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Drugs that are substrates of *MDR1*-P-glycoprotein (Pgp) such as doxorubicin and paclitaxel are key components in the current systemic treatment of breast cancer. We have investigated the expression of *MDR1* gene and its product, Pgp, in 3 groups of patients with breast cancer: 1) those entered in a clinical trial of paclitaxel for recurrences after chemotherapy (usually containing doxorubicin, 59 patients), 2) patients in a trial of 5-fluorouracil and radiation for locally advanced presentations (17 patients, of whom 14 biopsies were prior to any treatment) and 3) primary cancers within our tissue bank without any prior treatment (39 patients). In 115, expression was determined by immunocytochemistry employing the JSB-1 monoclonal antibody (and other Pgp immunostains for comparison). In 35 of these, the findings were confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR). Neither immunohistochemical staining nor RT-PCR demonstrated Pgp expression in any of the breast cancer patients. Because it is unlikely that Pgp is a frequent mechanism of drug resistance for the vast majority of untreated or minimally treated breast cancers, we have shifted our attention towards contrasting the mechanism of *MDR1* activation in breast versus ovarian cancers and seeking alternate drug-efflux pathways that may be exploited clinically. Within this perspective, our clinical trials have continued to focus on the modulation of paclitaxel activity, its pharmacokinetic consequences, and whether Pgp may be expressed after paclitaxel treatment. In the future we plan to explore non-invasive methods such as Technetium-99 sestamibi tumor clearance rates in order to detect reversal of drug-efflux. Such studies should provide important missing information on efflux mechanisms other than Pgp that may be operative in breast cancer.

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INTRODUCTION

We are summarizing year 2 of the grant, and the progress that has been made on our understanding of the relevance of multidrug resistance mediated via P-glycoprotein (Pgp) in breast cancer. The systemic treatment of breast cancer has been hampered by the eventual emergence of drug-resistant populations, in spite of initial responses in the majority of patients. In addition to anthracyclines, alkylating agents, fluoropyrimidines and other antimetabolites, in the past 5 years the taxanes have emerged as drugs with major antitumor activity both in the absence and the presence of previous treatment. Since paclitaxel is an excellent substrate of the Pgp-efflux pump encoded by the multidrug resistance gene *MDR*1and its role in breast cancer treatment is undergoing extensive evaluation, trials with this drug represented an excellent opportunity to delineate the importance of this drug-resistance mechanism in this disease. Therefore, the grant was structured to focus on determinations of Pgp before and after treatment with anthracycline-containing regimens, and on clinical trials of paclitaxel with drugs known to reverse Pgp action and restore sensitivity. These MDR1-reversal drugs have included calcium-channel blockers, steroid hormone-related derivatives, cyclosporins, and a number of other drug classes. The four tasks of the grant included 1) delineating the distribution of Pgp in the various subsets of patients with breast cancer (premenopausal, postmenopausal, stage related, hormone receptor related), 2) developing a paclitaxel plus a resistance reversal drug regimen in the treatment of advanced breast cancer, 3) performing a randomized clinical trial to assess the strategy of adding a multidrug resistance reversal to paclitaxel, and 4) investigating new methods to identify and reverse multidrug resistance.

The identification of the circumstances surrounding the occurrence of multidrug resistance in breast cancer has become even more important since the grant funding began in October 1994. Two of the drug classes that are key components in the current systemic treatment of breast cancer, the taxanes and the anthracyclines have been the focus of much clinical work, both in the adjuvant and in the advanced disease setting. Cooperative group trials are investigating adjuvant doxorubicin as sequentially versus combined with cyclophosphamide in high risk patients with 0 to 3 lymph nodes involved (SWOG-9313), or in arms with escalating doses, with or without paclitaxel for node positive disease (SWOG 9410, intergroup *study*). In advanced breast cancer, combinations of doxorubicin and paclitaxel are being compared to either drug alone (Intergroup study, coordinator Dr. George Sledge). While awaiting the results of this study, pilot studies have indicated not only striking activity, but also the likelihood of pharmacokinetic interactions between the two drugs with the potential of leading to higher AUCs of both doxorubicin and its metabolite, doxorubicinol. Presumably, in part related to this interaction, a propensity to enhanced cardiotoxicity has been described[1,2]. The pharmacokinetic interactions are likely to occur because both doxorubicin and paclitaxel are excellent substrates for *MDR1*-P-glycoprotein (Pgp). In the development of future therapeutic strategies it is highly desirable that the circumstances surrounding the expression of *MDR1* in these cancers be well characterized.

Accordingly, during the first year we devoted time to assemble pathologic specimens and accompanying clinical data for the eventual study of Pgp and prognostic correlates. Forms for clinical data retrieval were developed (Appendix 1). Specimens were obtained from primary surgical sources and from clinical trials that were designed to prospectively study the relation of Pgp immunostaining and response to paclitaxel treatment. These clinical trials were initially developed to explore the relevance of dose-schedules and modulators of drug resistance in achieving objective responses. The ultimate intent was to eventually select a paclitaxel dose-schedule and a modulator of drug resistance for phase II/III clinical study. Such study would be expected to shed further light on the importance of Pgp and drug-resistance modulation as a treatment strategy. These trials would potentially also contribute to optimize doxorubicin-paclitaxel regimens.

EXPERIMENTAL METHODS

a) Laboratory Methods:

Specimen Acquisition. Frozen tissue specimens were immediately embedded in Optimal Cutting Tissue® Compound (Tissue Tech Laboratories, Elk Hart, IN) and snap-frozen in liquid nitrogen before storage at -80°C. Normal human adrenal gland, liver, and kidney were obtained from the USC Tissue and Tumor Bank as positive controls.

Immunohistochemistry of P-glycoprotein. A series of commercially available P-glycoprotein antibodies were compared for immunostaining sensitivity and specificity using control tissues and cell lines. Optimal concentrations were determined by serial dilution of each antibody. Fixation conditions were chosen according to the manufacturer's recommendations. The peroxidase anti-peroxidase immunohistochemical technique was used to localize P-glycoprotein (Pgp) in tissue. Cryostat sections (6 μm) were prepared and fixed with acetone or 4% formaldehyde at room temperature for 10 minutes. The section were then washed in phosphate buffered saline (PBS) and treated with 0.5% hydrogen peroxide-PBS for 15 minutes to inactivate endogenous peroxidase activity. After 2 washings with PBS, 10% normal rabbit serum in PBS was applied to the sections to block the free binding sites. Monoclonal Pgp antibodies JSB-1 (Signet, Boston, MA) and/or MRK16 (Kamiya Biomedical Company, Tukwila, WA) were applied to the sections for 1 h at a concentration of 10μg/ml and 5μg/ml respectively in 10% rabbit serum. The concentrations were chosen after testing with positive control tissue and cell llines to ensure that low levels of staining were not missed. Specimens were then washed in PBS, 3 changes for 3 minutes each. Rabbit anti-mouse IgG (anti-H+L chains, ZYMED) bridging antibody at a 1 to 50 dilution was applied to the tissue section for 30 minutes in 10% normal rabbit serum. After again washing three times with PBS rabbit anti-mouse IgG conjugated with peroxidase at a dilution of 1:50 was applied for another 30 minutes. After a third wash with PBS, DAB solution was applied to the sections and reacted for 10

minutes as recommended by the manufacturer to identify the sites of immunoprecipitate formation. After a final wash with PBS, the preparations were counterstained with ethyl green, mounted in Permount, and cover-slipped.

Negative control for each sample were performed as above but with normal mouse isotype control immunoglobulins (ZYMED, S. San Francisco, CA) instead of primary antibody. The histology of each specimen was confirmed by review of one section stained with hematoxylin and eosin. Doxorubicin-resistant myeloma cell lines 8226/6 and /40 (Dr. T.M. Grogan, U of Arizona), ovarian cancer cell lines OVCAR4/ADR 100 (generated by stepwise selection: Yang X, Page M Cancer Lett 88:171-8, 1995), normal adrenal gland, kidney and liver were used as positive control samples and assayed with each group of tumor samples. OVCAR4/ADR100 cell lines were continuously maintained in the presence of 6ng/ml, 40 ng/ml and 100 ng/ml doxorubicin and grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY) 2 mM L-glutamine, 100 units penicillin and 100μg streptomycin at 37°C in a humidified atmosphere of 5%CO2. Cell lines were evaluated by two observers without knowledge of the clinical data. Immunohistochemistry was scored on a four point basis: 0 = no staining, 1+ = weak staining, 2+ = moderate staining and 3+ = strong staining with the percentage of positive tumor cells in each category.

Detection of *MDR1* mRNA. *MDR1* mRNA was characterized by RT-PCR of total RNA prepared from frozen tissues. Total RNA was prepared by TRIzol method (GIBCO) as recommended by the manufacturer. RNA yield was determined by spectrophotometry at 260 nm. cDNA was synthesized with 2.5 μg of total RNA and 250 ng of poly (A) oligo (dT) 15 primer (Promega) in 50 μl of a solution containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, 500 μM each dNTP, and 500 units of Moloney murine leukemia virus reverse transcriptase (GIBCO). After 1h at 40°C, the reaction was stopped by heating to 95°C for 5 minutes to inactivate the reverse transcriptase. cDNA was stored at -20°C until used.

PCRwas carried out with cDNA derived from 100 ng of RNA, 2 units of Taq polymerase and reaction kits (Promega) in a final volume of 50 ul. Each cycle of PCR included 1 minute of denaturation at 94°C, one minute of primer annealing at 55°C, and 2 minute of extension/synthesis at 72°C. PCR primers were synthesized by using an Applied Biosynthesis DNA synthesizer (model 394) at Norris Comprehensive Cancer Center, USC. Beta-2-microglobulin (β2m) was selected as an internal control and suitable PCR primers were synthesized: sense primer 5'-ACCCCCACTGAAAAAGATGA-3' corresponding to β2m cDNA 1544-1563 and antisense primer 5'-ATCTTCAAACCTCCATGATG-3' corresponding to cDNA sequence 2253-2262 and 3508-3517 (Gussow D et al, J Immunol 139:3132-3138, 1987). *MDR1* cDNA sense (5'-CCCATCATTGCAATAGCAGG-3') and antisense (5'-GTTCAAACTTCTGCTCCTGA-3') primers were used to amplify cDNA segment (157bp) from 2596 to 2752 (Noonan et al, PNAS 87:7160-4, 1990). Each primer was added at 55 pmol per reaction. PCR was carried out in a DNA thermal cycler (Crocodile III, Appligene, Oncor). Amplification was carried out for 40 cycles. PCR products were separated on 2% agarose gel.

Electrophoresis was performed (45 mM Tris-borate buffer, 1 mM EDTA) at 100 V for 2 h. Gels were stained with ethidium bromide, examined on a UV transilluminator (Gel Documentation System, ULTRA-LUM) and photographed. Negative controls were prepared with water instead of cDNA.

b) Clinical Methods:

Clinical data acquisition. A nine-page form is being completed on all USC subjects whose specimens constitute part of the UCLA/USC Breast Tumor Bank (supported under DAMD 17-94-J-4234). This form includes demographic information, history of prior malignancies, diagnosis information/staging and pathology, treatment following the initial diagnosis, subsequent treatments, and status at last follow-up (Appendix 1; modified from 1995-year 1 annual report).

Paclitaxel (Taxol) in patients with locally advanced or metastatic breast cancer (1B-92-3). This protocol consisted of two sequential phase II studies of two dose-schedules of paclitaxel, and was begun under Cancer Therapy Evaluation Program, National Cancer Institute sponsorship (no funding). It was completed in August 1996 as 1B-95-4 was opened. All patients had histologically proven advanced breast cancer, with tumor accessible for biopsy and failure of one or more prior standard chemotherapy. Other requirements included performance status (Zubrod scale) of 2 or better, satisfactory hematologic, renal, and hepatic functions, adequate interval from preceding therapies, negative pregnancy test if applicable, and signed informed consent..

