaluable
__ not applicable (e.g. adjuvant therapy)
__ information not available
__ too early to assess

Reason ET stopped:
__ completed course
__ disease progression
__ complications
__ other_____
__ too early to evaluate

Summary: Was patient considered to be free of disease at completion of treatment following initial diagnosis?

__yes (medical records available)
__probably (based on patient's statement, medical records not available)
__no (medical records available)
__probably not (based on patient's statement, medical records not available)
__information not available

Total Number of Recurrences/Progressions following Initial Therapy:_____
(If no progressions or recurrences, then go to the last page for follow-up information.)

----------FIRST RECURRENCE/PROGRESSION: DIAGNOSIS ----------

Date of First Recurrence or Progression __ __ / __ __ / 19 __ __
(if patient was NED after initial therapy, then: recurrence
if patient was never NED then: progression)

If date of first recurrence or progression is not known, date that the patient was last seen free of disease: __ __ / __ __ / __ __

Site(s) of first recurrence/progression

__Local (list):_____

__Regional (list):_____

__Distant (list):_____

Was tumor rebiopsied: __yes __no __information not available

Path. #:_____ Tumor Procure. #:_____
__LACH __Norris Hospital

Response to Treatment after First Recurrence/Progression
__surgically free of disease
__CR following systemic treatment or radiotherapy
__PR
__stable disease
__progression
__no treatment

-------TREATMENT FOLLOWING FIRST RECURRENCE/PROGRESSION OF BREAST CANCER--------

Surgical Procedure for Treatment of First Recurrence/Progression: __no __yes.
If yes, date __ __ / __ __ / 19 __ __

Surgery performed at __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Surgical Procedures for Local Disease (check all that apply):
__radical/extended radical mastectomy
__simple extended/modified radical mastectomy
__total mastectomy
__partial mastectomy/lumpectomy
__excisional surgery

Other Surgical Procedure: __yes __no. If yes_____

Radiation Therapy (RT) for First Recurrence/Progression __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Date start __ __ / __ __ / 19 __ __
Date finish __ __ / __ __ / 19 __ __

Cumulative RT Dose Delivered: __ __ __ __ cGy

Was RT Completed? __yes __no __information not available
Number of Days of RT Planned: __ __
Number of Days Actually Given: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

---

Response to Surgery or Radiation Treatment after First Recurrence/Progression
__surgically free of disease
__CR following radiotherapy
__PR
__stable disease
__progression
__no treatment

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------TREATMENT FOLLOWING FIRST RECURRENCE/PROGRESSION OF BREAST CANCER--------

Chemotherapy (CX) for First Recurrence/Progression __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Date start __ __ / __ __ / 19 __ __
Date finish __ __ / __ __ / 19 __ __

Purpose of CX: __adjuvant (Adj)
__front line (F-L)
__other (Oth)_____

Chemotherapy Agents Received (check all that applied):

CIC Protocol Number (if applicable): _____

Adj	F-L	Oth	
__	__	__	Doxorubicin (Adriamycin)
__	__	__	5-FU (5-Fluorouracil)
__	__	__	Ifosfamide
__	__	__	Cyclophosphamide (Cytoxan)
__	__	__	Methotrexate
__	__	__	Vinca alkaloids (Vinblastine, Vincristine, Navelbine)
__	__	__	Paclitaxel (Taxol)
__	__	__	other Taxanes
__	__	__	other alkylating agents (specify)_____
			_____
__	__	__	other drugs (specify)_____
			_____

Number of Kept Appointments for Chemotherapy: __ __
Number of Missed Appointments for Chemotherapy: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

Response to Chemotherapy
__ CR
__ PR
__ Stable
__ Progression
__ Not Evaluable
__ not applicable (e.g. adjuvant therapy)
__ information not available
__ too early to assess

Reason CX stopped:
__ completed course
__ disease progression
__ complications
__ other_____
__ too early to evaluate

Endocrine Therapy following First Recurrence/Progression (ET) __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Purpose of ET: __adjuvant (Adj)
__front line (F-L) __other (Oth)_____

CIC Protocol Number (if applicable): _____

Endocrine Therapies Received (check all that apply and list the agent):

Adj	F-L	Oth		
__	__	__	Tamoxifen	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Progestins (e.g. Megace) _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Other Hormones _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Aromatase Inhibitors _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	LH/RH agonists _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Surgical ablation procedure:_____	Date __ __ / __ __ / 19 __ __

Number of Kept Appointments for Endocrine Therapy: __ __
Number of Missed Appointments for Endocrine Therapy: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

Response to Endocrine Therapy
__ CR
__ PR
__ Stable
__ Progression
__ Not Evaluable
__ not applicable (e.g. adjuvant therapy)
__ information not available
__ too early to assess

Reason ET stopped:
__ completed course
__ disease progression
__ complications
__ other_____
__ too early to evaluate

----------SECOND OR LATER RECURRENCE/PROGRESSION: DIAGNOSIS----------

Recurrence/Progression Number: __2nd __Later (specifiy number):_____

Date of This Recurrence or Progression __ __ / __ __ / 19 __ __

If date of this recurrence is not known, date that the patient was last seen free of disease: __ __ / __ __ / __ __

Site(s) of This Recurrence
__Local (list):_____

__Regional (list):_____

__Distant (list):_____

Was tumor rebiopsied: __yes __no __information not available

Path. #:_____ Tumor Procure. #:_____
__LACH __Norris Hospital

Response to Treatment after This Recurrence
__surgically free of disease
__CR following systemic treatment or radiotherapy
__PR
__progression
__SD
__no treatment

For third and subsequent recurrences/progression, copy and use these pages (8 and 9) as often as necessary.

--TREATMENT FOLLOWING SECOND OR LATER RECURRENCE/PROGRESSION OF BREAST CANCER--

Surgical Procedure for This Recurrence/Progression: __no __yes.
If yes, date __ __ / __ __ / 19 __ __

Surgery performed at __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Type of Surgical Procedure:_____
_____

Radiation Therapy (RT) for This Recurrence __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Date start __ __ / __ __ / 19 __ __
Date finish __ __ / __ __ / 19 __ __

Cumulative RT Dose Delivered: __ __ __ __ cGy

Was RT Completed? __yes __no __information not available
Number of Days of RT Planned: __ __
Number of Days Actually Given: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

---

Response to Surgery or Radiation after This Recurrence
__surgically free of disease
__CR following radiotherapy
__PR
__stable disease
__progression
__no treatment

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--TREATMENT FOLLOWING SECOND OR LATER RECURRENCE/PROGRESSION OF BREAST CANCER--

Chemotherapy (CX) for This Recurrence/Progression __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Date start __ __ / __ __ / 19 __ __
Date finish __ __ / __ __ / 19 __ __

Purpose of CX: __adjuvant (Adj)
__front line (F-L)
__other (Oth)_____

Chemotherapy Agents Received (check all that applied):

CIC Protocol Number (if applicable): _____

Adj	F-L	Oth	
__	__	__	Doxorubicin (Adriamycin)
__	__	__	5-FU (5-Fluorouracil)
__	__	__	Ifosfamide
__	__	__	Cyclophosphamide (Cytoxan)
__	__	__	Methotrexate
__	__	__	Vinca alkaloids (Vinblastine, Vincristine, Navelbine)
__	__	__	Paclitaxel (Taxol)
__	__	__	other Taxanes
__	__	__	other alkylating agents (specify)_____
__	__	__	other drugs (specify)_____

Number of Kept Appointments for Chemotherapy: __ __
Number of Missed Appointments for Chemotherapy: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

Response to Chemotherapy
__ CR
__ PR
__ Stable
__ Progression
__ Not Evaluable
__ not applicable (e.g. adjuvant therapy)
__ information not available
__ too early to assess

Reason CX stopped:
__ completed course
__ disease progression
__ complications
__ other_____
__ too early to evaluate

Endocrine Therapy following This Recurrence/Progression (ET) __no __yes
If yes, delivered at: __LACH __NH __Other (in U.S.)_____
__Other (outside U.S.)_____

Purpose of ET: __adjuvant (Adj)
__front line (F-L) __other (Oth)_____

CIC Protocol Number (if applicable): _____

Endocrine Therapies Received (check all that apply and list the agent):

Adj	F-L	Oth		
__	__	__	Tamoxifen	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Progestins (e.g. Megace) _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Other Hormones _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Aromatase Inhibitors _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	LH/RH agonists _____	Date Started __ __ / __ __ / 19 __ __ Date Stopped __ __ / __ __ / 19 __ __
__	__	__	Surgical ablation procedure:_____	Date __ __ / __ __ / 19 __ __

Number of Kept Appointments for Endocrine Therapy: __ __
Number of Missed Appointments for Endocrine Therapy: __ __

Maximum Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Maximum Non-Hematologic Toxicity Observed (NCI Common Toxicity) __
type of toxicity: _____

Was Patient Hospitalized as a Results of Side Effects: __yes __no __ not sure

Response to Endocrine Therapy
__ CR
__ PR
__ Stable
__ Progression
__ Not Evaluable
__ not applicable (e.g. adjuvant therapy)
__ information not available
__ too early to assess

Reason ET stopped:
__ completed course
__ disease progression
__ complications
__ other_____
__ too early to evaluate

INTERPRINT

----------STATUS AT LAST FOLLOW-UP----------

Date of last follow-up: __ __ / __ __ / 19 __ __

Status at last follow-up
__ alive and continuously without evidence of disease
__ alive and NED following a recurrence
__ alive - never free of disease
__ alive - with disease following a recurrence
__ alive - disease status not known
__ dead

If patient died, date of death: __ __ / __ __ / 19 __ __

Cause of Death
__ Breast Cancer
__ Treatment Related Complications
__ Other cancer (but not breast)_____
__ Other non-cancer cause_____
__ Cause unknown (but NOT breast cancer)
__ Cause unknown (specify)_____
_____
_____

Site of Death
__ LACH
__ Norris Hospital
__ Other_____

Was Autopsy Performed? __Yes __No

Quality of Survival:

