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Our data show that MDR1 gene expression is important in breast cancer resistance. The role of the MDR1 gene in breast cancer treatment will be further defined by sequentially determining MDR1 gene expression pre and post treatment with doxorubicin in the context of three prospective clinical trials. In addition, this study will allow a correlation of MDR1 gene expression and clinical outcome. To determine what level of MDR1 gene expression is clinically significant, various molecular methods of determining MDR1 gene expression, including immunohistochemistry and quantitative reverse transcription followed by polymerase chain reaction, will be evaluated.

MDR can be reversed *in vitro* and we will test this hypothesis in a Phase I study of Cyclosporine A and quinine as MDR reversers of Vinblastine resistance. Together these studies will address the major goal of circumventing drug resistance in breast cancer. When the data of the MDR1 gene expression in breast cancer specimens from this proposal are available, clinical trials incorporating the modulators of MDR, Cyclosporine and quinine, will be designed for breast cancer as well. An alteration in drug efflux potentially may have an impact on response to chemotherapy and may result in improved survival for breast cancer patients. During the period between March 15, 1993 and March 14, 1997, we have outfitted our laboratory with staff, equipment, supplies and reagents, have been performing control experiments and have been pursuing activation of the various clinical trials to support this project. We now have some preliminary data on the expression of MDR1 and MRP (Multidrug resistant associated protein) in breast cancer specimens and normal adjacent breast tissue. Further follow-up of these patients is needed to determine the clinical significance of expression of these drug resistance genes.

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Lou J. Goldstein pp 8/6/97

PI - Signature Date

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INTRODUCTION

Drug resistance is a major obstacle in the treatment of cancer. The multidrug resistance gene (MDR1) encodes an energy dependent drug efflux pump, P-170, that confers cellular resistance to multiple therapeutic agents such as anthracyclines, vinca alkaloids, epipodophyllotoxins, taxol, and actinomycin-D. MDR1 gene expression is tumor specific in both *de novo* resistant tumors and those that acquire drug resistance following chemotherapy. The central role of P-170 in this multidrug resistance (MDR) phenotype suggests that modulation of either MDR1 gene expression or the function of P-170 may provide an effective means of clinically reversing drug resistance.

Our data show that MDR1 gene expression is important in breast cancer resistance. The role of the MDR1 gene in breast cancer treatment will be further defined by sequentially determining MDR1 gene expression pre- and post-treatment with doxorubicin in the context of prospective clinical trials. In addition, these studies will allow a correlation of MDR1 gene expression and clinical outcome. To determine what level of MDR1 gene expression is clinically significant, various molecular methods of determining MDR1 gene expression, including immunohistochemistry and quantitative reverse transcription followed by polymerase chain reaction, will be evaluated. We have also had the opportunity to analyze our samples for expression of the MRP gene (Multidrug Resistant Related Protein). The MRP gene is a new member of the ATP-binding cassette transporter superfamily. It is overexpressed in a variety of cell lines which exhibit acquired drug resistance, yet do not express P-glycoprotein. In addition, recent work has shown that this gene confers a multidrug resistant phenotype when transfected into sensitive cells. Currently, little is known of the mechanism by which MRP confers drug resistance. Furthermore, the clinical relevance of MRP is unknown. We have had the opportunity to develop methodologies to examine the expression of this gene in breast cancer specimens and compare this expression with MDR1 data.

When the data of the MDR1 and MRP gene expression in breast cancer specimens from this proposal are available, clinical trials incorporating modulators of MDR, cyclosporine and quinine, will be designed for breast cancer as well. An alteration in drug efflux potentially may have an impact on response to chemotherapy and may result in improved survival for breast cancer patients.

BODY

The aim of this project is to test the hypothesis that drug resistance in breast cancer is mediated by the MDR1 gene. Moreover, once MDR1 gene expression has been established in breast cancer and correlated with response and resistance to chemotherapy, such data may be used to predict drug resistance and design clinical trials to overcome such resistance using pharmacologic agents proven to reverse MDR *in vitro* and *in vivo.*

To accomplish the tasks outlined in the initial proposal, we have accomplished the following from March 15, 1993 to March 14, 1997:

1. Personnel: a) Scientific Technician, Rajashekar K. Reddy, MS. b) Post-Doctoral Associate, Dwayne Dexter, Ph.D. c) Research Fellow, Jack Leighton, M.D.

2. Space and Facilities: With the assistance of the personnel above, our 500 sq. ft. laboratory space has been fully equipped to perform the molecular experiments described in our original proposal.