Phase II randomized study of paclitaxel versus paclitaxel + PSC833 for advanced hormonally insensitive breast cancer (1B-95-4). This protocol began for patient entry in July 1996 replaced the prior study and consists of identical eligibility criteria, except for the requirement that the prior treatment must have consisted of doxorubicin (Appendix 2). PSC833 is a non-immunosuppressive cyclosporin analog provided by the Sandoz company, who has allowed crossfiling of their IND for this study. Pharmacologic studies of paclitaxel alone and with PSC833, as outlined in Appendix 2, are being performed under a National Cancer Institute cooperative agreement that includes the City of Hope and University of California at Davis, as well as USC. These institutions are also participating in this randomized study under support from their cooperative agreement. The biopsy specimens will be studied for Pgp and RT-PCR under this grant. The study will be amended to allow patients without biopsable material into the study, and lead to an earlier completion.

Phase II study of paclitaxel with megestrol acetate for *MDR1* reversal in recurrent and metastatic breast cancer (1B-93-8). This study employed a 96-hour schedule that proved impractical for the randomized trial that was originally conceived. Patient entry requirements are identical to those of protocol 1B-92-3, except that a biopsy is not required, and a central venous catheter must be placed prior to treatment. The study has

only 8 patient entries, but remains open as a lower priority protocol to 1B-95-4, in case suitable patients are referred that would allow completing accrual of 14 patients total.

Phase I dose escalation study of estramustine phosphate (EMP) given to enhance paclitaxel action (0C-93-4). This study has been performed under USC-Norris Core Grant (NCI) sponsorship, with the pharmacology being supported from industry gifts. It has completed accrual to the dose-escalation phase I portion, and is under analysis for the pharmacologic portion that was to accrue 10 patients at paclitaxel 225 mg/m2 over 3 h, and EMP 900 mg/m2 in 3 divided doses. Criteria for patient entry included confining eligibility to women who had been previously treated with paclitaxel. Men were excluded in order to have clear delineation of estrogen-related EMP toxicity in women.

Multimodality protocol of continuous infusion 5-fluorouracil and concomitant radiation therapy for potentially resectable, locally advanced breast cancer (1B-93-3). This study is conducted by Dr. Silvia Formenti, Associate Professor of Radiation Oncology, under R01 support from the National Cancer Institute (Dr. Peter Danenberg, principal investigator, Professor of Biochemistry). Breast cancer samples obtained during the course of these studies are also being studied for Pgp.

RESULTS

a) Laboratory Studies:

Testing of P-glycoprotein antibodies. Several commercially available anti-Pgp monoclonal antibodies were compared by immunohistochemical staining of Pgp-positive normal human tissues (adrenal gland, kidney, liver) and multidrug-resistant cell lines (myeloma 8226/40 and an ovarian cancer cell line OVCAR4/ADR100). JSB-1, C219 and C494 react with intracellular epitopes of Pgp, whereas MRK16, UIC2 and 4E3 recognize external epitopes of Pgp. Although all of the antibodies showed immunostaining in each of the *MDR1*-expressing tissues or cells, the intensity of this staining varied with the antibody and tissue type. JSB-1 and MRK16 were selected as the antibodies of choice for our studies because they provide strong staining across the spectrum of positive-control tissues (Table 1)

Table 1. Comparison of immunohistochemical detection of P-glycoprotein with six monoclonal antibodies

Tissue and cells	JSB-1	MRK16	UIC2	C219	C494	4E3
Nl adrenal	3+	3+	3+	2+	2+	3+
Nl kidney	3+	3+	3+	1+	2+	2+

Nl liver	2+	1+	1+	3+	1+	1+
OVCAR4 *	3+	3+	1+	1+	2+	3+

*subline ADR100

Immunohistochemical analysis of P-glycoprotein expression. None of the 115 tumors including 39 primary untreated, 17 entered in the locally advanced protocol (1B-93-3), and 59 entered in protocol 1B-92-3 showed membrane or cytoplasmic immunostaining for Pgp. The prior therapy of these patients included doxorubicin in 54, tamoxifen in 38, 5-fluorouracil + radiation in 3, and autologous bone marrow regimens in 5. No patients previously treated with paclitaxel have been studied as yet. Pgp membrane staining was strongly (3+) positive in normal adrenal cortex and proximal kidney tubules, the doxorubicin-resistant myeloma cell line, 8226/40 and the doxorubicin-resistant ovarian cancer cell line OVCAR4/ADR100. Moderate immunostaining (2+) was observed in the bile canaliculi of normal liver and 8226/6.

Detection of *MDR1* mRNA by RT-PCR. Total RNA from 35 advanced breast cancer specimens was analyzed by RT-PCR. *MDR1* mRNA was not observed in any case. In contrast, 157 bp of *MDR1* cDNA was detected in multidrug-resistant myeloma cell line, 8226/40, multidrug-resistant ovarian cancer cell line, OVCAR4/ADR100 and normal adrenal cortex. The internal control gene (β2m) product (120 bp) was detected in all breast cancer samples confirming that mRNA in these samples was intact. Figure 1 shows a representative RT-PCR containing 6 treated breast tumors and two positive controls.

b) Clinical studies:

Therapeutic responses to paclitaxel in patients previously treated with doxorubicin. (protocols 1B-92-3, 1B-95-1)

Trial 1B-92-3 designed to evaluate the activity of paclitaxel in previously treated advanced breast cancer and its correlation with Pgp began in 1992 under NCI-Cancer Therapy Evaluation Program sponsorship before paclitaxel became commercially available, and completed accrual in August 1996. To be eligible patients had to have failed one prior chemotherapy for advanced disease **and** to have a biopsy accessible for determination of Pgp by immunostaining and, if possible, RT-PCR. Thirty seven patients were accrued on a 24h paclitaxel schedule and 35 patients have been accrued on a 3h paclitaxel schedule. The specimens have been recently evaluated for Pgp and only 59 are evaluable (see above). **However, the absence of immunostaining and RT-PCR (in 35 of these 59) for Pgp is a noteworthy feature**. Objective responses are undergoing critical reevaluation at this time, since we are also evaluating p53 and Her2/neu immunostaining in these specimens and wish to perform blinded correlates with response. The activity of paclitaxel on either

schedule has been consistent with observations by others in previously treated populations (approximately 25%), most of whom had been pretreated with doxorubicin.

Trial 1B-95-4 (Appendix 2) opened in July 1996 and has accrued 3 patients to date. Three other institutions are soon also to be contributing specimens to this randomized trial utilizing paclitaxel 3h infusion without or PSC833. Because of the findings in 1B-92-3, the focus of studies employing resistance-reversal agents has shifted to issues relating to the delay in the acquisition of the MDR phenotype by drugs such as PSC833. Although Pgp may not be an important mechanism of resistance in this population of patients with advanced breast cancer failing doxorubicin, the possibility that PSC833 is able to modulate other mechanisms of drug efflux cannot be excluded and phase II efficacy endpoints are sought. Accordingly, we have refined our objectives to study the activity and pharmacokinetics of the combination, and to assess the presence of Pgp **after** treatment with paclitaxel. Reflecting this shift, we have amended the study to include patients without biopseable lesions, but continue to encourage entry of patients with such lesions and seek post-paclitaxel treatment biopsies as well. The multi-institutional arrangement and the study design will ensure completion of this randomized study in a timely fashion, and enable the formulation of subsequent trials employing this combination.

Therapeutic responses to paclitaxel in protocols investigating the potential for overcoming paclitaxel-resistance. (protocols 1B-93-8, OC-93-4).

The 96-hour infusion of paclitaxel proved to be impractical in the setting of patients entered at the Los Angeles County Hospital and the Norris Cancer Center. Two episodes of catheter related sepsis were documented early on, and prompted an ongoing study of whether long infusions with paclitaxel predispose to catheter complications. One of these patients, and one other patient appeared to benefit from the prolonged infusion and megestrol acetate, but both had to interrupt treatment because of catheter problems. The study had interruption in patient accrual to review catheter-related complications, but is now planned to reopen to accrual for selected patients without accessible biopsies, not meeting eligibility to the 1B-95-4.

Estramustine phosphate (EMP), on the other hand, is well tolerated by women with breast cancer and has resulted in responses to paclitaxel, and the administration of greater number of cycles than with paclitaxel alone in the same patient (Table 2). Abstracts have been published in 1995 and 1996, and one manuscript concerning responses in breast cancer has been published. Another manuscript including paclitaxel pharmacokinetics is near completion. Pharmacologic findings suggest no interaction between EMP and paclitaxel, and no enhancement of paclitaxel toxicity. Therefore, therapeutic enhancement is unlikely to be solely mediated by inhibition of Pgp.

Table 2. Therapeutic effects observed with paclitaxel + EMP in paclitaxel-pretreated patients with breast cancer

Initials/Age	Site of disease	Response	Duration (months)	Prior Paclitaxel-Response
MC/62	breast, Lns	CR	3+	CR (3m)
MS/49	chest wall	PR	14+	CR (11m)
BG/51	chest wall, LNs	PR	8	PR (6m)
SH/56	chest wall, lung	PR	5	PD
VD/62	liver	SD	20	SD (12m)
ER/42	breast	SD	9	PR (6m)
IT/68	chest wall, lung	SD	12	SD (10m)
KG/63	pleura	SD	3+	mixed response
TL/25	lung, breast	SD	6+	1 dose only

CR=complete response PR=partial response SD=stable disease PD=progressive disease LN=lymph nodes

CONCLUSIONS AND FUTURE DIRECTIONS

Our experience indicates *MDR1* does not play a major role in the drug resistance of untreated or minimally treated breast cancer. This conclusion is consistent with some studies [3,4] and at variance with a number of other reports (reviewed in the initial application) providing a correlation of enhanced expression of *MDR1* with poor clinical prognostic features of breast cancer. It is now very important to provide some explanations for this discrepancy and seek to find Pgp expression in the most likely of clinical circumstances, i.e. after paclitaxel treatment. In addition, since these studies are already underway, the effects of emergence of Pgp when paclitaxel is modulated by PSC833 are being investigated. Work from Sikic's laboratory utilizing a fluctuation analysis of drug resistance patterns indicates that PSC833 is capable of delaying the emergence of drug resistance mediated by *MDR1* after exposure to doxorubicin and paclitaxel[5] . For the ensuing two years we are pursuing studies in the following directions:

1) broadening the sampling to patients with metastatic breast cancer in sites other than locoregional, and to patients receiving additional treatments including paclitaxel,

2) studying correlates of *MDR1* expression and drug resistance in ovarian cancer, and comparing these to analogous findings in breast cancer. We shall seek information as to

mechanism of drug resistance and circumstances associated with activation of *MDR1, MRP, LRP* or other genes conferring resistance to chemotherapy.

3) continuing to evaluate methods to modulate drug resistance through clinical trials. Efforts to correlate presence of MDR1 expression and resistance to taxanes, anthracyclines, and vinca alkaloids represent an initial step towards guiding drug selection based on specific tumor characteristics. Studies have also been proposed evaluating chemosensitivity assays in order to exclude agents associated with extreme drug resistance in a particular tumor. A safe *in vivo* method to indicate resistance to the above noted drugs, and the ability to modulate such resistance would add immeasurably to what is now a static determination on a tumor utilizing *in vitro* conditions for clinical correlative effects.

SESTAMIBI (hexakis (2-methoxyisobutyl isonitrile)) --see reference listed immediately below-- is a cationic complex that serves as a radioligand for technetium (I), and has been extensively used as a myocardial perfusion imaging agent (D. Piwnica-Worms et al, 1988 and 1989). Tc-99m-SESTAMIBI (SM) was subsequently found useful in tumor imaging (IM Hassan et al, 1989; LA O'Tuama et al, 1990), and soon thereafter was adopted by several groups for scintimammography, as a complementary study to conventional mammmography in patients with palpable masses and dense breasts (I Kalkhali et al, 1994, 1996; CH Kao et al, 1994; R Taillefer et al, 1996; K Nguyen et al, 1996). More recently, it has also been used to image axillary lymph nodes with a sensitivity of 78.6% and a specificity of 83.3% (R Taillafer et al, 1996) --somewhat below what is achieved for primary lesions, but nonetheless deemed useful.