At 6 Months AFTER Diagnosis or AFTER Last Recurrence\Progression (whichever occurred last)	At 12 Months AFTER Diagnosis or AFTER Last Recurrence\Progression (whichever occurred last)
0	0 Normal Activity
1	1 Symptomatic and Ambulatory
2	2 Ambulatory > 50%; Occasionally Needs Assistance
3	3 Ambulatory < 50%; Nursing Care Needed
4	4 Bedridden, May Require Hospitalization
6	6 Alive but status unknown
9	9 Expired
-8	-8 Symptoms and Status are probably due to Non-Cancer Cause (describe briefly): _____ _____
-9	-9 Not yet 6 (12) months since last recurrence/ too early

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UNIVERSITY OF SOUTHERN CALIFORNIA
NORRIS COMPREHENSIVE CANCER CENTER

CLINICAL INVESTIGATIONS SUPPORT OFFICE
1441 Eastlake Avenue, Room 501
Los Angeles, California 90033-0804
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****

PROTOCOL NUMBER:	1B-95-4
TITLE:	Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Hormonally Insensitive Breast Cancer (Recurring Less Than One Year Since Adjuvant or as Second-Line for Advanced Disease).
SITE:	Breast
HISTOLOGY:	Breast CA
STAGE:	Advanced
MODALITY:	Chemotherapy
TYPE:	Phase II
ARMS:	Randomized
PRINCIPAL INVESTIGATOR:	Franco Muggia, M.D.
CO-INVESTIGATORS:	Christy Russell, M.D. Valerie Israel, M.D. Susan Groshen, Ph.D. James Doroshow, M.D. (City of Hope) David Gandara, M.D. (UC Davis)
DATE OF IRB APPROVAL:	9-21-95
DATE OF ACTIVATION:	
PARTICIPANTS:	LAC+USC Medical Center USC+Norris Cancer Hospital City of Hope UC Davis
RESEARCH COMMITTEE NUMBER:	#959031
GRANT NUMBER:	
DATE OF AMENDMENTS/REVISIONS	

****

THE UNIVERSITY OF SOUTHERN CALIFORNIA ASSUMES NO
RESPONSIBILITY FOR THE USE OF THIS EXPERIMENTAL PROTOCOL
OUTSIDE THE PARTICIPATING INSTITUTIONS

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1B-95-4

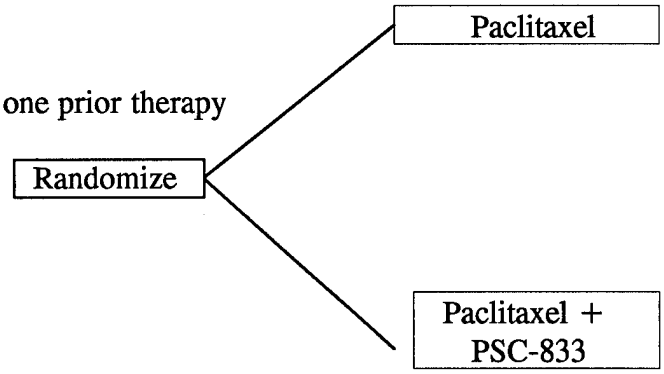
Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Hormonally Insensitive Breast Cancer (Recurring Less Than One Year Since Adjuvant or as Second-Line for Advanced Disease)

SCHEMA

Biopsy of tumor tissue for MDR1-Pgp and advanced breast cancer (One prior therapeutic chemo or < 6 months from end of adjuvant chemotherapy)

Stratify

- Recurrence < 6 months vs progression after one prior therapy
- Measurable vs evaluable
- Institution



1.0 OBJECTIVES

Specific Aims

1. To evaluate the response rate and time to treatment failure of paclitaxel without and with PSC-833 in advanced hormonally-insensitive breast cancer.

2. For each treatment arm, to relate paclitaxel AUC, and/or time above .05 μm, to myelosuppression and/or to response.

3. To estimate the association of efficacy to paclitaxel or to paclitaxel + PSC-833 with respect to MDR as measured by MDR1-Pgp immunostaining in pre-treatment biopsies.

4. To make preliminary comparisons of the benefit from PSC-833 in patients who do and do not show MDR1 Pgp immunostaining.

2.0 BACKGROUND AND HYPOTHESIS

2.1 General Breast cancer is sensitive to a number of different cytotoxic chemotherapeutic agents, but cures remain elusive when treatment is applied after the development of overt metastatic disease (1). Endocrine therapies are favored for metastatic disease when the disease is known to be hormone-sensitive (60% of ER + and/or PR + tumors, and < 10% ER/PR negative tumors). For hormone-insensitive tumors and for those eventually refractory to endocrine therapies, combination chemotherapy has been the mainstay of treatment. Most often such treatment has consisted of cyclophosphamide, Adriamycin (doxorubicin), and 5-Fluorouracil (CAF) or methotrexate substituting for the more toxic Adriamycin in the combination CMF. With these combinations, objective responses for an average duration of less than a year take place in more that 50% of patients, as long as there has been no prior exposure to chemotherapy or the metastatic disease has become manifest one year or more from completion of adjuvant therapy (1,2).

2.2 Drug resistance. The causes of early relapse and eventual drug failure, or primary refractoriness of breast cancer are largely unknown. Overexpression of P-glycoprotein (Pgp) encoded by the Multidrug resistance gene, MDR1, has been found in association with multiple prior treatments, and in some instances of shorter survival (3-10). Moreover, it is reasonable to expect that a percentage of patients treated with doxorubicin will fail because of overexpression of Pgp. Such overexpression may also affect the responsiveness of breast cancer to the new anticancer drug, paclitaxel.

2.3 Paclitaxel in breast cancer. Paclitaxel has shown striking antitumor activity against untreated breast cancer (11). The response to Taxanes, both paclitaxel and docetaxel declines significantly in previously treated patients, as compared to untreated patients (13-15). In our paclitaxel Phase II trial in doxorubicin refractory breast cancer (protocol #1B-92-3), no responses were observed among nine patients who had Pgp positive immunostaining, whereas all the objective responses were seen among 35 with negative immunostaining (15). Analysis of prior therapy indicated no relation with cumulative doxorubicin dose (Appendix I, Figure 1), although a history of recent exposure to doxorubicin was most common in Pgp positive patients (Appendix I, Figure 2). A trend towards unfavorable survival was noted among Pgp positive patients (Appendix I, Figure 3). Others have noted no correlation between prior doxorubicin and paclitaxel response (16).

2.4 Reversal of MDR1-mediated resistance. Cells exhibiting the MDR phenotype can become responsive to anti-cancer drugs by treatment with MDR reversing agents. These agents come from diverse groups of drugs that include various membrane active agents, calmodulin antagonists, calcium channel blockers, local anesthetics and cyclosporine A (CsA). CsA has been extensively studied in the clinic as a modulator of MDR but its utility is limited by nephrotoxicity and other adverse effects.

In a search for more potent and less toxic modulators of MDR expression, several hundred cyclosporine analogs were screened. This search yielded a non-immunosuppressive analog, PSC 833, which is approximately 10-fold more potent than cyclosporine A in its ability to modulate MDR *in vitro*(18). While CsA binds to both P-glycoprotein and to cyclophilin, the

latter accounting for its immunosuppressive effects, PSC 833 appears to interact specifically with P-glycoprotein. PSC 833 is a member of the cyclosporine family, an analogue of cyclosporine D, with the chemical formula: [3'-keto-BMT^1]-[Val^2]-cyclosporine. In vitro resistance to paclitaxel is clearly related to MDR1 overexpression (19). Refractoriness to paclitaxel's action may also be related to cytokinetic factors (20), and to mutations in the β-tubulin binding site of paclitaxel (21). Also, prolonged exposure may restore sensitivity to paclitaxel, and this could be related to the insensitivity of the S-phase to its actions (20). Nevertheless, strategies to overcome resistance associated with MDR1 overexpression deserve testing in improving the outlook of prior chemotherapy-treated patients with advanced breast cancer (17,22-27,29). Paclitaxel is an excellent choice for second-line therapy and the ability to increase its activity and/or duration of choice for second-line therapy and the ability to increase its activity and/or duration of effect makes study of paclitaxel + an MDR1 reversal agent also worthy of exploration. PSC-833 has been combined with paclitaxel and has restored cytotoxicity patterns following anticancer drug exposure in MDR1 overexpressing cell lines (17).

Pharmacologic changes with MDR1 reversal: *In vitro* studies with cancer cell lines showed that PSC 833 is approximately one order of magnitude more potent in reversing chemotherapy resistance than CSA, which itself is about one order of magnitude more active than equimolar concentrations of other known chemosensitizers (including verapamil, quinidine and amiodarone). *In vitro* studies also suggest that MDR reversal can be achieved at approximate concentrations of 1000-2000 ng/mL of PSC 833. *In vivo* studies demonstrated that PSC 833 reversed the resistance to vinca alkaloids and doxorubicin in MDR-tumor bearing mice. On the other hand, PSC 833 does not possess cytotoxic, cytostatic or immunosuppressive effects (18).

Clinical studies indicate interference of paclitaxel disposition when the two drugs are combined so that 40% of the dose of paclitaxel yields equivalent AUCs to paclitaxel when given alone. An equitoxic schedule of paclitaxel + PSC 833 to paclitaxel alone by 3 h infusion has been worked out by Sikic et at (26). A Phase III study of these two regimens in advanced breast cancer represents a test of the hypothesis that MDR1 overexpression is in large part responsible for treatment failure or early relapse. Of additional interest is whether prior treatment with doxorubicin contributes to such overexpression (27).

Selection of PSC 833 dose schedule: In general, reversible cerebellar dysfunction as manifested by ataxia and dysmetria appears to be the dose limiting toxicity in patients receiving PSC 833 by either the IV or oral route of administration. With the IV formulation, dose limiting ataxia (grade 3 or 4) has occurred in patients receiving 12.5 or 15 mg/kg/d. The ataxia reportedly required several weeks to completely resolve in one patient. No serious or severe adverse events due to SDZ PSC 833 have occurred in patients receiving intravenous doses up to and including 10 mg/kg/d. At this dose, blood concentrations of PSC 833 have ranged from 2,200-3,500 ng/ml. These concentrations are sufficient to modulate P-gp in highly resistant cell lines in vitro. Hence, 10 mg/kg/d when administered as a continuous infusion is considered the MTD for the IV formulation.

Dose limiting ataxia and dysmetria of PSC 833 have been encountered at 33 mg/kg/d divided into Q 12 hour dosing using the old drink solution and at 24 mg/kg/d divided into Q 8 hour

or Q 6 hour dosing using the soft gelatin capsule. These symptoms vary in intensity throughout the dosing interval and appear to be maximal one to three hours after dosing thus suggesting a peak concentration effect. In at least one patient, moderate hypertension was clearly associated with the above symptoms and recurred upon rechallenge but was completely reversible and without clinical sequelae.

The dose of PSC 833 chosen for this trial is based on clinical tolerability and achievement of blood concentrations known to reverse MDR1. In phase I studies B151 (PSC 833 + Etoposide) and B153 (PSC 833 + Paclitaxel) one in six patients receiving 18 mg/kg/day (6 mg/kg, q 8 hr) developed grade 3 ataxia while 6 of eight patients experienced grade 3 ataxia at a dose of 24 mg/kg/day (three at 8 mg/kg, q 8 hr and three at 6 mg/kg, q 6 hr). These events were transient and recovery was complete in all patients (28).

Preliminary data on 31 patients from protocols B151 and B 153 who have received PSC 833 for at least one cycle at 20 mg/kg/day (5 mg/kg, q 6 hr or four times daily) indicate that 12 patients have experienced grade 1 and 14 have experienced grade 2 reversible ataxia. No grade 3 ataxia has been seen at this dose level. Other toxicities which have been reported in this group of patients are summarized in the following table (without regard to assessed drug relationship):

No. Patients with PSC 833 Related Toxicities, 5mg/kg, PO, q 6 hr or 4x daily

N=31

	Grade of Toxicity			
Adverse Event	1	2	3	4
Peripheral Neuropathy	31	0	0	0
Ataxia	12	14	0	0
Nausea and Vomiting	15	1	0	1
Increased Bilirubin	0	6	6	2
Myalgia	6	0	0	0
Chest Tightness	5	0	0	0
Hypotension	1	2	2	0
Cough	3	0	0	0
Anxiety	2	2	0	0
Pain	3	0	0	0
Arthralgia	2	0	0	0
Fatigue	2	0	0	0
Dizziness	2	0	0	0
Perioral Numbness	2	0	0	0

Two patients have experienced pre-syncopal episodes associated with hypotension and one patient experienced an episode of syncope which lasted approximately 5 minutes. Other single occurrences of adverse events observed in this group of patients (all grade 1) include the following: tachycardia, pruritis, chills, weakness, dyspnea, lightheadedness, epistaxis, sinus drainage, constipation, upper respiratory infection, heartburn and hyperglycemia.

Severe elevations of alkaline phosphatase, SGOT, bilirubin, creatinine, BUN and LDH were observed in one patient who died of progressive disease while on study.

At the present time, 20 mg/kg/d administered Q 6 hours or 4 times daily represents the MTD for the oral formulation of PSC 833 when administered as the microemulsion-based soft gel capsule. In these ongoing studies in cancer patients, 5 mg/kg/dose of PSC 833 given Q 6 hours or 4 times daily has been well tolerated with average blood PSC 833 concentrations estimated to be at least 2,000 ng/ml.

A study of the absolute and relative bioavailability of PSC 833 in 20 normal human volunteers, when given as the labrafil-based drink solution (ODS), as the soft gelatin capsule (SGC) and as the new microemulsion-based drink solution (NDS) showed that the SGC and NDS were bioequivalent. The bioavailability of the microemulsion-based formulations was approximately two times higher than that of the ODS formulation used in tolerability studies. The new drink solution will be used in this study.

It must be emphasized that the use of potent MDR reversal agents such as PSC 833 will result in inhibition of the clearance of anticancer drugs principally due to modulation of P-gp in the kidney and biliary tract. The dose of paclitaxel used in combination with PSC 833 was chosen based upon the clinical tolerability and pharmacokinetic interaction profiles as determined in the phase I studies.

The starting dose of paclitaxel in this study will be reduced by approximately 60% of standard in order to achieve equal exposure and equal myelosuppression as compared to the standard paclitaxel dose (175 mg/m^2) administered without PSC 833. There is wide variability in the way patients respond to treatment with the paclitaxel/PSC 833 regimen in terms of myelotoxicity. Of the 3 patients in study B153 who received 1 cycle each at the 40% paclitaxel dose (70 mg/m^2) with PSC 833 at 5 mg/kg, q6h, one had a granulocyte nadir $< 500/mm^3$ and 2 patients had nadirs between $500/mm^3$ and $1000/mm^3$ and 2 patients had nadirs between $500/mm^3$ and $1000/mm^3$. No one experienced fever during their therapy.

Ten patients have received 11 cycles of treatment at the 50% paclitaxel (87.5 mg/m^2)/5 mg/kg PSC 833 dose level. During 7 cycles, patients experienced granulocyte nadirs $< 500/mm^3$ (range = 20-456, median = 299); four of these events were accompanied by fever. A granulocyte nadir between 500 and $1000/mm^3$ was seen during one cycle while during 3 cycles, nadirs never fell below $1500/mm^3$ (one patient had a nadir $> 1500/mm^3$ during one cycle and $< 500/mm^3$ during the subsequent cycle). Three patients to date have had their paclitaxel doses increased to 60% (105 mg/m^2) and all maintained nadirs > 1000 granulocytes/mm^3.

Hypothesis 1: MDR1 overexpression contributes to treatment failure in advanced breast cancer treated with paclitaxel,

Hypothesis 2: Reversal of MDR1 Pgp activity with PSC-833 may lead to activity in this disease even if MDR1-Pgp is expressed in the tumor.

Hypothesis 3: MDR1-P-glycoprotein immunostaining may identify a patient population with overexpression and that is particularly likely to benefit from the combined treatment of paclitaxel with PSC-833.

Hypothesis 4: Responses in the Pgp negative cohorts will be more frequent with higher paclitaxel AUCs or with time above a .05 μm threshold. On the other hand, in the Pgp positive cohort such responses and correlations will only be seen in the PSC-833 arm.

3.0 DRUG INFORMATION

3.1 Taxol

3.1.1 Formulation: Taxol is a poorly soluble plant product from the Western Yew, *taxus brevifolia*. Improved solubility requires a mixed solvent system with further dilutions of either 0.9% sodium chloride or 5% dextrose in water.

3.1.2 Supplier/How Supplied: Bristol Myers, Oncology Division. A sterile solution concentrate, 6 mg/ml in 5 ml vials (30 mg/vial) in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol, USP, 50%. The contents of the vial must be diluted just prior to clinical use.

3.1.3 Solution Preparation: Taxol will be prepared by diluting the total dose with the appropriate volumes of either 0.9% sodium chloride injection, USP, or 5% dextrose injection, USP (D5W). Taxol must be prepared in glass or polyolefin containers due to leaching of diethylhexylphthalate (DEHP) plasticizer from polyvinyl chloride (PVC) bags and intravenous tubing by the Cremophor vehicle in which Taxol is solubilized. Each bag/bottle should be prepared immediately before administration.

NOTE: Formulation of a small number of fibers in solution (within acceptable limits established by the USP Particle Matter Test for LVPs) have been observed after preparation of Taxol. Therefore, in-line filtration is necessary for administration of Taxol solutions. In-line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 microns (e.g., MillexGV, Millipore Products) into the IV fluid pathway distal to the infusion pump. Although particulate formation does not indicate loss of drug potency, solutions exhibiting excessive particulate matter formation should not be used.

3.1.4 The intact vials should be stored under refrigeration (2-8°C).

3.1.5 Shelf-life surveillance of the vials is ongoing. All solutions of Taxol exhibit a slight haziness directly proportional to the concentration of drug and the time elapsed after preparation, although when prepared as described above, solutions of Taxol (0.3-1.2 mg/ml) are physically and chemically stable for 24 hours.

3.1.6 Administration of Taxol: Taxol, at the appropriate dose and dilution, will be given as a 3-hour continuous IV infusion. Taxol will be administered via an infusion

control device (pump) using non-PVC tubing and connectors, such as the IV administration sets (polyethylene or polyolefin) and through a .22 micron filter. Nothing else is to be infused through the line where Taxol is being administered.

3.1.7 Adverse Effects:

A comprehensive listing may be found in the package insert. The most frequent effects include the following:

Hematologic: Myelosuppression

Gastrointestinal: Nausea and vomiting, diarrhea, stomatitis, mucositis, pharyngitis.

Heart: Asymptomatic bradycardia is common.

Neurologic: Sensory (taste), peripheral neuropathy, seizures, mood swings.

Allergy: Anaphylactoid and urticarial reactions (acute), flushing, rash, pruritus.

Liver: Increased bilirubin alkaline phosphatase and SGOT.

Other: Alopecia, fatigue, arthralgia, myalgia.

3.2 SDZ PSC-833

3.2.1 Formulation: SDZ PSC 833 is available in an oral solution. It will be supplied by Sandoz in 50 mL bottles containing 5000 mg at a concentration of 100mg/mL. Bottles will be labeled with tear off labels which meet the FDA criteria for investigational drug packaging.

3.2.2 Storage: SDZ PSC 833 must be stored in a secure location and must be carefully controlled in accordance with regulations governing Investigational New Drugs. PSC 833 must be stored between 15°C and 25°C in a secure location and must be carefully controlled in accordance with regulations governing Investigational New Drugs.

3.2.3 Adverse Effects: (Also See Background Section)

3.2.3.1 Neurologic: Numbness and tingling in the lips, tongue, and fingers and reversible cerebellar ataxia
3.2.3.2 Liver: Increased bilirubin and tranaminases
3.2.3.3 Other: Light-headedness, dizziness, urge to cough, chest tightness or pressure

3.2.4 Supplier: SDZ PSC 833 is an investigational new drug supplied by Sandoz Pharmaceuticals Corporation.

3.2.5 Administration of PSC 833: PSC 833 dosing should be on an empty stomach, i.e., at least one hour before and two hours after a meal. The oral solution should be diluted (ie. 1:10) preferably with orange juice or apple juice, however, other non-alcoholic drinks such as soft drinks can be used according to each patient's individual taste. Grapefruit juice should be avoided as a diluent.

To dilute, withdraw the prescribed amount of solution from the bottle using the syringe (supplied) and add it to the beverage. Each dose will be rounded to the nearest 50 mg. Stir well and administer to the patient within 10 minutes after preparation.