Interest in SM use in oncology was further stimulated by the identification of Pgp expression as a determinant of its efflux (D Pinwica-Worms et al, 1993; VV Rao et al, 1994). The rate of efflux was recently determined to be 3 times higher in the 7 of 21 patients with untreated breast cancer that had detectable Pgp in their primary tumors (S Del Vecchio et al, 1996). Moreover, these patients showed a lesser responsiveness to epirubicin treatment than patients with the slower clearances (A Ciarmiello et al, 1996). Another group has reported similar findings in relation to relative lack of response to chemotherapy if the clearance is high (E Fukuma et al, 1995).

These initial diagnostic and therapeutic observations with SM point towards a practical *in vivo* assay of Pgp in breast cancer. Immunocytochemical and biochemical determinations of MDR1 have limitations, and do not address the functionality of Pgp. We had initially proposed *in vitro* studies to investigate the functional status of Pgp in breast cancer cell lines (Task four). With the availability of SM, it is particularly attractive to not only verify its value in diagnosis and therapeutic prognostication, but to extend observations to modulation or reversal of Pgp-mediated efflux *in vivo*. Estramustine phosphate (EMP) is an attractive agent for the study of SM-efflux modulation. EMP interacts with Pgp, but does not appear to be a substrate for Pgp-mediated efflux (LA Speicher et al, 1994; C-PH Yang et al, 1994). Nevertheless, it potentiates both taxane and vinca-mediated cytotoxicity --an effect that may be mediated by its disruption of microtubules (ME Stearns & K Tew,

1985, 1988; B Hartley-Asp, 1984; B Dahllof et al, 1993; LA Speicher et al 1994). Additionally, EMP is easily administered by the oral route, and has been found to be tolerable for 3 days at total daily doses of 900 mg/m2 in 3 divided doses for 3 days given together with paclitaxel to patients with breast cancer achieving several responses as noted above. Moreover, no significant effect has been found on paclitaxel pharmacokinetics in 8 patient profiles that have been obtained to date comparing paclitaxel with EMP + paclitaxel at the same dose (R Koda and FM Muggia, unpublished).

In collaboration with Elissa Kramer, Associate Professor, NYU Department of Radiology, therefore, we seek not only to establish the value of SM scintimammography as a diagnostic method, but also to evaluate the modulation with EMP in patients with breast cancer. This information will be used to assess its predictivity with respect to the quality and rate of response to paclitaxel and to the expression of Pgp. Our main hypothesis is that SM scintimammography and its modulation will be able to more accurately predict the outcome from paclitaxel therapy than currently available methods. Not only may SM clearance provide a direct *in vivo* assessment of drug resistance, but it also has the potential to establish the role of resistance modulators and possibly uncover other efflux mechanism involved in multidrug resistance.

We plan to complete assessment of PSC833, and to initiate study of technetium-99m-sestamibi clearance rates as a method to evaluate the effects of modulators such as PSC833, and estramustine phosphate. If these modulators exert effects on such clearance rates, it is likely that efflux pumps other than *MDR1* are operative.

We conclude, therefore, that the study of multidrug resistance in breast cancer is key to optimizing its systemic treatment with anthracyclines, taxanes, vinca alkaloids, and likely other drugs. Differences in activation of drug-resistance associated genes between breast and ovarian cancer may prove illuminating. In the next two years we plan to more precisely define the genes involved in drug resistance during breast cancer treatment, and to explore new clinical methods to detect drug resistance and methods to overcome it.

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FIGURE I

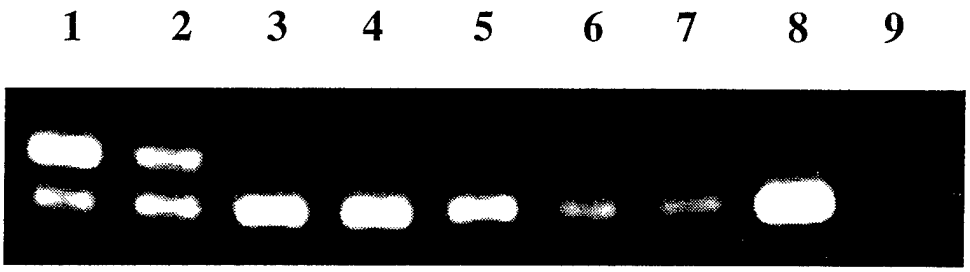

Figure 1. PCR products from *MDR1* and β_2m-specific primers are identified with RNA from OVCAR4/ADR100 (lane 1) and normal adrenal cortex (lane 2). However, RNA from six breast cancer specimens (lanes 3-8) show PCR products for only the smaller 120 bp product corresponding to β_2m message. This indicates that the mRNA is present and intact but *MDR1* sequences are not present. A negative control (lane 9) lacking RNA demonstrates no reaction product.

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 known:_____

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

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: __ __

APPENDIX I

----------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

----------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
__ well differentiated / Grade 1
__ moderately differentiated / Grade 2
__ poorly differentiated / Grade 3
__ 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_____

----------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

----------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 yet available (treatment is still ongoing)
__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

------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

--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

27

----------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

10/26/95
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PROTOCOL NUMBER:	**1B-95-4 (Ph II-06)**
TITLE:	Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Breast Cancer (Recurring Less Than Six Months Since Adjuvant or as Second Line for Advanced Disease)
SITE:	Breast
HISTOLOGY:	All
STAGE:	Advanced
MODALITY:	Chemotherapy/MDR Modulator
TYPE:	Phase II
ARMS:	Randomized Arm I: Paclitaxel Arm II: Paclitaxel + PSC 833
PRINCIPAL INVESTIGATOR:	Darcy Spicer, M.D.
CO-INVESTIGATORS:	Christy Russell, M.D. Valerie Israel, M.D. Ellen Warner, M.D. Franco Muggia, M.D. James Doroshow, M.D. (COH) Tim Synold, Pharm.D. (COH) David Gandara, M.D. (UCD)
IRB ANNIVERSARY DATE:	**09/21/95**
IRB FINAL APPROVAL DATE:	**10/27/95**
PARTICIPANTS:	LAC+USC Medical Center USC/Norris Comprehensive Cancer Center & Hospital City of Hope University of California, Davis Toronto Sunnybrook Regional Cancer Center
RESEARCH COMMITTEE NUMBER:	**959031-CC**
AMENDMENTS/REVISIONS:	**(A1)**2/29/96; **(A2)**4/24/96; **(A3)**5/17/96; **(A4)**10/1/96

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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

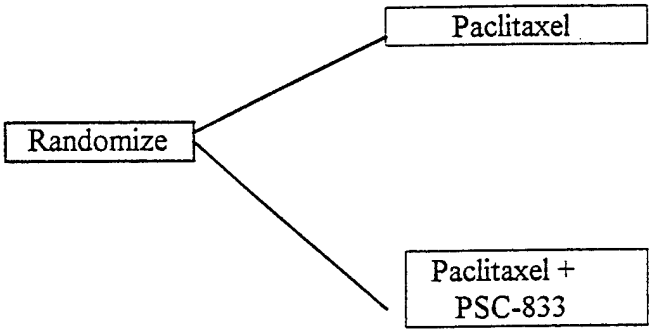
Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Breast Cancer (Recurring Less Than Six Months Since Adjuvant or as Second-Line for Advanced Disease)

SCHEMA

Biopsy of tumor tissue for MDR1-Pgp (in patients with accessible tumor)
(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 breast cancer.

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

3. To obtain preliminary estimates of MDR in this group of patients by measuring MDR1 - Pgp immunostaining in pre-treatment biopsies in 20 patients and biopsies taken at the time of progression.

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 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 randomized Phase II 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 analysis of data from 41 patients who received PSC 833 for at least one cycle at 20 mg/kg/day (5 mg/kg, q 6 hr or qid) on protocols B151 and B153 reveals 12 patients (29%) with a maximum grade of ataxia of 1, 18 (44%) with a maximum grade of 2, and 1 (2%) with a maximum grade of 3. Ataxia was not noted in the remaining 9 patients.

Twenty-six patients have received PSC 833 for at least one cycle at a dose of 5 mg/kg qid on protocol B251, a phase II study of paclitaxel in combination with PSC 833 in patients with paclitaxel-refractory ovarian cancer. To date, 1 patient (4%) has experienced grade 3 ataxia.

Eighteen patients have received PSC 833 for at least one cycle at a dose of 5 mg/kg qid on protocol B154, a phase I study of vincristine, doxorubicin, and dexamethasone in combination with PSC 833 in patients with refractory or relapsed multiple myeloma or low grade non-Hodgkin's lymphoma. Preliminary data on these 18 patients indicate that 4 patients (22%) have experienced grade 3 ataxia, an observation that prompted dose reduction of PSC 833 in the final cohort to 4 mg/kg qid. To date, 11 patients have been accrued to this final cohort, with no grade 3 ataxia having been observed in the 10 patients who were actually treated with the correct dose. The single incident of grade 3 ataxia in this cohort was seen in a patient to whom PSC 833 was inadvertently administered at the 5 mg/kg qid dose level.

Finally, a total of 30 patients have been treated at the 5 mg/kg qid PSC 833 dose level in 4 ongoing phase I and II investigator IND studies. In this heterogeneous population, 7 episodes of grade 3 ataxia have been observed, for a cumulative incidence of 23%.

With the exception of 1 patient, all cases of grade 3 ataxia in these studies have been rapidly reversible, with patients often being able to receive the next PSC 833 dose at the scheduled time. No patient to whom PSC 833 was administered at the protocol-mandated 25% reduced dose experienced a recurrence of grade 3 ataxia. In the 1 case where improvement in grade 3 ataxia was more gradual, 2 confounding factors were noted: (1) the patient had a gait abnormality of unclear etiology at baseline, and (2) instead of waiting for the ataxia to resolve prior to resuming PSC 833 at the reduced dose (as required by the protocol), the patient continued to take PSC 833 on schedule, although he was compliant with the 25% dose reduction.

In summary, 115 patients in phase I and II studies in the U.S. have received PSC 833 at a dose of 5 mg/kg qid, among whom 13 (11%) have experienced grade 3 ataxia. No episodes of grade 4 ataxia have been recorded.

Additional preliminary toxicity data are available in a more limited number of patients from protocols B151 and B153 and 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.

On balance, based upon the above toxicity data accumulated to date from phase I and II studies in the U.S., it appears that 5 mg/kg every 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 or as the new drink solution. Trough concentrations of PSC 833 in these trials as determined by radioimmunoassay have generally been greaterr than the 1000 ng/mL level deemed necessary to achieve MDR reversal *in vitro.*

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: General MDR contributes to treatment failure in advanced breast cancer treated with paclitaxel,

Hypothesis 2: Reversal of MDR with PSC-833 may lead to increased activity of paclitaxel in this disease.

Hypothesis 3: Responses will be more frequent with higher paclitaxel AUCs or with time above a .05 μm/L threshold.

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 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 labels and study medication bottles, together with a copy of a completed drug disposition form, 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 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 No radiation therapy within 3 weeks, and evaluable or measurable disease in at least one non-irradiated area.

5.1.4 No hormonal therapy within 2 weeks.

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

5.1.6 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.

5.1.7 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 any of the following agents and treatment cannot discontinued at the specified time relative to SDZ PSC 833 administration. All of these drugs are well substantiated to interact with cyclosporine A.

<u>Drugs that increase cyclosporine A concentration</u>
The following drugs must not be administered for 48 hours before SDZ PSC 833 is started, during the course of its administration, or up to 48 hours after the last dose of SDZ PSC 833 in a cycle.