For each cycle, on treatment Day 1 patients will receive PSC 833, 5 mg/kg/dose, on a four times daily schedule with no two doses being administered less than 5 hours apart. On Day 2, approximately 2 hours after the fifth or sixth dose of PSC 833 (depending on convenience), and subsequent to the patient receiving prophylactic premedications, paclitaxel will be administered as a 3 hour IV infusion at a dose of 70 mg/m^2. PSC 833 oral dosing will continue on a four times daily schedule until the patient has received 12 doses, ending on either day 3 or 4 of the cycle. The doses of PSC 833 and paclitaxel will be adjusted according to the tolerance of each patient as defined in sections 8.1 (PSC 833) and 8.2 (paclitaxel).

3.2.6 Drug Accountability Records: The Principal Investigator will maintain an accurate record of receipt, disposition, and return of all study medication on the Drug Disposition form supplied by the sponsor. Drug supplies are to be used only in accordance with this protocol under the supervision of the Principal Investigator. The Principal Investigator agrees not to destroy any labels, empty bottles or unused drug supply.

At the completion of the study, the Principal Investigator will ship all used and unused tear-off labels and study medication bottles to the sponsor at the following address:

J. Dana Associates
11 Princess Road; Suite A
Lawrenceville, N.J. 08648
Attn. Jack Yarin

A written explanation will be provided for missing bottles of medication and for any missing tear-off labels.

4.0 STAGING

4.1 Staging of breast cancer: UICC/AJCC System

Primary tumor (T)

- Tx Primary tumor cannot be assessed
- T0 No evidence of primary tumor
- Tis Carcinoma in situ
- T1 tumor 2 cm or less in greatest diameter
  - T1a 0.5 or smaller
  - T1b larger than 0.5 cm, but less than 1 cm.
  - T1c Larger than 1 cm., but less than 2 cm.
- T2 tumor larger than 2 cm but less than 5 cm in greatest diameter
- T3 tumor larger than 5 cm. in greatest diameter
- T4 tumor of any size with extension to chest wall or skin
  - T4a fixation to chest wall
  - T4b edema, ulceration of the skin of the breast, or satellite skin nodules confined to the same breast
  - T4c both 4a and 4b
  - T4d inflammatory breast cancer

Regional Nodes (N)

- Nx nodes cannot be assessed clinically
- N0 no regional lymph node metastases
- N1 metastases to moveable ipsilateral axillary nodes
- N2 metastases to ipsilateral axillary lymph nodes fixed to one another or to other structure
- N3 metastases to ipsilateral internal mammary lymph node(s)

Distant Metastases (M)

- Mx presence of distant metastases cannot be assessed
- M0 no distant metastases
- M1 distant metastases (including metastases to ipsilateral supraclavicular node(s))

Stage Grouping

Stage 0	TisN0M0
Stage I	T1N0M0
Stage IIA	T0N1M0, T1N1M0, T2N0M0
Stage IIB	T2N1M0, T3N0M0
Stage IIIA	T0N2M0, T1N2M0, T2N2M0, T3N1M0, T3N2M0
Stage IIIB	T4 any N M0, any T N3 M0
Stage IV	any T any N M1

5.0 ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

5.1.1 Metastatic disease within 6 months of an adjuvant anthracycline-based chemotherapy and no chemotherapy for advanced disease, or failure of one prior anthracycline-based chemotherapeutic regimen for advanced breast cancer. (Exception: When anthracyclines are contraindicated, metastatic disease within 6 months of any adjuvant cytotoxic regimen, or failure of one prior cytotoxic chemotherapeutic regimen for advanced breast cancer also qualifies.)

5.1.2 Evaluable or measurable disease, with indicator lesion not radiated.

5.1.3 Presence of a lesion readily accessible for biopsy.

5.1.4 No radiation therapy within 3 weeks, and evaluable or measurable disease in at least one non-irradiated area.

5.1.5 No hormonal therapy within 2 weeks.

5.1.6 Performance Status 0, 1, or 2. (Appendix II).

5.1.7 Patients of childbearing potential must have a negative serum beta HCG pregnancy test within two weeks prior to study entry and agree to employ a barrier method of birth control for the duration of this clinical study.

5.1.8 Patients must give written informed consent to participate in the study.

5.2 Exclusion Criteria

Exclusion from the study will be required if:

5.2.1 Prior Taxol.

5.2.2 Patient has impairment of hepatic, renal or hematologic function as defined by the following baseline laboratory values:

a) Serum SGOT and/or SGPT > 2 times the institutional upper limit of normal (IULN).
b) Total serum bilirubin > 1.5 mg/dL.
c) History of chronic active hepatitis or cirrhosis.
d) Serum creatinine > 2.0 mg/dL.
e) Platelets < 100,000/mm^3
f) Absolute neutrophil count (ANC) < 1500/mm^3
g) Hemoglobin < 8.0 g/dL.

5.2.3 Patient has severe or uncontrolled concurrent medical disease (e.g. uncontrolled diabetes, unstable angina, myocardial infarction within 6 months, congestive heart failure, etc.).

5.2.4 Patient has known HIV infection (pre-study testing is not mandatory).

5.2.5 Patient has impairment of gastrointestinal function which might significantly alter the absorption of PSC 833. This includes uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome or bowel obstruction.

5.2.6 Patient has been treated with myelosuppressive chemotherapy within four weeks prior to study entry or within six weeks if administered nitrosoureas.

5.2.7 Patient is pregnant or breast feeding.

5.2.8 Patient has not recovered from previous surgery.

5.2.9 Patient has received investigational therapy within four weeks of study entry.

5.2.10 Patient has a known hypersensitivity to ingredients of the study medication or cyclosporine A.

5.2.11 Patients with a history of a second malignancy (with the exception of non-melanoma skin cancer or carcinoma in situ of the cervix).

5.2.12 Patient is currently receiving treatment with the following agents known to decrease the blood concentration of cyclosporine A (Column 2. Table 1) and treatment cannot be discontinued.

5.2.13 Patient is currently receiving *oral* administration of agents known to increase cyclosporine A blood concentrations (Column 1. Table 1) and treatment cannot be withheld *during* PSC 833 administration.

5.2.14 Brain metastases or other neurologic problems requiring treatment.

5.2.15 Unable to reliably follow instructions.

Table 1. Drugs Well Substantiated to Interfere with Cyclosporine A Pharmacokinetics

Drugs Increasing the Serum Concentrations of CSA	Drugs Decreasing the Serum Concentrations of CSA
Allopurinol	Carbamazepine
Amiodarone	Metamizole
Bromocriptine	Nafcillin
Cholic acid and derivatives	Octreotide
Danazol	Phenobarbital
Diltiazem	Phenytoin
Doxycycline	Probucol
Erythromycin	Rifampin
Ethynglestradiol	Sulfadimidine i.v.
Fluconazole	Ticlopidine
Glybenclamide	Trimethoprim i.v.
Itraconazole	
Josamycin	
Ketoconazole	
Lovastatin	
Methylprednisolone	
Metoclopramide	
Nicardipine	
Nifedipine	
Ondansetron	
Oral Contraceptives	
Ponsinomvcin	
Pristinamycin	
Propafenone	
Verapamil	

6.0 STRATIFICATION/DESCRIPTIVE FACTORS/RANDOMIZATION SCHEME

This is a multi-centered Phase II study. The coordinating center is the City of Hope. The participating centers are the City of Hope, USC and UC Davis. All patients will be registered at the City of Hope through the Biostatistics Office. (See Section 13.0).

6.1 This is a randomized study.

6.2 Stratifications:

6.2.1 Treatment within six months of adjuvant chemotherapy vs. progression on chemotherapy for advanced disease.

6.2.2 Measurable vs. evaluable disease.

6.2.3 Institution.

6.3 Randomization: To be done centrally at CISO, USC (213) 764-0450.

7.0 TREATMENT PLAN AND PHARMACOKINETIC STUDIES

7.1 Treatment will be randomized

All patients will be pre-treated during cycle 1 (premedication dosages may be modified for subsequent cycles) with:

1. Dexamethasone, 20 mg orally or intravenously, 12 and 6 hours before paclitaxel.
2. Diphenhydramine, 50 mg, 30 to 60 minutes before paclitaxel.
3. Cimetidine, 300 mg or Ranitidine, 50 mg, 30 minutes before paclitaxel.

7.1.1 Regimen I

Paclitaxel 175 mg/m^2 by 3-hour continuous infusion Day 1, every 21 days.

7.1.2 Regimen II

Patients should be advised that the use of alcohol, sedatives or sleeping medications should be avoided during administration of PSC 833 as this could increase the likelihood of falling. Patients should also be advised not to drive a car or other vehicle during initial treatment with PSC 833 until effects on coordination in that patient have been determined.

SDZ PSC 833 - 5 mg/kg po qid for 12 doses, Day 1, and ending Day 3 or 4, every 21 days. Paclitaxel 70 mg/m^2 by 3-hour continuous infusion, Day 2,

every 21 days. Patients may continue to receive paclitaxel or PSC 833 and paclitaxel as long as their absolute neutrophil count (ANC) is ≥ 1500/mm^3 and platelet count is ≥ 100,000/mm^3 prior to the start of each cycle.

NOTE: Because of possible anaphylactic reactions, patients should be closely observed and vital signs monitored during the first 15 minutes of paclitaxel infusion.

7.2 Pharmacokinetic Studies: To determine the disposition of paclitaxel with and without concomitant PSC 833, pharmacokinetic studies will be performed with the first course of therapy in a subset of patients from both regimens I and II. For these studies, 5 ml of peripheral blood will be collected from a vein contralateral to the drug infusion at the following times; immediately before paclitaxel, at 1, 2, and 3 hours during the paclitaxel infusion, then at 0.25, 0.5, 1, 2, 3, 6, 10, and 24 hours after the end of infusion. Plasma will be separated by centrifugation and stored at -20°C until analysis. Paclitaxel concentrations in plasma will be determined by an HPLC-ultraviolet detection assay adapted from a previously described method (30).