Calcium channel blockers: diltiazem, nicardipine, verapamil
Antifungals: fluconazole (dose < 200 mg/day *allowed*), itraconazole, ketoconazole
Antibiotics: clarithromycin, erythromycin
Other Drugs: bromocriptine, danazol

Drugs that decrease cyclosporine A concentration
The following drugs must not be administered 14 days before SDZ PSC 833 is started or during the course of its administration. They may be restarted immediately after the last dose of SDZ PSC 833.

Antibiotics: nafcillin, rifampin
Anticonvulsants: carbamazepine, phenobarbital, phenytoin

5.2.14 Brain metastases or other neurologic problems requiring treatment.

5.2.15 Unable to reliably follow instructions.

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, UC Davis and Toronto Sunnybrook . 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 Biostatistics Office, City of Hope.

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 $\geq$ 1500/mm^3 and platelet count is $\geq$ 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 In patients with accessible tumor, biopsies will be obtained pretreatment and at the time of progression (see Appendis III for details). A minimum of 20 patients with accessible tumor will be studied.

7.3 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 in a green top tube 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. In addition the buffy coat will be removed from the pre-taxol sample and stored for future research. This will be done only at USC. Upon drawing, blood will be placed on ice until separated by centrifugation and plasma will be 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). Batched samples (Appendix VIII) will be sent on dry ice to the following address:

Dr. Tim Synold
City of Hope
Main Medical Building, Wing IV
1500 E. Duarte Rd.
Duarte, Ca. 91010

7.4 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. In the event of ≥ grade 3 neurotoxicity due to PSC, the PSC dose should be reduced by 20%.

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 4 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 (4 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 of the Taxol alone arm and may be increased by 7mg/m^2 (approximately 40% of 17.5 mg/m^2 - 10% of 175 mg/m^2) in subsequent cycles of the Taxol + PSC 833 arm, 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 ≥ 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, fractionated Bili, 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 III for method of preservation & transport.)
***** Mandatory on cycle 1 unless approved by PI (exceptions will be made for poor venous access).
@ In 20 patients with accessible tumor.
@@ In as many patients as possible.

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.

10.1.3 Nonevaluable disease: Pleural effusions, ascites, disease documented by indirect evidence only (i.e. CEA and CA-15-3).

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.

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 day of randomization 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.

10.4.2 Progression-free survival. Defined as the time from day of randomization 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.

10.4.3 Time to treatment failure. Defined as the time from day of randomization 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.

10.4.4 Time to progression. Defined as the time from day of randomization 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.

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.

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.

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), the University of California at Davis (UCD) and Totonto Sunnybrook Regional Cancer Centre.

The primary objective of this study is to assess the response to paclitaxel with PSC-833 and to paclitaxel alone in similar groups of women with advanced breast cancer who have failed anthracycline-based therapy or for whom anthracyclines are contraindicated.

The secondary objectives of this study are (a) to describe the plasma pharmacokinetics of paclitaxel with and without PSC-833, and (b) to estimate proportion of women who are MDR+ (as measured by immunohistochemical staining for MDR1 Pgp) prior to the start of paclitaxel therapy and at the time of progression after paclitaxel therapy.

Pharmacokinetics will be done on all patients. Biopsy of tumor for MDR evaluation will be taken on a minimum of 20 patients prior to start of paclitaxel treatment and on all available patients at the time of progression.

12.1. Study Design

For the paclitaxel + PSC-833 arm, a modification of Simon's optimal two-stage design (34) will be used. Patients will be randomized to the paclitaxel alone arm, only as long as the paclitaxel + PSC-833 arm is open.

In the first stage, 21 evaluable patients will be entered onto each arm. If no or only one response is observed on the paclitaxel + PSC-833 arm **and if** the paclitaxel alone arm has two more responders compared to the PSC-833 arm (i.e. 2+ responders if the PSC-833 arm has no responders, or 3+ responders if the PSC-833 arm has one responder), then accrual will stop with the conclusion that the PSC-833 regimen is not promising enough for further study. Otherwise, an additional 20 patients will be accrued to each arm during the second and final stage. At the completion of the second stage, five or more responses out of 41 patients on the PSC-833 arm, **or** as many or more responders on the

PSC-833 arm compared to the paclitaxel alone arm, will be taken as evidence that this regimen of PSC-833 + paclitaxel warrants consideration for further study providing other factors, such as toxicity and survival appear favorable.

12.1.1.1 Rationale for the Design

This design is a modification of Simon's optimal two-stage design. The design selected is based on the following assumptions: a true response rate less than 5% would not warrant further study of the PSC-833 at this dose with paclitaxel; a response rate of 20% would be considered promising for further studies in these patients; if the observed response rate of paclitaxel + PSC-833 is not less than that of paclitaxel alone, then further studies may be of interest. A two-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.

The modifications were adopted because a low observed response rate in both arms (i.e. paclitaxel alone and paclitaxel + PSC-833) might suggest a low responsiveness in the group of patients, rather than poor activity of paclitaxel + PSC-833. Simon's design was modified in two ways: (1) at the end of the first stage, continuation to the second stage is permitted in the event that the paclitaxel alone arm (a well-studied regimen with a 20% or better response rate in this group of patients) is also observed to have very few responders - i.e. 0 or 1 (if the PSC-833 arm had no resonders) or 0, 1, or 2 (if the PSC-833 arm had one response); and (2) at the end of the second stage, if the paclitaxel + PSC-833 arm has as many or more responders compared to the paclitaxel alone arm (regardless of the number of PSC-833 responders), then further study of PSC-833 with paclitaxel would not be ruled out .

With Simon's optimal design, the probability of correctly concluding that an agent with a 5% response rate does not warrant further study, is 0.95 ($1-\alpha$), and the probability of incorrectly declaring a regimen with a 20% response rate as **not** warranting further study is 0.10 (β). Table 2 below lists the probability of early stopping and the probability of not recommending PSC-833 for further study for a variety of response rates for paclitaxel + PSC-833 and paclitaxel alone (based on exact binomial calculations). Inspection of Table 2 below reveals that the overall probabilities for the modified Simon design are similar to the unmodified design, except when the response rate of paclitaxel + PSC-833 is low, but not as low as the response rate of paclitaxel alone. In this situation there is an improved probability that further study will be recommended.

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 trial.

Table 2: Probability of Not Recommending this Regimen of PSC-833 for Further Study

Using the Modified Simon Design

Response Rate for Paclitaxel + PSC-833	Response Rate for Paclitaxel Alone	Probability of Stopping at First Stage	Probability of Not Recommending PSC-833 for Further Study	Probability of Not Recommending PSC-833 for Further Study Based on Simon's Design
5%	5%	0.1284	0.408	0.954
5%	10%	0.3487	0.747	0.954
5%	15%	0.5247	0.896	0.954
5%	20%	0.6301	0.941	0.954
5%	25%	0.6824	0.952	0.954
5%	35%	0.7132	0.954	0.954
10%	5%	0.0526	0.150	0.647
10%	10%	0.1593	0.400	0.647
10%	15%	0.2532	0.560	0.647
10%	20%	0.3128	0.623	0.647
10%	30%	0.3572	0.646	0.647
10%	40%	0.3641	0.647	0.647
15%	5%	0.0197	0.046	0.292
15%	10%	0.0639	0.154	0.292
15%	15%	0.1047	0.237	0.292
15%	25%	0.1453	0.287	0.292
15%	35%	0.1539	0.292	0.292
15%	45%	0.1550	0.292	0.292
20%	5%	0.0067	0.012	0.098
20%	10%	0.0229	0.047	0.098
20%	15%	0.0383	0.076	0.098
20%	20%	0.0485	0.090	0.098
20%	25%	0.0539	0.096	0.098
20%	30%	0.0563	0.098	0.098
20%	40%	0.0575	0.098	0.098
20%	50%	0.0576	0.098	0.098
25%	15%	0.0125	0.020	0.027
25%	25%	0.0177	0.026	0.027
25%	35%	0.0189	0.027	0.027
30%	20%	0.0047	0.006	0.007
30%	30%	0.0054	0.007	0.007
30%	40%	0.0056	0.007	0.007

Shaded lines indicate response rates for PSC-833 and palcitaxel which might be of interest for further study.

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, Toronto Sunnybrook). A stratified fixed randomization algorithm will be used to ensure that the two treatment arms will be balanced for the three potential prognostic factors (36), using a small blocking factor.

12.3 Analysis of Results

12.3.1. 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 - whichever occurs first) will be used to evaluate efficacy. For each treatment arm separately, 95% confidence intervals will be constructed for the response rates (36) and for the median time to failure and survival. For the preliminary 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.

We will estimate the difference in response rates between the two treatment arms and construct 95% confidence intervals. The overall survival and time to treatment failure curves will be drawn for each arm separately, based on the Kaplan-Meier product limit estimates (38), and will be compared using the logrank test.

Toxicity as classified by the NCI Common Toxicity Criteria (Appendix VI) 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.

12.3.2 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) (37). Descriptive analysis will be performed to compare these values among patients who received PSC-833 (n=41) or not (n=41). 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 received (≈41/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.

12.3.3 **Analysis of MDR Status**

The proportion of patients who are MDR+ prior to treatment with paclitaxel will be estimated and 95% confidence intervals will be constructed. The same will be done for the proportion of patients who are MDR+ at the time of progression. For those patients who provided tumor samples at both times, the proportion of patients whose MDR status changes will also be estimated. With a minimum of 20 patients, and assuming that not more than 20% of patients will be MDR+ (based on work by Xiaowei Yang in Michael Press's group - manuscript in preparation), 95% confidence intervals will have half-widths of no more than +/- 0.18. If none of the 20 patients whose biopsies are obtained prior to treatment are classified as MDR+, then we can conclude that fewer than 14% of patients with advanced breast cancer are likely to be MDR+ (using a 0.05-level test).

12.4 <u>Analysis of Ethnic Subgroups</u>

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, because of our predominant ethnic mix 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 randomization.

13.2 Other imaging studies (scans, etc) must be completed within 4 weeks prior to randomization.

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 and randomized assignment. See appendix (Registration Procedures for Phase II Trials) for details.

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

Patients of both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria outlined in Section 5.0. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to accrue a representative sample. However, since this is a Phase I trial, considerations for patient safety and a reluctance to expose patients either to a potentially toxic and/or ineffective treatment, will limit the total number of patients entered. If differences in outcome appear to be associated with gender or ethnic identity, then a follow-up study will be designed to investigate those differences more fully. The cachement area for patients with cancer for USC and for COH is L.A. County; the cachement area for UCD is Sacramento County. The tables below summarize the ethnic and gender distribution of cancer patients in these counties.