7.3 Frequency of Therapy: Treatment cycles are to be repeated at 3 week intervals and can be delayed for up to two weeks if a reversible toxicity develops. If delay of treatment interval is greater than two weeks because of drug toxicity the patient is to be discontinued from the study. Treatment will be discontinued due to progression of disease, unacceptable toxicity, death or at the investigator's discretion. Patients will be considered to have completed study when the above conditions have been met and the patient is off protocol treatment. Patients off study because of CR should be followed at every 9 week intervals.

8.0 TOXICITIES TO BE MONITORED AND DOSAGE MODIFICATIONS

8.1 Dose Modification for PSC 833 Related Toxicity

8.1.1 Hepatic toxicity

If end of cycle SGOT or SGPT concentrations rise to > 3 times the Institutional Upper Limit of Normal (IULN), the patient will be discontinued from the study.

8.1.2 Neurotoxicity

PSC 833 may cause reversible cerebellar dysfunction (ataxia, dysmetria) or paresthesias. If these occur during PSC 833 administration and are of ≥ grade 3 in severity, the dose of PSC 833 should be reduced by 25%.

Cerebellar dysfunction is the dose limiting toxicity of PSC 833. Since the definitions used in the Common Toxicity Criteria (CTC) are ill-suited to classifying the actual dysfunction observed in patients, the following definitions for grades 1 through 4 ataxia will be utilized.

Grade 1: Slight subjective sense of incoordination. No difficulty walking. Physical examination normal or equivocally normal.

Grade 2: Definite subjective incoordination on walking but able to walk without assistance. On examination, evidence of cerebellar dysfunction, such as broad-based gait, mild dysmetria, difficulty walking heel-to-toe or difficulty with rapid alternating movements.

Grade 3: Unable to walk without assistance from another person or a walker. On examination, markedly abnormal gait and inability to walk heel-to-toe.

Grade 4: Unable to walk because of incoordination, even with assistance.

If ≥ grade 3 neurotoxicity occurs before paclitaxel is administered, treatment should be discontinued until the toxicity resolves. The patient can then be restarted at 3.75 mg/kg/dose of PSC 833 and should receive the complete cycle of therapy (12 doses PSC 833 / paclitaxel, 3 hr., d2) at this dose.

If ≥ grade 3 neurotoxicity occurs after paclitaxel has been administered, treatment should be delayed until the toxicity resolves completely. Continued treatment with the reduced dose of PSC 833 (3.75 mg/kg/dose) may be resumed at the dose number the patient would have received if the cycle had gone uninterrupted. For example, if grade 3 ataxia occurs in a patient after dose #6 and she recovers completely by the time dose #9 would have been given, treatment should resume with dose #9 and continue through dose #12. Doses #7 and #8 would not be administered.

8.1.3 **Patients should be advised that the use of alcohol, sedatives or sleeping medications should be avoided during administration of PSC 833 as this could increase the likelihood of falling. Patients should also be advised not to drive a car or other vehicle during initial treatment with PSC 833 until effects on coordination in that patient have been determined.**

8.2 Dose Modifications for Paclitaxel

After the first cycle of paclitaxel alone or PSC 833 and paclitaxel, **the paclitaxel dose:**

8.2.1 **- may be increased by 10%** (of 175 mg/m^2) in subsequent cycles if the patient's nadir ANC was ≥ 1000/mm^3 and nadir platelet count was ≥ 100,000/mm^3 during the previous cycle.

8.2.2 - **should be attenuated** in subsequent cycles if during the previous cycle any of the following occurred:

the patient's nadir ANC was $< 500/mm^3$ for ≥ 7 days

nadir platelet count was $< 50,000/mm^3$

the patient experienced febrile neutropenia

the ANC fails to recover for retreatment by day 1 of the next cycle (ie. day 22)

8.2.2.1 For patients who have not had their paclitaxel dose escalated beyond the starting dose of 70 mg/m^2 (PSC 833 arm), the dose should be attenuated by 20% to 56 mg/m^2.

8.2.2.2 For patients who have had their paclitaxel dose escalated beyond the starting dose of 70 mg/m^2, the dose should be attenuated to the next lower level that the patient previously tolerated.

8.2.2.3 In neutropenic patients who have already been dose-reduced and experienced infectious complications during the previous cycle, we suggest G-CSF should be given beginning on day 3 (5 μg/kg/d, SC), approximately 24 hours after the completion of the paclitaxel infusion and continuing until hematopoietic recovery (ANC $\geq 1500/mm^3$). If G-CSF cannot be provided, another grade 4 neutropenia extending beyond day 14 will mandate a second 20% dose reduction to be carried out, and dose re-escalated to the previous baseline if an infectious complication is no longer encountered.

9.0 STUDY CALENDAR

	Cycle 1			Cycle 2 and subsequent cycles	At Progression
	Day 1	8	15	Day 1 (22)	
Ht, Wt, BSA	X			X	
Performance Status	X			X	
Physical Exam	X			X	
CBC diff., plat.	X	X	X	X	
SMAC panel*	X			X	
Urinalysis	X				
PT, PTT	X				
CEA, CA 15-3	X			X (if initially possible)	
CXR**	X			X	
EKG	X				
Scans***	X				
Biopsy for PCR of MDR gene expression****	X				X
Pharmacokinetic Studies*****	X (day 1, reg I; day 2, reg II)				
Taxol administration	X (day 1, reg I; day 2, reg II)			X (day 1, reg I; day 2, reg II)	
PSC 833 administration	X (regimen II)			X (regimen II)	

*Includes electrolytes, BUN, creatinine, glucose, calcium, albumin, LDH, alkaline phosphatase, SGOT, and SGPT. If used for treatment, should be performed within 4 days.
** CXR to be repeated every other cycle if used for disease assessment; otherwise every 4 months.
*** Scans to be repeated every 3 cycles if used for disease assessment.
**** (See Appendix VIII for method of preservation & transport.)
***** Mandatory on cycle 1 unless approved by PI (exceptions will be made for poor venous access).

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

10.1 Disease status

10.1.1 Measurable disease: Bidimensionally measurable lesions with clearly defined margins by 1) medical photograph (skin or oral lesions) or plain x-ray, with at least one diameter .5 cm or greater (bone lesions not included) or 2) CT, MRI, or other imaging scan, with both diameters greater than the distance between cuts of the imaging study or 3) palpation, with both diameters 2 cm or greater.

10.1.2 Evaluable disease: Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with both diameters less than 0.5 cm, lesions on scan with either diameter smaller than the distance between cuts, palpable lesions with either diameter less than 2 cm, bone disease. (Markers which have been shown to be highly correlated with extent of disease are also considered to be evaluable. Disease committees must specify markers considered to be evaluable. 'Committees may also clarify sites with margins not clearly defined.'

10.1.3 Nonevaluable disease: Pleural effusions, ascites, disease documented by indirect evidence only (e.g., by lab values).

10.2 Objective status

(To be recorded at each evaluation.) If an organ has too many measurable lesions at each evaluation, choose three to be followed before the patient is entered on study. The remaining measurable lesions in that organ will be considered evaluable for the purpose of objective status determination.

10.2.1 Complete response (CR): Complete disappearance of all measurable and evaluable disease. No new lesions. No disease-related symptoms. No evidence of nonevaluable disease, including normalization of markers and other abnormal lab values. All measurable, evaluable, and nonevaluable lesions and sites must be assessed using the same technique as baseline. Refers to clinical CR. (When restaging surgery is required, a separate pathologic response variable is incorporated in the response data).

10.2.2 Partial response (PR): Applies only to patients with at least one measurable lesion. Greater than or equal to 50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions. No progression of evaluable disease. No new lesions. All measurable and evaluable lesions and sites must be assessed using the same techniques as baseline.

10.2.3 Partial response in nonmeasurable disease (PRNM). Disease specific. Defines specific types of evaluable disease that may be followed for partial response if there are no measurable lesions. Defines response for these types. Patients with both measurable and evaluable disease are assessed according to the definition in 10.2.2, partial response.

10.2.4 Stable/No response: Does not qualify for CR, PR, or progression, All measurable and evaluable sites must be assessed using the same techniques as baseline.

10.2.5 Progression: 50% increase OR an increase of 10 cm^2 (which ever is smaller) in the sum of products of all measurable lesions over smallest sum observed (over baseline if no decrease) using the same techniques as baseline, OR clear worsening of any evaluable disease, OR reappearance of any lesion that had disappeared, OR appearance of any new lesion/site, OR failure to return for evaluation due to death OR deteriorating condition (unless clearly unrelated to this cancer). For "scan-only" bone disease, increased uptake does not constitute clear worsening. Worsening of existing nonevaluable disease does not constitute progression. Lesions that appear to increase in size due to presence of necrotic tissue will not be considered to have progressed unless associated with clear symptomatic progression in evaluation by attending MD.

Exceptions: In cases for which initial tumor flare reaction is possible (hypercalcemia, increased bone pain, erythema of skin lesions), either symptoms must persist beyond 4 weeks or there must be additional evidence of progression.

10.2.6 Unknown Progression has not been documented and one or more measurable or evaluable sites have not been assessed.

Notes

1) Nonevaluable disease does not affect objective status except in determination of CR (all disease must be absent - a patient who otherwise has a CR, but who also has had nonevaluable disease present or not assessed, will be classified as having a PR) and in determination of progression (if NEW sites of nonevaluable disease develop). Patients with only nonevaluable disease cannot be assessed for response.

2) For evaluable disease other then types specified in 2.3, the only objective statuses which apply are CR, stable/no response, progression and unknown.

3) Objective statuses must stay the same or improve over time until progression (unknown excepted).

4) PR and PRNM cannot apply to the same patient.

10.3 Best Response: Best response is determined from the sequence of objective statuses.

10.3.1 Disease assessment every 3 weeks. Two objective status determinations of CR before progression are required for a best response of CR. Two determinations of PR or better before progression, but not qualifying for a CR, are required for a best response of PR. Two determinations of PRNM or better before progression, but not qualifying for CR, are required for PRNM.

Two determinations of stable/no response or better before progression, but not qualifying as CR, PR or PRNM, are required for a best response of stable/no response; if the first objective status is unknown, only one such determination is required. Patients with an objective status of progression on or before the second evaluation (second AFTER the prestudy evaluation) will have a best response of increasing disease. Best response is unknown if the patient does not qualify for a best response of increasing disease and if all objective statuses after the first determination and before progression are unknown.