TABLE 1

Ethnic and Gender Distribution of Patients Diagnosed with Cancer in L.A. County in 1993

	Gender		Ethnic Distribution			
Primary Site of Tumor	% Males	% Females	% Hispanic	% Black	% White	% Asian & Other
Bones & Joints	53.5	46.5	34.3	10.1	47.5	8.1
Brain & Other Nervous Systems	55.5	44.5	16.9	7.4	68.3	7.4
Breast	0.7	99.3	11.4	10.0	71.3	7.4
Digestive System	53.0	47.0	13.4	11.7	64.1	10.8
Endocrine System	27.7	72.3	23.7	6.3	55.7	14.3
Eye & Orbit	51.0	49.0	9.6	1.0	83.7	5.8
Female Genital System	0.0	100.0	26.0	10.1	55.3	8.5
Head and Neck	70.0	30.0	11.6	12.3	68.5	7.5
Leukemias	56.0	44.0	24.1	8.6	58.7	8.7
Lung & Bronchus	55.7	44.3	8.6	13.6	71.4	6.4
Lymphomas	59.3	40.7	17.8	7.5	67.9	6.8
Male Genital System	100.0	0.0	12.1	13.7	68.9	5.3
Multiple Myeloma	51.7	48.3	14.6	20.1	58.7	6.6
Skin (excl Basal & Squamous)	68.0	32.0	12.0	3.9	82.7	1.4
Soft Tissue	54.5	45.5	19.8	8.9	63.0	8.3
Urinary System	70.7	29.3	12.1	7.4	75.3	5.2
All sites combined	48.6	51.4	14.5	10.9	67.3	7.4

TABLE 2

Ethnic and Gender Distribution of Patients Diagnosed with Cancer in Sacramento County in 1991

	Gender		Ethnic Distribution			
Primary Site of Tumor	% Males	% Females	% Hispanic	% Black	% White	% Asian & Other
Bones & Joints	47.4	52.6	5.6	0.0	77.8	16.7
Brain & Other Nervous Systems	61.4	38.6	6.6	2.6	82.9	7.9
Breast	0.5	99.5	5.1	4.4	86.8	3.7
Digestive System	51.1	48.9	6.5	5.2	82.1	6.2
Endocrine System	32.1	67.9	11.1	2.6	77.1	9.2
Eye & Orbit	50.0	50.0	0.0	3.7	96.3	0.0
Female Genital System	0.0	100.0	7.0	3.6	83.0	6.4
Head and Neck	70.6	29.4	4.8	5.1	84.5	5.6
Leukemias	66.7	33.3	9.4	5.7	79.2	5.7
Lung & Bronchus	57.2	42.8	4.1	4.4	88.3	3.2
Lymphomas	56.8	43.2	8.3	5.1	81.3	5.3
Male Genital System	100.0	0.0	5.6	5.7	85.0	3.7
Multiple Myeloma	48.5	51.5	7.6	10.7	75.6	6.1
Skin (excl Basal & Squamous)	59.7	40.3	5.3	3.7	90.1	0.9
Soft Tissue	51.7	48.3	5.4	5.4	87.5	1.8
Urinary System	70.3	29.7	5.7	3.1	88.6	2.6
All sites combined	52.0	48.0	6.0	4.7	84.9	4.5

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 the respective pharmaceutical and agency sponsors.

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.

17.5 In each ADR case, a note should be made that Sandoz should be copied on these reports. The relevant Sandoz contact is as follows:

Manny Litchman, M.D.
Sandoz Research Institute
59 route 10
East Hanover, New Jersey 07936-1080
Telephone: (201) 503-5844
Fax: (210) 503-6598

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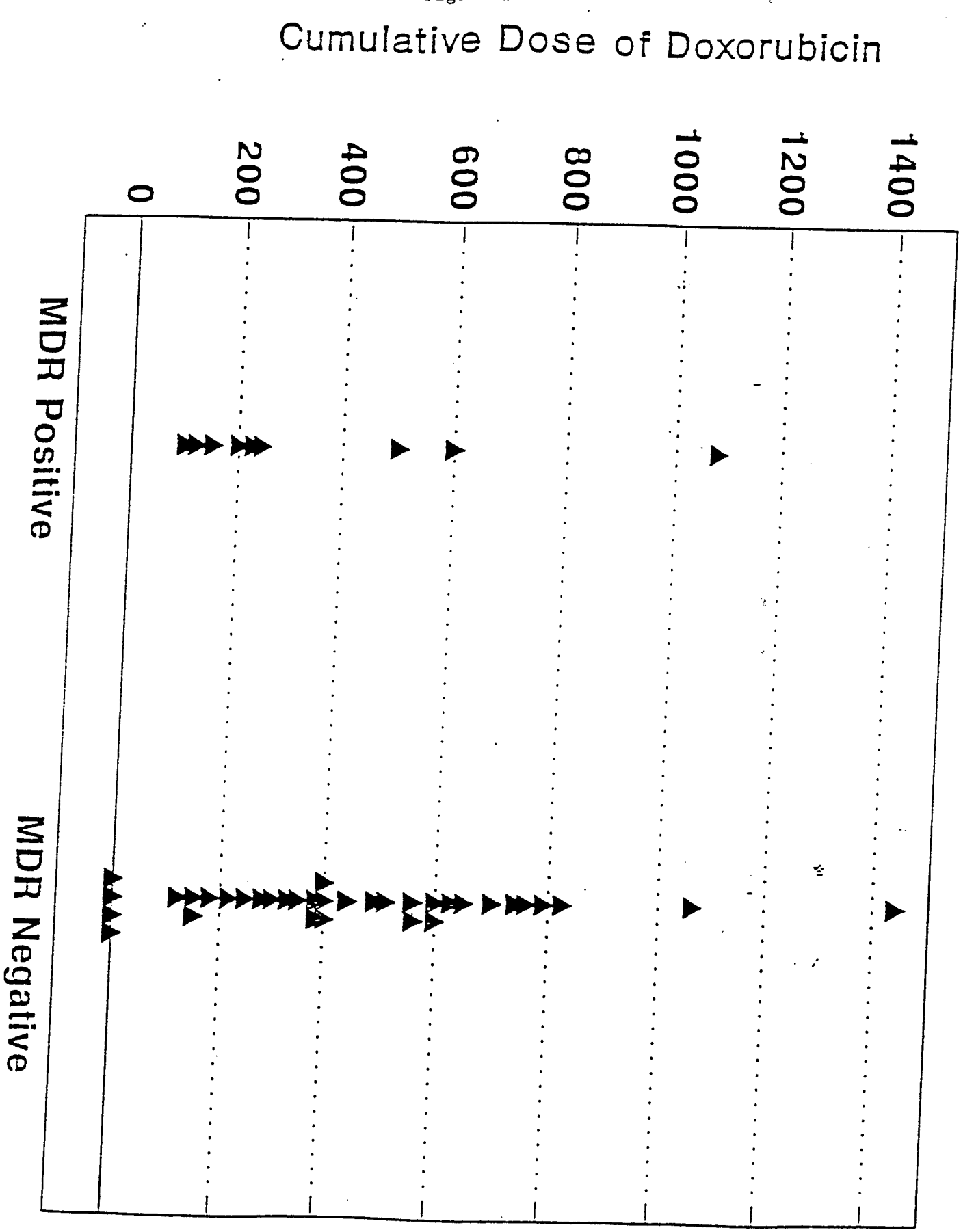
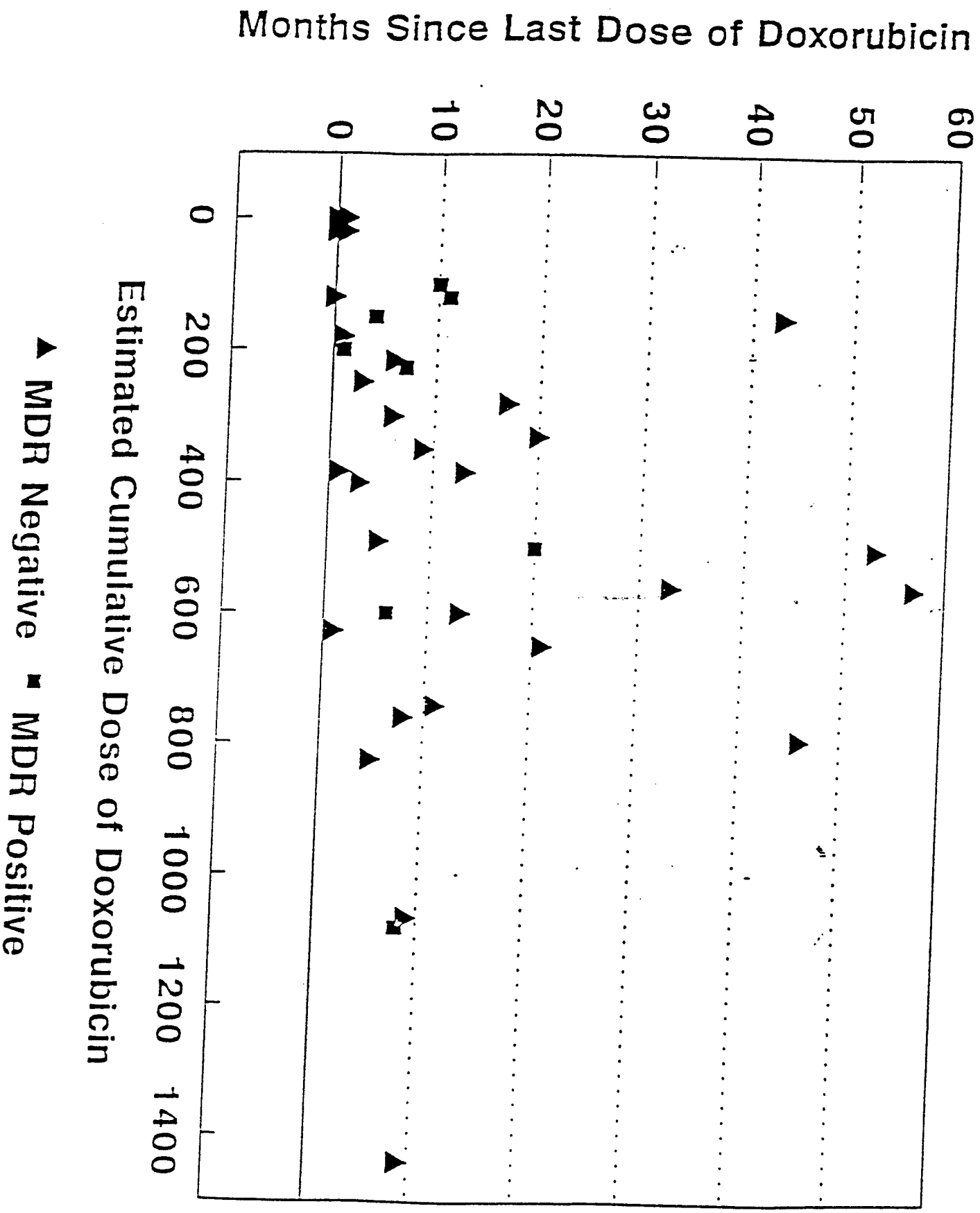
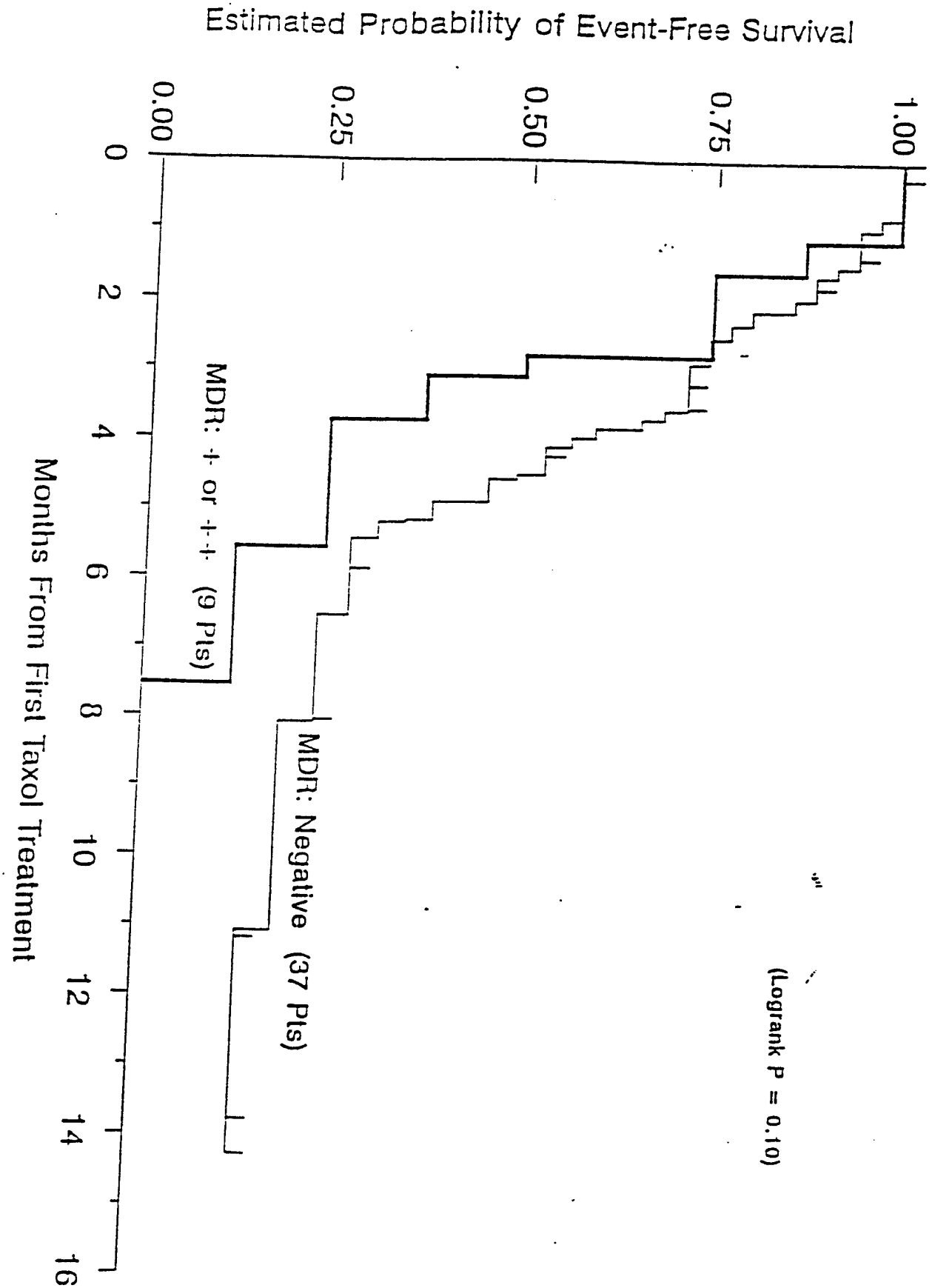
19.0 APPENDICES