Use of the definition is illustrated in Table 1 with several sequences of objective statuses and the corresponding best response.

Table 1. Sequences of objective statuses with corresponding best response

1st objective status	2nd objective status	3rd objective status	Best response
3-6 week assessment interval			
Progression			Progression
Stable, PR, CR, unk	Progression		Progression
Stable[a]	Stable	Progression	Stable
Stable, unk[a]	PR, CR	Progression	Stable[e]
Stable, unk	Unknown[d]	Progression	Unknown
PR[b]	PR	Progression	PR
PR[b]	CR	Progression	PR
PR, CR	Unknown[d]	Progression	PR (Unconfirmed)
CR[c]	CR	Progression	CR
Unknown[a]	Stable	Progression	Stable

[a]Best response is the same if these sequences are preceded by the objective statuses of unknown or stable, or if unknowns separate the first objective status from the second.
[b]Best response is the same if these sequences are preceded by the objective statuses of unknown, stable or PR, or if unknowns separate the first objective status from the second.
[c]Best response is the same if these sequences are preceded by the objective statuses of unknown, stable, PR or CR, or if unknowns separate the first CR from the second.
[d]Best response is the same if followed by additional unknowns.
[e]Evaluation codes allow identification of these patients with best response of stable or unknown who had unconfirmed PR or CR.

10.4 ENDPOINT DEFINITIONS

10.4.1 Overall Survival. Defined as the time from first day of treatment to time of death due to any cause. If a patient is still alive, survival time is censored at the time of last follow-up.[1]

10.4.2 Progression-free survival (or relapse-free survival for adjuvant studies). Defined as the time from first day of treatment to the first observation of disease progression or death due to any cause. If a patient has not progressed or died, progression-free survival is censored at the time of last follow-up.[2]

10.4.3 Time to treatment failure. Defined as the time from first day of treatment to the first observation of disease progression, death due to any cause, or early discontinuation of treatment. If failure has not occurred, failure time is censored at the time of last follow-up.[3]

10.4.4 Time to progression. Defined as the time from first day of treatment to the first observation of disease progression or death due to disease. If failure has not occurred, failure time is censored at the time of last follow-up.[4]

10.4.5 Duration of response (CR/PR). Defined as the time from first objective status assessment of CR/PR to the first time or progression or death due to any cause. If a responding patient has not progressed or died, duration is censored at the time of last follow-up.[5]

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1 Time to death to disease (defined the same way, except for censorship at the time of death, if the death is due to other causes) generally is not used, since unbiased estimation is possible only if deaths due to other causes are independent of the cancer being studied, and since cause of death information is often unreliable.

2 This endpoint is preferred to "time to progression" (censorship at the time of death, if the death is due to other cause) for reasons similar to those noted above.

3 This endpoint is often appropriate in studies of advanced disease where early discontinuation typically is related to poor patient response or tolerance.

4 This endpoint is often appropriate in studies of advanced disease where early discontinuation typically is related to poor patient response or tolerance.

5 Since it can be misleading to report failure times only in a subset of patients, this endpoint is used less often that the other four.

10.5 Definition of performance status levels. (Appendix II)

11.0 SPECIAL INSTRUCTIONS:

The concomitant use of investigational agents other than PSC 833 will **NOT** be permitted during this study.

Antiemetic agents may include intravenous ondansetron. *Orally* administered metoclopramide or ondansetron should be avoided during the administration of PSC 833.

Other medications required to maintain the patient's baseline condition or to treat a coexistent condition may be administered at the discretion of the Principal Investigator. Patients who require a concomitant medication for a chronic condition may continue to use that medication if it is agreed upon by the sponsor's medical expert and provided that the medication's use is not contraindicated.

Information regarding the administration of all concomitant prescription medications used during the course of this study should be entered on the appropriate case report form.

12.0 STATISTICAL CONSIDERATIONS

This is a multi-center Phase II study. The co-ordinating center is located at the City of Hope National Medical Center. The participating centers are the City of Hope (COH), the USC-Norris Cancer Center (USC), and the University of California at Davis (UCD).

The primary objective of this study is to assess the response to paclitaxel alone and to paclitaxel with PSC-833 in similar groups of women with advanced hormonally-insensitive breast cancer who have failed anthracycline-based therapy or for whom anthracyclines are contraindicated. The first period of this study is designed to meet this goal and is a randomized Phase II trial which will accrue a maximum of 86 patients (43/treatment arm).

The secondary objectives of this study are to describe the plasma pharmacokinetics of paclitaxel with and without PSC-833, to estimate the response rates for patients with MDR+ and MDR- tumors, and to obtain (preliminary) information regarding the difference or similarity of these in the treatment arms. For the objectives involving MDR, additional patients will be accrued to a second period of this study (requiring approximately 60 additional patients to be randomized, for a total of 116 patients with completed MDR determinations).

12.1 Study Design and Justification

12.1.1 First Period of the Trial

For each arm separately, a design with three stages of accrual suggested by Ensign et al (34) will be used. The design selected is based on the following assumptions: a true response rate less than 5% would not warrant further study of the agent(s) at the proposed dose and schedule; a response rate of 20% would be considered promising for further studies in these patients. A three-stage design was selected since paclitaxel+PSC-833 has not been studied

extensively in this group of patients and the dose of paclitaxel is lower in the paclitaxel+PSC-833 arm; although it is not anticipated, should the combination be ineffective, this design will allow for early termination.

In the first stage, 14 evaluable patients will be entered. If no responses are observed, then accrual will stop with the conclusion that the regimen is not promising for further study. If 1 or more responses are observed in the first 14 patients, then an additional 15 patients will be accrued during the second stage. If 2 or fewer responses out of 29 patients are observed, then accrual will stop with the conclusion that the regimen is not promising for further study. If 3 or more responses are observed in the 29 patients, then an additional 14 patients will be accrued during the third and final stage. Five or more responses out of 43 patients will be taken as evidence warranting further study of the regimen providing other factors, such as toxicity and survival appear favorable. If 4 or fewer responses out of 43 patients are observed, further study of the regimen would not be considered.

12.1.1.1 Rationale for the Design of the First Period

With this proposed design, the probability of falsely declaring an agent with a 5% response rate as warranting further study, is 0.05 (alpha), and the probability of correctly declaring a regimen with a 20% response rate as warranting further study is 0.90 (power). With 43 patients, the true probability of response can be estimated with a maximum standard error equal to 0.076.

Assuming that biopsies will be evaluable and analyzable in at least 80% of patients enrolled on the trial, there will be approximately 34 patients per treatment arm for which MDR status will be available. Twenty percent of patients enrolling in this study are expected to be MDR+, resulting in approximately 7 MDR+ and 17 MDR- patients in each treatment arm. This estimate of MDR positivity is conservative; the actual rate may be higher. Pharmacokinetics will be required of all patients in the first stage of the trial. Descriptive comparisons will be performed to examine the relationship between the pharmacokinetic parameters, treatment, and MDR status.

Based on current projections expected accrual is approximately 70 patients per year; it should take slightly longer than one year to complete accrual to this portion of the trial.

12.1.2 Second Period of the Trial

At the completion of the first period of the trial, the members of the Phase I/II Consortium will discuss whether to continue or to terminate the trial. In consultation with the NCI staff, a decision will be made, based on the proportion of patients with biopsy specimens which were adequate for analysis (at least 80%), the proportion of patients who were MDR+ (at least 20%), the response rates and toxicity observed in the first stage, and the difference in response rates between the two arms (not significantly different at the 0.05 level - i.e. an observed difference of less than 21%).

If a decision is made to continue, accrual will proceed until a total of 116 of the randomized patients have been treated and are evaluable for response and MDR, and until 10 patients per arm are found to be MDR+. Approximately 60 more patients will be randomized in order to have 116 patients evaluable for the MDR analyses. It should take approximately one more year to complete the second period of this study.

12.1.2.1 Rationale for the Design of the Second Period

Once accrual is completed for the second period, the odds ratio for the association between MDR status and response will be estimated for each treatment arm and 95% confidence intervals will be constructed. The odds ratios will be formally compared using the (one-sided) test for a three-way interaction in a loglinear model. (The odds ratio is the ratio of the odds of responding to treatment for patients who are MDR+ compared to the odds of responding to treatment for patients who are MDR-.) If this test is not significant at the 0.05 level and if the ratio of the odds ratio is less than 3.00, then we will combine the two treatment arms and compare the response rates for patients with MDR+ tumors to patients with MDR- tumors (one-sided 0.05-level test). If the observed ratio of the odds ratios is greater than 3.0, but the formal test is not significant at the 0.05 level, the individual odds ratios and response rates will be examined in order to decide whether to combine the two treatment arms.

Two of the underlying biological hypotheses of this trial are (1) patients whose tumors are tested to be MDR+ are less likely to respond to paclitaxel and (2) PSC-833 will reverse MDR1 Pgp activity and lead to responses to paclitaxel in patients whose tumors are tested to be MDR+. If the formal test to compare the odds ratios between the two treatments, is significant at the 0.05 level, then this will be strong evidence to support the second hypothesis. However, with 116 evaluable patients, there will be little power (see column 12 in Table 2) for this test. If

the formal test is not significant at the 0.05 level, then the confidence intervals and the ratio of the odds ratios will be used to decide whether to combine the results of the two treatment arms in order to compare the response rates of MDR+ and MDR- tumors. In this situation, although we will not conclude formally that the odds ratios are similar or different, we will be able to present estimates that are compatible or incompatible with the second hypothesis. For example, if PSC-833 reverses MDR1 Pgp activity, then depending on the response rate in patients who are MDR-, there will be a reasonable chance (i.e. greater than 70%, if the overall response rate to paclitaxel is 25% or better) that the observed ratio of the odds ratios will be 3.0 or greater (see column 13 in Table 2).

If we do not find the differences in the odds ratios to be strong, then we will combine the results of both treatment arms to compare the MDR+ ($n \approx 23$) and MDR- ($n \approx 93$) tumors in terms of response rates. In this situation (if PSC-833 has no effect), 116 patients will insure that we have at least 0.80 power using a one-sided 0.05-level test when the true response rates are 5% and 30% for MDR+ and MDR- tumors respectively, and 20% of patients have MDR+ tumors (35). In certain situations, when PSC-833 only has a moderate effect, there will also be reasonable power (ie. greater than 0.80, see column 16 in Table 2) to observe a significant difference in response rates.

12.2 Stratification and Randomization

Prior to randomization, each patient will be stratified according to (a) whether she recurred within 6 months of completion of adjuvant therapy or progressed after one prior therapy for advanced disease, (b) whether she has measurable vs. evaluable disease, and (c) the responsible institution (USC, COH, UC Davis). An adaptive randomization algorithm will be used to ensure that the two treatment arms will be balanced for the three potential prognostic factors (36).

12.3 Analysis of Results

12.3.1 First Period of the Trial

12.3.1.1 Analysis of Clinical Endpoints

Objective tumor response (CR, PR or Improvement), survival, and time to treatment failure (disease progression, termination of treatment due to toxicity, or death due to any cause - whatever occurs first) will be used to evaluate efficacy. 95% confidence intervals will be constructed for the response rates (37) and for the median time to failure and survival. For the

comparison of paclitaxel alone and paclitaxel+PSC-833, an intent-to-treat analysis will be performed; all patients randomized will be included in the analyses. For response, patients who go off-study prior to the evaluation of response will be included in the group of non-responders. Patients who terminate treatment early will be followed for progression; patients who begin another therapy will be counted as having failed this protocol.

Toxicity as classified by the NCI Common Toxicity Criteria will be used to assess the side effects except for neurotoxicity (see Section 8.1.2). Since the dose of paclitaxel is lower in the paclitaxel+PSC-833 arm, all tests performed and all p-values reported will be two-sided. All toxicities, the time of onset, severity, duration and reversibility will be examined and summarized for each arm separately. In addition to the toxicity grades, the nadir WBC, ANC, and platelet counts will be compared, as well as maximum bilirubin, SGOT, and alkaline phosphatase determinations. Primarily, chi-square tests and t-tests (or Wilcoxon tests) will be used for these comparison.

12.3.1.2 **Analysis of Pharmacokinetic Studies**

For each patient who undergoes the pharmacokinetic studies, the estimate of the paclitaxel AUC and the estimate of the time that the serum paclitaxel levels are above 0.05 μm will be computed (for the first course) (38). Descriptive analysis will be performed to compare these values among patients who are MDR+ ($n \approx 14$) and MDR- ($n \approx 55$), and who received PSC-833 ($n=43$) or not ($n=43$). In addition, the association of these levels with response and grade 4 myelosuppression (during the first course) will be examined. Finally, the WBC and ANC nadirs will be plotted as a function of the pharmacokinetic determinations to further describe the association. A regression analysis (after transformation, if necessary) will be used to compare the pharmacokinetic values among patients who show Pgp immunostaining or not, and who received PSC-833 or not.

To compare the pharmacokinetic determinations in terms of whether or not the patient showed Pgp immunostaining (≈ 14 MDR+; ≈ 54 MDR-) there will be 90% power, and in terms of the treatment received (≈ 43/group) there will be greater than 95% power, for detecting a difference of 1.0 standard deviations, using a two-sided 0.05-level t-test.

12.3.2 **Second Period of the Trial**

Response rates and 95% confidence intervals will be estimated for MDR+ and MDR- subgroups within the treatment arms; for each treatment arm the odds ratio for the association between MDR status and response will be estimated and 95% confidence intervals constructed. If the odds ratios are found to be similar (see Section 12.1.2.2) then treatment groups will be combined for overall estimates.

For the paclitaxel and paclitaxel+PSC-833 arms separately, the objective response rates will be reported, and the survival and time to treatment failure will be summarized using Kaplan-Meier plots (39). Confidence intervals will be constructed for the response rates and the median times to failure.

12.4 **Analysis of Ethnic Subgroups**

No differences between ethnic groups are known in terms of MDR expression or efficacy of paclitaxel with or without PSC-833. At completion of this study, we will summarize the results by ethnicity - per NIH requirements. In particular, we will compare the Hispanic patients and the non-Hispanic white patients in terms of toxicity experienced, response rate, time to treatment failure, and the results of the Pgp immunostaining.

13.0 REGISTRATION GUIDELINE

13.1 Baseline serum measurements, CXR (PA and L), performance status, height, and weight must be performed within 1 week of treatment.

13.2 Other imaging studies must be completed within 2 weeks prior to initiation of therapy.

13.3 Once signed informed consent has been obtained and all pretreatment evaluations have been performed, patients will be entered on study. To register a patient the research nurse or data manager must complete the Eligibility Checklist and FAX a copy of this and the informed consent including the Patient Human Rights to the Phase II coordinator at City of Hope (FAX # 818-301-8393). The research nurse or data manager will call the coordinator at 818-359-8111 x2468, and after verifying the eligibility, the coordinator will register the patient onto the study and assign a patient accession number. See appendix (Registration Procedures for Phase II Trials) for details.

COH patients may be screened for registration by calling the Department of Biostatistics, 818-359-8111, extension 2468.

The individual accepting registrations should ascertain the date of IRB approval at each participating institution before registering the first patient from that institution.

Multicenter study records at each participating institution will be randomly selected for audit.

14.0 DATA SUBMISSION SCHEDULE

All data will be collected using COH Biostatistics Information Tracking System (BITS) data collection forms. According to the submission schedule specified in Appendix V (forms submission for Phase II Trials) completed forms should be submitted to the City of Hope Department of Biostatistics c/o the assigned COH data manager. The original data collection forms will be stored in a secure location at COH. USC and UCD will store a copy of all forms and mail the originals to COH.

15.0 MINORITIES AND WOMEN STATEMENT

TABLE - ETHNIC AND GENDER DISTRIBUTIONS OF CANCER PATIENTS IN 1992 IN LOS ANGELES COUNTY

		ETHNIC DISTRIBUTION				GENDER DIST	
PRIMARY SITE OF TUMOR	TOTAL	WHITE %	HISPANIC %	BLACK %	ASIAN/OTHER %	FEMALE %	MALE %
BONES & JOINTS	66	48	40	6	6	50	50
BREAST	4584	67	15	11	7	99	1
EYE & ORBIT	62	75	18	5	2	44	56
SOFT TISSUE INCLUDING HEART	205	57	27	10	6	48	52
CERVIX	526	34	46	12	8	100	0
OVARY	662	68	18	7	8	100	0
URINARY SYSTEM	1569	72	14	9	5	33	67
LEUKEMIAS	864	60	23	9	8	44	56
NON-HODGKINS LYMPHOMA	1224	67	18	8	7	42	58
LUNG & BRONCHUS	4274	70	10	14	6	42	58
UNKNOWN PRIMARY	993	67	15	12	6	54	46
TOTAL	15025	67	15	11	7	64	36

The numbers above reflect the ethnic and gender distribution of cancer patients in the County of Los Angeles. Although distributions may vary by disease type, our recruitment procedures have been developed to enroll patients who are representative of the target population.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

All institutional, NCI, Department of Defense and Federal regulations concerning the Informed Consent form will be fulfilled.

17.0 REPORTING REQUIREMENTS

17.1 Any life-threatening and/or unexpected and serious (grade 3 or 4) toxicity will be reported immediately to the study chairman who, in turn, must notify the IRB (and, if applicable, the sponsoring agency).

17.2 Report by phone to Investigational Drug Branch (IDB) within 24 hours (301-230-2330), available 24 hours, recorder after hours) all life-threatening and lethal (grade 4 and 5) unknown reactions. Written report to follow within 10 working days. Information may be FAXED to 301-230-0159.

17.3 Report in writing within 10 working days:

17.3.1 Life-threatening and lethal (grade 4 and 5) known reactions (except grade 4 myelosuppression).

17.3.2 Grade 2 and 3 unknown reactions.

Address for submitting ADR reports:

Investigational Drug Branch
Box 30012
Bethesda, MD 20824-9998

17.4 ADRs will be reported as outlined in the Appendix (Reporting Guidelines for Adverse Drug Reactions). Questions regarding ADR reporting should be directed to the COH data manager at 818-359-8111 extension 2468.