APPENDIX I
Figure 1




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

APPENDIX I
Figure 3



APPENDIX II

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

Appendix III

Tumor Tissue Sampling

1.0 General procedure:

Tumor tissue may be obtained by endoscopic biopsy, radiologic biopsy, or by surgery for analysis of gene expression and histopathologic examination. Pleural and ascites fluids should be obtained if adequate tumor cells are available in these fluids for analysis.

If the tissue is needed for a pathologic diagnosis, a tumor sample of at least 5-10 mg in excess of the pathologist's needs should be frozen as soon as possible. If a pathologic diagnosis has been made previously, a tumor biopsy of at least 5-10 mg should be obtained and the tissue should be divided immediately **(within 60 seconds)** in the operating room into specimens for pharmacodynamics (biochemical and gene expression assays), and histopathologic examination. Approximately 10 to 15% of the tissue should be divided from the rest of the sample and preserved as requested by the pathologist for histopathologic examination. This aliquot should be taken from the portion of the biopsy which appears most likely, by gross examination, to contain tumor; the approximate percentage of the tissue which has the same gross appearance should be noted. The remaining tissue, for pharmacodynamics, should be frozen immediately in the operating room. In either case, the tumor specimen for pharmacodynamics should be placed in a labeled 2.0 ml polypropylene sterile cryogenic vial (Corning # 25705 or equivalent), the vial capped, and submerged in liquid nitrogen for at least 15 seconds. The samples can then be transported or shipped on dry ice as required in Section 3.0 Protocol Specific Information.

Cells obtained by thoracentesis or paracentesis will be collected on wet ice(4°C) in sterile containers containing 10,000 units heparin per liter. The entire sample should be centrifuged at 4°C. The cell pellet should be resuspended in a minimum amount of the supernatant fluid and the suspension transferred to a labeled 2.0 ml polypropylene sterile cryogenic vial (Corning # 25705 or equivalent). The sample should be centrifuged again at 4°C and the supernatant fluid removed. The vial should be capped and submerged in liquid nitrogen for at least 15 seconds. The samples can then be transported or shipped on dry ice as required in Section 3.0 Protocol Specific Information.

The vial must be labeled with the following:

the USC-COH-UCD **protocol #**
the **patient accession #**
the **date** and **time** of sampling
the patient's **initials** and the **sample #** (if there are multiple samples).

Complete the Tumor Tissue Sample Form. Include a copy of the form with the sample. **Before shipping the sample**, please call the receiving laboratory to facilitate tracking and proper handling. At the same time, transmit copies of the form by FAX to both the receiving laboratory and the Data Coordinating Center (Phase I studies, USC - Phase I Data Manager 213/764-0089; Phase II studies, COH - Phase II Data Coordinator 818/301-8393). The Data Coordinating Centers will distribute copies of the form to the involved investigators. Samples transported between institutions should be shipped by overnight courier with 10 lbs of dry ice. When the biopsy sample

arrives, the receiving laboratory should mark "RECEIVED - date" on a copy of the form and transmit it by FAX to the Data Coordinating Center.

2.0 Institution Specific Information:

2.1 City of Hope:

Tumor specimen(s) must be handled according to the document "DRUG RESISTANCE MARKER PROTOCOL, Tumor Tissue Sampling — 4/95" agreed to by the Division of Pathology and the Department of Medical Oncology. Before NOON on the day PRIOR to the biopsy procedure, notify the pathologist on call for surgicals of the time and place of the procedure so that the patient's file can be reviewed to determine if additional tissue is needed for diagnostic review. At the same time, notify the Medical Oncology research nurse (Mary Carroll, R.N.). AGAIN notify the pathologist on call for surgicals and the Medical Oncology research nurse ONE HOUR PRIOR to the procedure, so that a physician's assistant from the Division of Pathology can be present to divide and process the specimen(s) appropriately depending on whether or not diagnostic tissue is required, and the research nurse can be present with liquid nitrogen and vials for freezing samples. The physician's assistant will transport tissue to the Division of Anatomic Pathology (Northwest Building, 2nd Floor, Room 2241, ext 2456) and the research nurse will transport frozen tissue to the Analytical Pharmacology Facility (Wing IV, Room 415, ext 2110). The specimen retained in pathology will be fixed for determination of the histological extent of the tumor in the tissue and for potential future immunohistochemistry. The specimen received by the Analytical Pharmacology Facility will be stored at or below -70 C and will be distributed to participating laboratories as indicated in Section 3.0 Protocol Specific Information.

If there are questions or problems during the tissue sampling or processing procedure, contact Dr. James Doroshow (ext 2727, page 5204), Dr. Timothy Synold (ext 2110, page 5793), Dr. Edward Newman (ext 2566, page 5716), or Mary Carroll, R.N. (ext 2307, page 5335).

2.2 USC:

ONE DAY PRIOR to the procedure, notify the specimen nurse, Ginny Kortes (phone 226-6381, page 701-9816) of the time and place of the procedure. ONE HOUR PRIOR to the procedure page the specimen nurse again. USC investigators, with the surgeon, will divide the sample as necessary for routine pathology and freezing for pharmacodynamics. The specimen nurse will transport or ship the pharmacodynamics sample on dry ice or in liquid N_2 as indicated in Section 3.0 Protocol Specific Information. Storage should be at -70° such as in Revco; routine -20° freezing is not acceptable.

2.2 UCD:

ONE DAY PRIOR to the procedure, notify Dr. Paul Gumerlock (phone 916/734-8614) of the time and place of the procedure. ONE HOUR PRIOR to the procedure call Dr. Gumerlock again. He will arrange for dividing the sample as necessary for histopathology

and freezing the remainder for RT-PCR. He will arrange transport or shipping of the RT-PCR sample on dry ice as indicated in Section 3.0 Protocol Specific Information.

3.0 Protocol Specific Information:

For the Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Breast Cancer (Recurring Less Than Six Months Since Adjuvant or as Second-Line for Advanced Disease) (PHII-06), MDR will be done in the laboratory of Dr. Michael Press at USC. Please call the laboratory at 213/342-1187 before shipping the tumor sample. Also, FAX a copy of the Tumor Tissue Sample Form to 818/301-8393 (Phase II coordinator).

The address for the laboratory is:

Dr. Michael Press
Hoffman Research Bldg.
Room 910
2011 Zonal Avenue
Los Angeles, Ca. 90033

c:\docs\dot\ts1b954

TUMOR TISSUE SAMPLE FORM
for pharmacodynamic, biochemical and drug resistance marker studies

Complete all information on this form. If the specimens are to be shipped to a laboratory at another institution, FAX the form in advance to the receiving laboratory. In all cases, a copy of the form should be transported with the specimen. If the specimen is divided, a copy of the form must accompany each portion. General and protocol-specific information on handling of the specimens is included as an appendix to each applicable protocol.

If there are any questions or problems, contact: _____

_____ or _____

TREATING INSTITUTION:_____

PATIENT NAME: _____

MEDICAL RECORD #: _____

PROTOCOL NAME: _____

PROTOCOL #s LOCAL:_____ CONSORTIUM/COOP. GROUP:_____

PATIENT ACCESSION NUMBER: _____

DATE OF BIOPSY: _____ TIME OF BIOPSY: _____

TIME PLACED IN LIQUID NITROGEN: _____

BIOPSY METHOD: _____

PORTION SENT FOR HISTOPATHOLOGY: _____YES _____NO

Indicate the site of the specimen. If more than one specimen was obtained, number the specimens and indicate the site for each specimen.

SPECIMEN 1 - SITE: _____

SPECIMEN 2 - SITE: _____

SPECIMEN 3 - SITE: _____

NAME AND PHONE NUMBER OF THE PERSON COMPLETING FORM AT THE ORIGINATING INSTITUTION: _____

The receiving laboratory is to complete the date received and then FAX the form to the Data Coordinating Center.

NAME AND PHONE NUMBER OF THE PERSON RECEIVING SPECIMEN: _____

_____

DATE RECEIVED: _____.

c:\docs\dot\tisform

APPENDIX IV

REGISTRATION PROCEDURES FOR PHASE II TRIALS

1. Registrations for Phase II protocols must be made through the Biostatistics office at the City of Hope between the hours of 8:30 a.m. to 4:30 p.m., Monday through Friday (except holidays).

2. Patients must be registered within 24 hours prior to initiation of protocol therapy.

3. A patient failing to meet all protocol requirements may not be registered. If you have any questions regarding eligibility, contact the City of Hope Phase II Coordinator, Catherine Christie, at (818) 359-8111 x 2468, pager number 5478.

4. Prestudy laboratory tests, scans and x-rays must be completed prior to registration according to study calendar.

5. Patients must sign an informed consent prior to registration.

6. Confirm that the patient meets all inclusion and exclusion eligibility criteria for a protocol.

7. Complete the Eligibility Checklist.

8. Verify that all required prestudy tests were performed.

9. Fax the completed Eligibility Checklist and the signed, dated informed consent to the City of Hope Phase II Coordinator. The FAX number is (818) 301-8393.

10. Call the City of Hope Phase II Coordinator at (818) 359-8111 x 2468 to confirm the FAX arrival. If the Phase II Coordinator is not in the office, have her paged.

11. If the patient qualifies, the City of Hope Phase II Coordinator will call the USC or UCD Data Manager to complete the registration/randomization procedure and assign the patient's study ID number.

12. Once a patient has been registered the COH Phase II Coordinator will fax a "Confirmation of Registration" to the center registering the patient.

For questions regarding eligibility call the COH Phase II Coordinator
at the Department of Biostatistics
(818) 359-8111 x 2468

APPENDIX V

FORMS SUBMISSION FOR PHASE II PROTOCOLS

ELIGIBILITY CHECKLIST:	The data manager will have completed and faxed this form at the time of registration.
ON-STUDY FORM (FORM OS):	Completed on-study forms due within two weeks of registration.
TREATMENT FORM (FORM RX):	Completed treatment forms are due within four weeks of completion of a cycle.
FLOW SHEETS:	Protocol specific flow sheets are to be submitted along with each treatment form.
RESPONSE/OFF-STUDY/FOLLOW-UP (FORM F/U):	Form F/U is to be submitted each time a patient is evaluated for response and/or new follow-up information is obtained.
SUPPLEMENTAL DATA FORM:	(The timeline for submission of the supplemental data form will be protocol specific)

For questions regarding forms submission call the City of Hope Biostatistics Department
(818) 359-8111 x2468

9/27/94

CANCER CLINICAL TRIALS

COMMON TOXICITY CRITERIA

	TOXICITY	GRADE 0	1	2	3	4
Blood/Bone Marrow	WBC	≥ 4.0	3.0 - 3.9	2.0 - 2.9	1.0 - 1.9	< 1.0
	PLT	WNL	75.0 - normal	50.0 - 74.9	25.0 - 49.9	< 25.0
	Hgb	WNL	10.0 - normal	8.0 - 10.0	6.5 - 7.9	< 6.5
	Granulocytes/ Bands	≥ 2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	< 0.5
	Lymphocytes	≥ 2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	< 0.5
	Hemorrhage (clinical)	none	mild, no transfusion	gross, 1-2 units transfusion per episode	gross, 3-4 units transfusion per episode	massive, > 4 units transfusion per episode
	Infection	none	mild	moderate	severe	life-threatening
Gastrointestinal	Nausea	none	able to eat reasonable intake	intake significantly decreased but can eat	no significant intake	—
	Vomiting	none	1 episode in 24 hrs	2-5 episodes in 24 hrs	6-10 episodes in 24 hrs	> 10 episodes in 24 hrs or requiring parenteral support
	Diarrhea	none	increase of 2-3 stools/day over pre-Rx	increase of 4-6 stools/day, or nocturnal stools, or moderate cramping	increase of 7-9 stools/day, or incontinence, or severe cramping	increase of ≥ 10 stools/day or grossly bloody diarrhea or need for parenteral support
	Stomatitis	none	painless ulcers, erythema, or mild soreness	painful erythema, edema, or ulcers, but can eat	painful erythema, edema, or ulcers, and cannot eat	required parenteral or enteral support
Liver	Bilirubin	WNL	—	< 1.5 x N	1.5 - 3.0 x N	> 3.0 x N
	Transaminase (SGOT, SGPT)	WNL	≤ 2.5 x N	2.6 - 5.0 x N	5.1 - 20.0 x N	> 20.0 x N
	Alk Phos or 5' nucleotidase	WNL	≤ 2.5 x N	2.6 - 5.0 x N	5.1 - 20.0 x N	> 20.0 x N
	Liver— clinical	no change from baseline	—	—	precoma	hepatic coma
Kidney/Bladder	Creatinine	WNL	< 1.5 x N	1.5 - 3.0 x N	3.1 - 6.0 x N	> 6.0 x N
	Proteinuria	no change	1+ or < 0.3 g% or < 3 g/l	2 - 3+ or 0.3 - 1.0 g% or 3 - 10 g/l	4+ or > 1.0 g% or > 10 g/l	nephrotic syndrome
	Hematuria	neg	micro only	gross, no clots	gross + clots	requires transfusion
	Alopecia	no loss	mild hair loss	pronounced or total hair loss	—	—
	Pulmonary	none or no change	asymptomatic with abnormality in PFT's	dyspnea on significant exertion	dyspnea at normal level of activity	dyspnea at rest

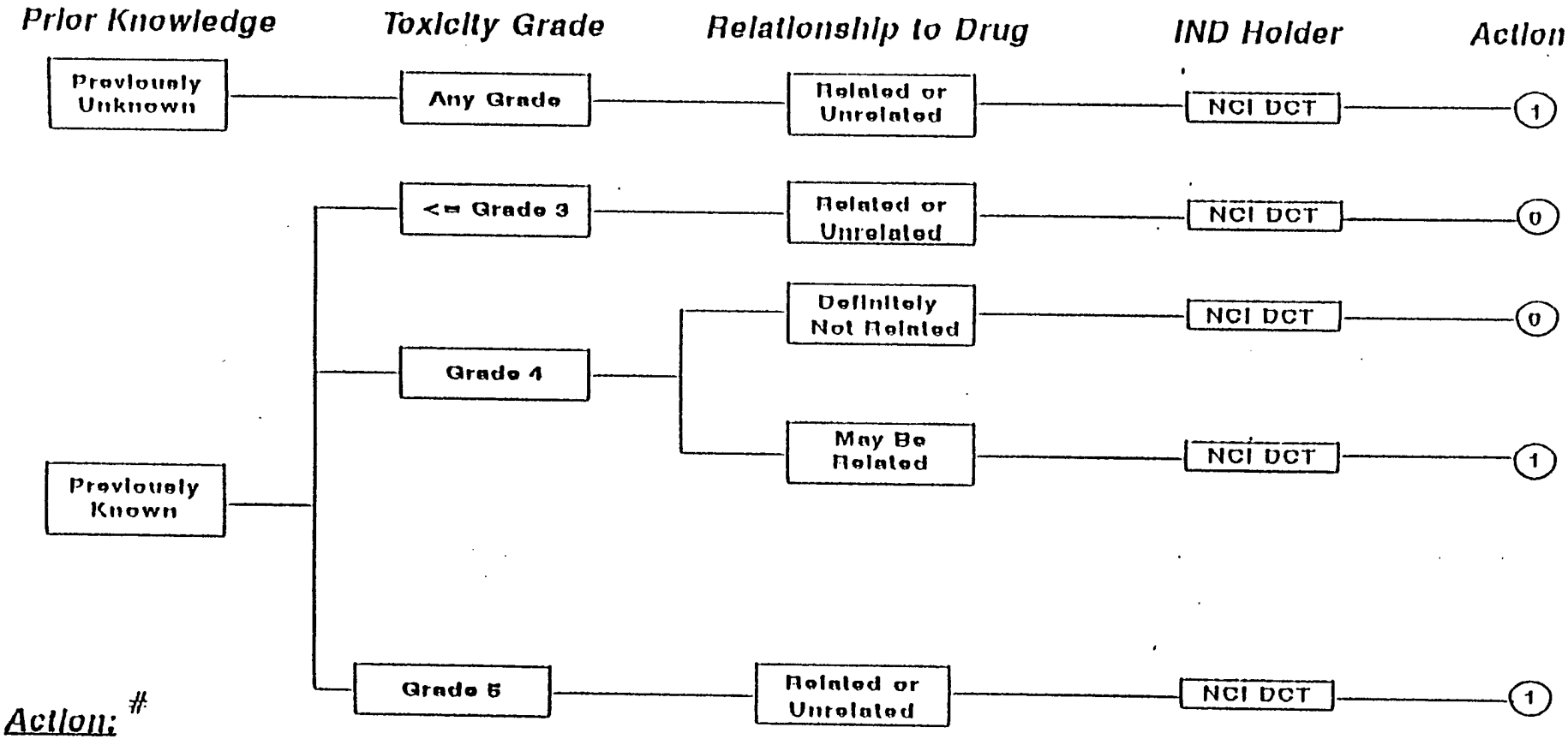
COMMON TOXICITY CRITERIA (continued)

	TOXICITY	GRADE 0	1	2	3	4
Heart	Cardiac dysrhythmias	none	asymptomatic, transient requiring no therapy	recurrent or persistent, no therapy required	requires treatment	requires monitoring; or hypotension, or ventricular tachycardia, or fibrillation
Heart	Cardiac	none	asymptomatic, decline of resting ejection fraction by less than 20% of baseline value	asymptomatic, decline of resting ejection fraction by more than 20% of baseline value	mild CHF, responsive to therapy	severe or refractory CHF
Heart	Cardiac--ischemia	none	non-specific T-wave flattening	asymptomatic, ST and T wave changes suggesting ischemia	angina without evidence for infarction	acute myocardial infarction
Heart	Cardiac--pericardial	none	asymptomatic effusion, no intervention required	pericarditis (rub, chest pain, ECG changes)	symptomatic effusion; drainage required	tamponade; drainage urgently required
Blood Pressure	Hypertension	none or no change	asymptomatic, transient increase by greater than 20 mm Hg (D) or to > 150/100 if previously WNL. No treatment required	recurrent or persistent increase by greater than 20 mm Hg (D) or to > 150/100 if previously WNL. No treatment required	requires therapy	hypertensive crisis
Blood Pressure	Hypotension	none or no change	changes requiring no therapy (including transient orthostatic hypotension)	requires fluid replacement or other therapy but not hospitalization	requires therapy and hospitalization; resolves within 48 hrs of stopping the agent	requires therapy and hospitalization for > 48 hrs after stopping the agent
Neurologic	Neuro--sensory	none or no change	mild paresthesias, loss of deep tendon reflexes	mild or moderate objective sensory loss; moderate paresthesias	severe objective sensory loss or paresthesias that interfere with function	—
Neurologic	Neuro--motor	none or no change	subjective weakness; no objective findings	mild objective weakness without significant impairment of function	objective weakness with impairment of function	paralysis
Neurologic	Neuro--cortical	none	mild somnolence or agitation	moderate somnolence or agitation	severe somnolence, agitation, confusion, disorientation, or hallucinations	coma, seizures, toxic psychosis
Neurologic	Neuro--cerebellar	none	slight incoordination, dysdiadokinesis	intention tremor, dysmetria, slurred speech, nystagmus	locomotor ataxia	cerebellar necrosis
Neurologic	Neuro--mood	no change	mild anxiety or depression	moderate anxiety or depression	severe anxiety or depression	suicidal ideation
Neurologic	Neuro--headache	none	mild	moderate or severe but transient	unrelenting and severe	—
Neurologic	Neuro--constipation	none or no change	mild	moderate	severe	ileus > 96 hrs
Neurologic	Neuro--hearing	none or no change	asymptomatic, hearing loss on audiometry only	tinnitus	hearing loss interfering with function but correctable with hearing aid	deafness not correctable
Neurologic	Neuro--vision	none or no change	-	-	symptomatic subtotal loss of vision	blindness

COMMON TOXICITY CRITERIA (continued)

TOXICITY	GRADE 0	1	2	3	4
Skin	none or no change	scattered macular or papular eruption or erythema that is asymptomatic	scattered macular or papular eruption or erythema with pruritus or other associated symptoms	generalized symptomatic macular, papular, or vesicular eruption	exfoliative dermatitis or ulcerating dermatitis
Allergy	none	transient rash, drug fever< 38c, 100.4F	urticaria, drug fever=38c, 100.4F mild bronchospasm	serum sickness, bronchospasm, req parenteral meds	anaphylaxis
Fever in absence of infection	none	37.1 - 38.0c 98.7 - 100.4F	38.1 - 40.0c 100.5 - 104.0F	> 40.0c > 104.0F for less than 24 hours	> 40.0c (104.0F) for more than 24 hrs or fever accompanied by hypotension
Local	none	pain	pain and swelling, with inflammation or phlebitis	ulceration	plastic surgery indicated
Weight gain/loss	< 5.0%	5.0 - 9.9%	10.0 - 19.9%	≥ 20.0%	—
Metabolic: Hyperglycemia	< 116	116 - 160	161 - 250	251 - 500	> 500 or ketoacidosis
Metabolic: Hypoglycemia	> 64	55 - 64	40 - 54	30 - 39	< 30
Metabolic: Amylase	WNL	< 1.5 x N	1.5 - 2.0 x N	2.1 - 5.0 x N	> 5.1 x N
Metabolic: Hypercalcemia	< 10.6	10.6 - 11.5	11.6 - 12.5	12.6 - 13.5	≥ 13.5
Metabolic: Hypocalcemia	> 8.4	8.4 - 7.8	7.7 - 7.0	6.9 - 6.1	≤ 6.0
Metabolic: Hypo-magnesemia	> 1.4	1.4 - 1.2	1.1 - 0.9	0.8 - 0.6	≤ 0.5
Coagulation: Fibrinogen	WNL	0.99 - 0.75 x N	0.74 - 0.50 x N	0.49 - 0.25 x N	≤ 0.24 x N
Coagulation: Prothrombin time	WNL	1.01 - 1.25 x N	1.26 - 1.50 x N	1.51 - 2.00 x N	> 2.00 x N
Coagulation: Partial thromboplastin time	WNL	1.01 - 1.66 x N	1.67 - 2.33 x N	2.34 - 3.00 x N	> 3.00 x N

Reporting Guidelines for Adverse Drug Reactions for Joint USC/ City of Hope/ UC Davis Studies: Trials of Investigational Drugs



Action: #

(0) *No action required.*

(1) *Report to IDB-CTEP by phone within 24 hrs; written report to IDB-CTEP within 10 days and to IRB within 5 days using DCT form; for Phase I trials, if ADR occurs at a center other than the Coordinating Center (CC), Fax short form to the CC immediately followed by DCT form within 5 days; for Phase II, Fax DCT within 5 days. Fax # (818)301-8393.*

 # IRB Reporting also requires submission of a Physician's narrative summary, counter-signed by the Principal Investigator or his/her designee, along with the ADR form.