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19.0 APPENDICES

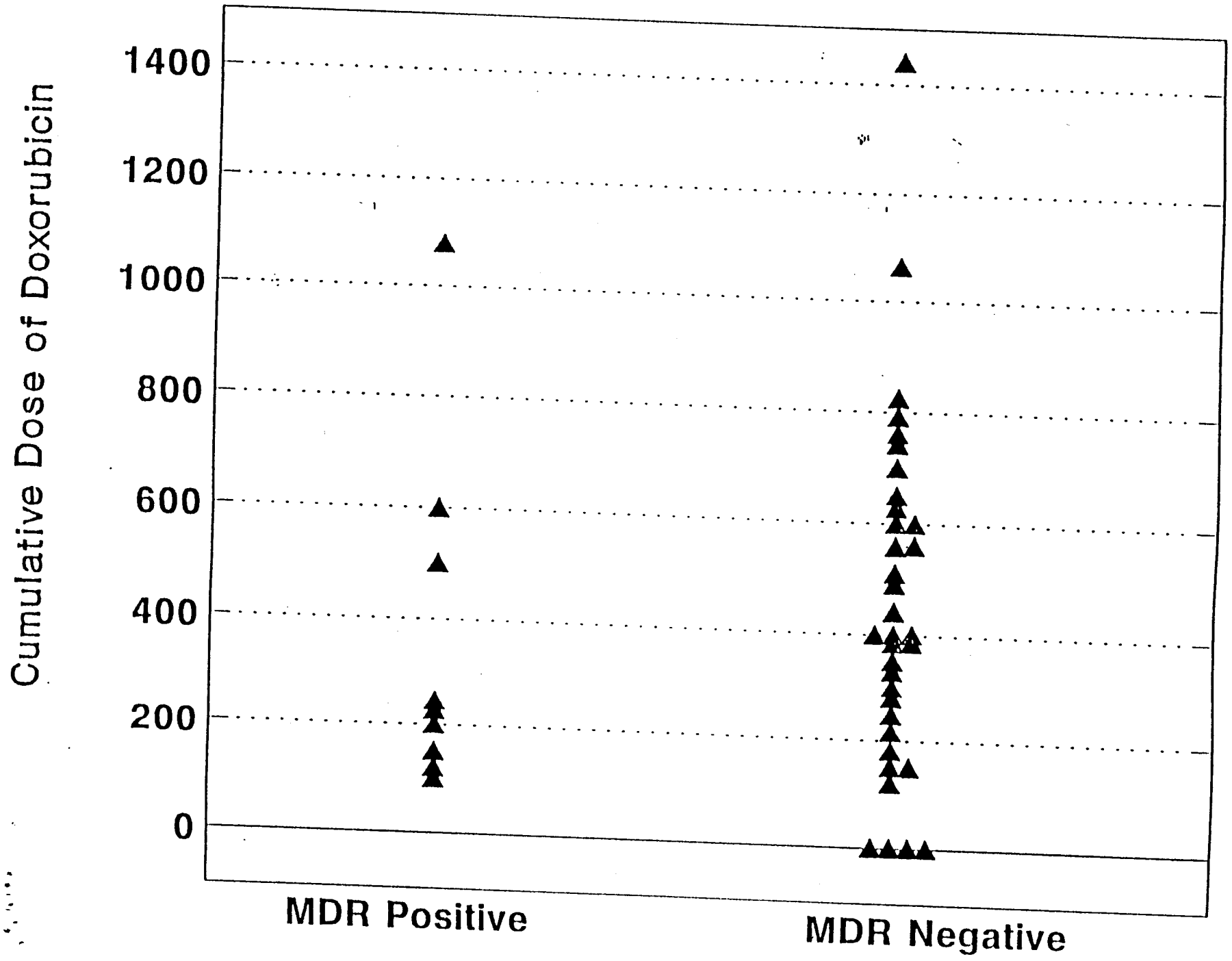

Cumulative Dose of Doxorubicin
1400
1200
1000
800
600
400
200
0
MDR Positive
MDR Negative

Figure 1

Figure 2

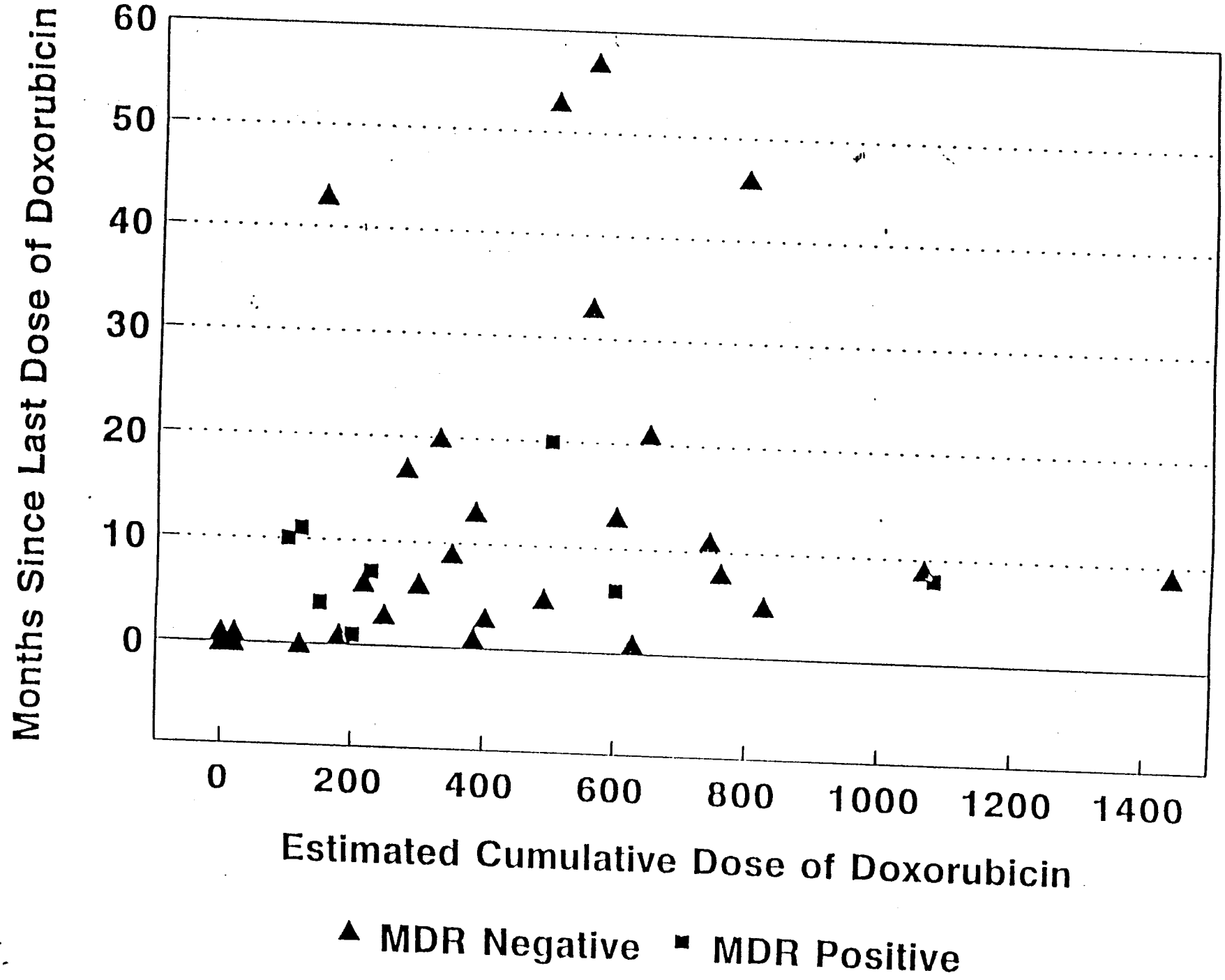
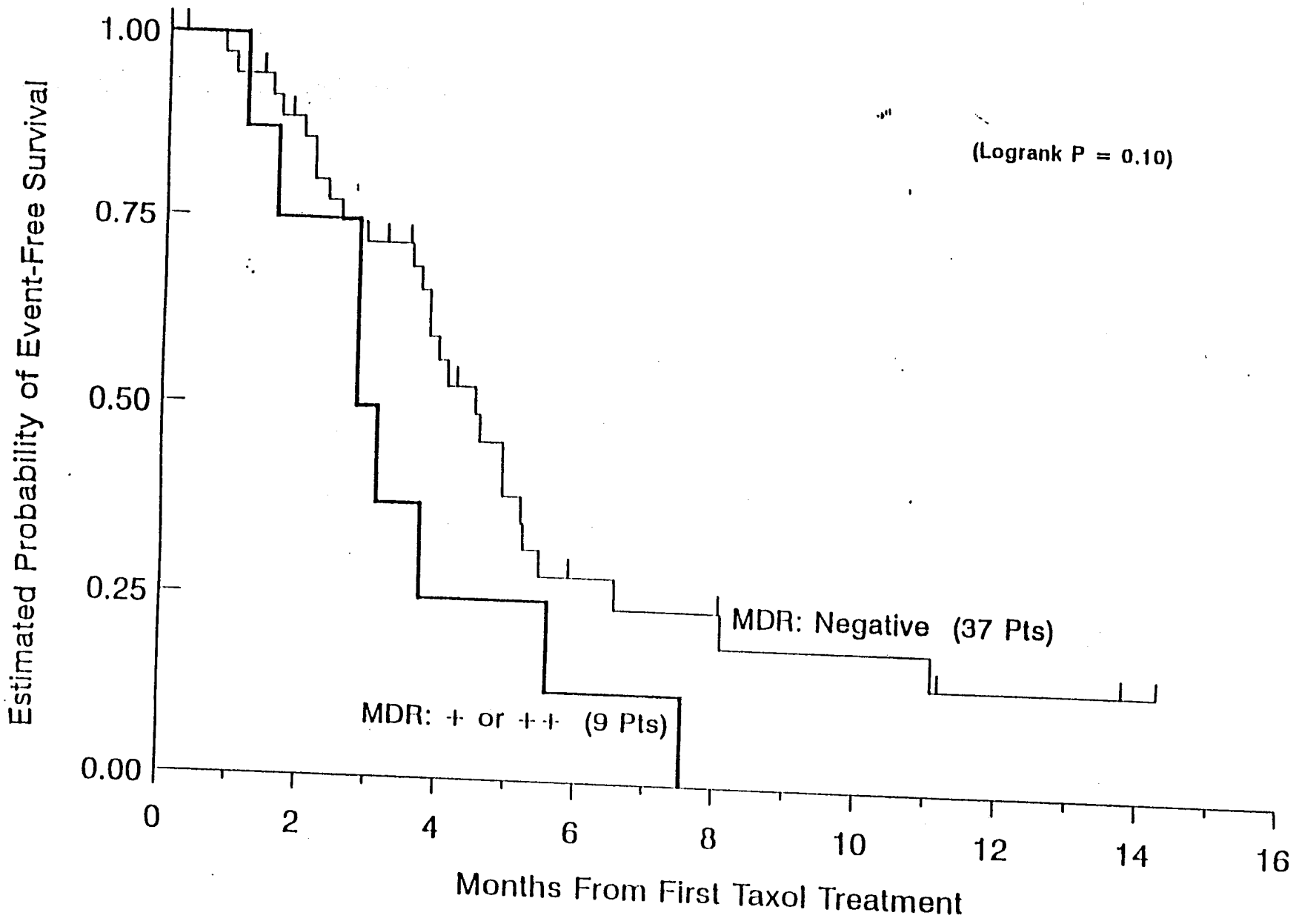

Months Since Last Dose of Doxorubicin
60
50
40
30
20
10
0
0
200
400
600
800
1000
1200
1400
Estimated Cumulative Dose of Doxorubicin
MDR Negative
MDR Positive

Figure 3


Estimated Probability of Event-Free Survival
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
14
16
Months From First Taxol Treatment
(Logrank P = 0.10)
MDR: Negative (37 Pts)
MDR: + or ++ (9 Pts)

STANDARD CRITERIA FOR ESTIMATION OF PERFORMANCE STATUS

SWOG Criteria Grade	Karnofsky Scale Grade	Scale Definition
0	90-100	Fully active, able to carry on all predisease performance without restriction.
1	70-80	Restricted in physically strenous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	50-60	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	30-40	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	10-20	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	0	Dead