Revised- 9/27/94

Attachment 1
December 1990

DCT ADVERSE REACTION FORM FOR INVESTIGATIONAL AGENTS

ADR # _____ (Assigned at NCI)

Person Completing this Form _____ Date _____
Physician Responsible for this Report _____
(Please print or type)

I. DEMOGRAPHICS

A. Patient Information

PT I.D. # _____ Age ____ Sex ____ Date of Initial Dx _____
Malignancy _____
Site of Primary _____ PS (at start of study) ____
Site(s) of Metastatic Disease

Concurrent Non-Malignant Disease and Non-Protocol Medications

B. Drug Information

Drug Name _____
Source of Drug: NCI _____ Other _____ (specify _____)
Type of Reaction _____ Toxicity Grade _____
Date of Reaction _____ Date IRB notified _____
NCI Protocol # _____ Attending Physician (Investigator) _____
Phase of Study _____ Institution _____ Phone ( ) -
Protocol Treatment (include all agents)

Drug	Dose	Schedule	Route

Date of First Course _____ Number of Courses _____
Date Last Course Started _____ Date of Therapy Associated with ADR _____
Prior Therapy (Drug, radiation, relevant surgery: Include dates of therapy)

II. DOCUMENTATION OF REACTION

A. Non-Myelosuppressive Toxicity and Previously Unknown Myelosuppression

1. Description of Reaction and Temporal Relationship to Investigational Drug Administration

2. Physical Findings and Laboratory Data (e.g. bilirubin, creatinine, including baseline, worst and recovery values) Documenting Toxicity

3. Treatment of Adverse Reaction

4. Complications and Sequelae (If death, was an autopsy obtained?)

5. Past History of Organ Dysfunction

6. Rechallenge with Agent ____No ____Yes
   If yes, describe outcome:

B. **Myelosuppression (Previously known or unknown)**
   1. Laboratory Data Documenting Myelosuppression

	Baseline Date/Value	Nadir Date/Value	Recovery or Latest Value Date/Value
WBC or PMN	_____ /_____	_____ /_____	_____ /_____
Platelets	_____ /_____	_____ /_____	_____ /_____
Hgb or Hct	_____ /_____	_____ /_____	_____ /_____

   2. Complications, Treatment and Sequelae (e.g. infections/hemorrhage)

C. **Grade of Toxicity and Reporting Requirements (Check one)**
   1. Previously Unknown Toxicities:
      a. Fatal _____ or Life-threatening ____ (Report by telephone within 24 hours: 301-496-7957) Date _____ NCI contact _____
      b. Grade I ____ II ____ III ____ (Send form within 10 days)
   2. Previously Known Non-Myelosuppressive Toxicities:
      a. Fatal ____ or Life-threatening ____ (Send form within 10 days)
   3. Previously Known Myelosuppressive Toxicities:
      a. Fatal ____ (Send form within 10 days)

   Send Forms to: Investigational Drug Branch, NCI
   P.O. Box 30012
   Bethesda, Maryland 20814

D. **Investigator's Assessment (If more than 1 investigational agent were used, give an assessment for each by writing the drug names on the appropriate lines)**

	IND Drug	Non-IND Drug	Disease
Unrelated	____	____	____
Unlikely	____	____	____
Possible	____	____	____
Probable	____	____	____
Definite	____	____	____

Action Taken:
None ____
Dose Reduced ____
Dose Withheld ____
Drug Discontinued ____

Therapy Required:
None ____
Symptomatic ____
Supportive ____
Intensive ____

E. I hereby certify that the information on this form is correct and complete to the best of my knowledge.

_____ M.D.
(Signature of Responsible Physician)

Specimen Collection Form for Pharmacodynamic Studies

Cycle # 1

Title: PH II- 06 Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Hormonally Insensitive Breast Cancer (Recurring Less Than Six Months Since Adjuvant or as Second-Line for Advanced Disease) (1B-95-4)

Patient's Name_____ _____ Patient Accession # USC - ___ ___ ___
(first) (last)

Hospital # _____ Height _____ cm Weight: ____ . __ kg BSA __ . __ __ m2

Check appropriate regimen:

_____ Regimen I:

Paclitaxel 175 mg/m2 = 175 x _____ (BSA) = _____ mg IV over 3 hours on day 1

_____ Regimen II:

PSC 833 5 mg/kg = 5 x _____ kg = _____mg PO qid for 12 doses, starting day 1

Paclitaxel 70 mg/m2 = 70 x _____ (BSA) = _____ mg IV over 3 hours on day 2

Exact Time of **Start** of Paclitaxel Infusion _____ Exact Time of **End** of Paclitaxel Infusion _____

Exact Time of start of PSC 833: Date ___/___/___ Time _____am/pm

Draw 5ml blood in green top tube each time. Spin, separate, store plasma at -20°C

DATE	BLOOD SAMPLE #	TIME DUE	EXPECTED TIME	ACTUAL TIME	Drawn By (initials)	Comments	ASSAY RESULTS (Lab Only Use)
	1	Time 0 *	am pm	am pm			
	2	1 hr post start (during infusion)	am pm	am pm			
	3	2 hrs post start (during infusion)	am pm	am pm			
	4	3 hrs post start (End of infusion)	am pm	am pm			
	5	0.25 hrs after end of infusion	am pm	am pm			
	6	0.5 hrs after end of infusion	am pm	am pm			
	7	1 hrs after end of infusion	am pm	am pm			
	8	2 hrs after end of infusion	am pm	am pm			
	9	3hrs after end of infusion	am pm	am pm			
	10	6 hrs after end of infusion	am pm	am pm			
	11	12hrs after end of infusion	am pm	am pm			
	12	24 hrs after end of infusion	am pm	am pm			

*Obtain buffy coat, freeze and send to Dr. Mike Press.

Rest of specimens to: Dr. Tim Synold's laboratory at COH _____ (# samples) by _____ on _____/_____/_____ (Date). Received by: _____ (Lab manager) on _____/_____/_____ (Date).

Revised 4/16/96

Specimen Collection Form for Pharmacodynamic Studies

Cycle # 1

Title: PH II- 06 Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Hormonally Insensitive Breast Cancer (Recurring Less Than Six Months Since Adjuvant or as Second-Line for Advanced Disease) (1B-95-4)

Patient's Name_____ _____ Patient Accession # COH - ___ ___ ___
(first) (last)

Hospital # _____ Height _____ cm Weight: ____ . __ kg BSA __ . __ __ m2

Check appropriate regimen:
_____ Regimen I:
Paclitaxel 175 mg/m2 = 175 x _____ (BSA) = _____ mg IV over 3 hours on day 1
_____ Regimen II:
PSC 833 5 mg/kg = 5 x _____ kg = _____mg PO qid for 12 doses, starting day 1
Paclitaxel 70 mg/m2 = 70 x _____ (BSA) = _____ mg IV over 3 hours on day 2

Exact Time of **Start** of Paclitaxel Infusion _____ Exact Time of **End** of Paclitaxel Infusion _____
Exact Time of start of PSC 833: Date ___/____/____ Time _____am/pm

Draw 5ml blood in green top tube each time. Spin, separate, store plasma at -20°C

DATE	BLOOD SAMPLE #	TIME DUE	EXPECTED TIME	ACTUAL TIME	Drawn By (initials)	Comments	ASSAY RESULTS (Lab Only Use)
	1	Time 0	am pm	am pm			
	2	1 hr post start (during infusion)	am pm	am pm			
	3	2 hrs post start (during infusion)	am pm	am pm			
	4	3 hrs post start (End of infusion)	am pm	am pm			
	5	0.25 hrs after end of infusion	am pm	am pm			
	6	0.5 hrs after end of infusion	am pm	am pm			
	7	1 hrs after end of infusion	am pm	am pm			
	8	2 hrs after end of infusion	am pm	am pm			
	9	3hrs after end of infusion	am pm	am pm			
	10	6 hrs after end of infusion	am pm	am pm			
	11	12hrs after end of infusion	am pm	am pm			
	12	24 hrs after end of infusion	am pm	am pm			

To Dr. Tim Synold's laboratory at COH _____ (# samples) by _____ on _____/_____/_____ (Date
Received by: _____ (Lab manager) on _____/_____/_____ (Date).

Revised 4/16/96

Specimen Collection Form for Pharmacodynamic Studies

Cycle # 1

Title: PH II- 06 Phase II Randomized Study of Paclitaxel Versus Paclitaxel + PSC 833 for Advanced Hormonally Insensitive Breast Cancer (Recurring Less Than Six Months Since Adjuvant or as Second-Line for Advanced Disease) (1B-95-4)

Patient's Name_____ _____ Patient Accession # UCD - ___ ___ ___
(first) (last)

Hospital # _____ Height _____ cm Weight: ____ . __ kg BSA __ . __ __ m2

Check appropriate regimen:

_____ Regimen I:

Paclitaxel 175 mg/m2 = 175 x _____ (BSA) = _____ mg IV over 3 hours on day 1

_____ Regimen II:

PSC 833 5 mg/kg = 5 x _____ kg = _____mg PO qid for 12 doses, starting day 1

Paclitaxel 70 mg/m2 = 70 x _____ (BSA) = _____ mg IV over 3 hours on day 2

Exact Time of **Start** of Paclitaxel Infusion _____ Exact Time of **End** of Paclitaxel Infusion _____

Exact Time of start of PSC 833: Date ___/____/____ Time _____am/pm

Draw 5ml blood in green top tube each time. Spin, separate, store plasma at -20°C

DATE	BLOOD SAMPLE #	TIME DUE	EXPECTED TIME	ACTUAL TIME	Drawn By (initials)	Comments	ASSAY RESULTS (Lab Only Use)
	1	Time 0	am pm	am pm			
	2	1 hr post start (during infusion)	am pm	am pm			
	3	2 hrs post start (during infusion)	am pm	am pm			
	4	3 hrs post start (End of infusion)	am pm	am pm			
	5	0.25 hrs after end of infusion	am pm	am pm			
	6	0.5 hrs after end of infusion	am pm	am pm			
	7	1 hrs after end of infusion	am pm	am pm			
	8	2 hrs after end of infusion	am pm	am pm			
	9	3hrs after end of infusion	am pm	am pm			
	10	6 hrs after end of infusion	am pm	am pm			
	11	12hrs after end of infusion	am pm	am pm			
	12	24 hrs after end of infusion	am pm	am pm			

To Dr. Tim Synold's laboratory at COH _____ (# samples) by _____ on _____/_____/_____ (Date

Received by: _____ (Lab manager) on _____/_____/_____ (Date).

Revised 4/16/96

List of Personnel

1995-96

Franco Muggia, M.D. - Principal Investigator

Michael Press, M.D., Ph.D. - Co-investigator

Susan Groshen, Ph.D. - Biostatistician

XiaWei Yang, Ph.D. - Lab tech

Rosalina Palaroan, R.N. - lab tech, specimen facilitator