3. Reagents and Supplies: With the assistance of the laboratory staff above, we have now fully equipped the laboratory with the necessary reagents and supplies. We have established reproducible, quality controlled experiments with regard to cell culture, RNA isolation, hybridization with our various cDNA probes and reverse transcription-PCR (RT-PCR) using our competitive templates for the MDR1/MRP gene as internal controls. In addition, the laboratory staff has been evaluated for proper handling of tissue specimens and RNA isolation by doing experiments on tumor bank specimens looking for MDR1/MRP gene expression.

4. Methods

A) RNA Isolation

Cellular lysis and RNA extraction were accomplished using a modified one-step guanidinium salt lysis procedure. Approximately 100 mg of fresh frozen tissue, stored at -80°C, was pulverized in the presence of dry ice and transferred directly to a sterile, polypropylene 50 ml conical centrifuge tube. The pulverized tissue was then transferred to a dounce vessel containing 1.5 ml of lysis solution (2 *M* guanidium thiocyanate, 12.5 m*M* sodium citrate, pH 7.0, 0.25% sarcosyl, 0.1 *M* 2-mercaptoethanol, 0.2 *M* sodium acetate, pH 5.2, and 50% phenol). The mixture was homogenized, on ice, using a Teflon or glass dounce until no visible tissue could be seen. The homogenate was transferred to a 2.0 ml microcentrifuge tube and 0.4 ml of chloroform:iso-amyl alcohol (24:1) were added. The sample was mixed by vortexing for 30 seconds and then incubated for 5 minutes at 4°C. The organic and aqueous phases were subsequently partitioned by centrifugation at 14,000 x g, 4°C for 15 minutes. The aqueous phase was removed to a fresh 1.5 ml microcentrifuge tube and 1 volume of 2-propanol was added to precipitate the RNA. The sample was incubated at -70°C for 15 minutes, then the precipitated RNA was pelleted by centrifugation for 15 minutes at 14,000 x g, 4°C. The 2-propanol was decanted, the RNA pellet washed once with 1 ml of 75% ethanol and then air dried. The pellet was resuspended in TE (10 m*M* Tris, pH 6.8, 1 m*M* EDTA, pH 8.0) and the RNA concentration determined by standard UV spectral analysis.

RNA integrity was determined by non-denaturing agarose gel electrophoresis. One to two micrograms of RNA were resolved in a 1.2% agarose gel prepared in 1X TBE (890 m*M* Tris, 890 m*M* boric acid, 20 m*M* EDTA, pH 8.0) containing 0.5 mg/ml of ethidium bromide. Visual examination of the resolved RNA was performed by UV transillumination. RNA integrity was judged on the quality of the 28S and 18S ribosomal RNA bands. Furthermore, integrity of sequence information was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers directed towards β-actin (See PCR Methods).

B) Slot Blot Filter Analysis

Preparation of Slot Blot Filter. Slot blot filters were prepared using standard techniques. To minimize variations in pipetting, serial dilutions of sample RNA were prepared in 10X SSC (1.5 *M* NaCl, 0.15 *M* sodium citrate, pH 7.0). These serial dilutions were applied to a nylon filter according to manufacturer's instructions (Minifold II, Schleicher and Schuell, Keene, NH). For quantitative purposes, RNA isolated from the KB 3-1 and KB 8-5 cell lines were also applied to each filter. Previous slot blot filter analysis of human tumors used RNA from these two cell lines as a benchmark for MDR1 expression in drug-sensitive (3-1) and drug-resistant (8-5) cells (Goldstein, et al). After the samples were applied to the filter, it was washed 2-3 times with 10X SSC and then the samples were covalently linked to the filter by UV irradiation (Stratalinker, Stratagene, La Jolla, CA).

Hybridization of Slot Blot Filters. Slot blot filters were probed with ^{32}P-labeled DNA fragments for MDR*1* and β-actin (See Probe Preparation). Before adding the probe, the filters were pre-hybridized for 4 hours at 42°C with PRE-HYB solution [50% formamide, 5X SSC, 5X Denhardt's (0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% BSA), 200 mg/ml of denatured salmon sperm DNA, and 50 m*M* sodium phosphate, pH 6.5]. After pre-hybridization, the PRE-HYB solution was replaced with hybridization solution (50% formamide, 5X SSC, 1X Denhardt's, 10% dextran sulfate, 100 µg/ml denatured salmon sperm DNA, and 20 m*M* sodium phosphate, pH 6.5) and denatured, radioactively-labeled probe was added. The filter was hybridized for 18-24 hours at 42°C at which time the hybridization solution was removed and the filter washed. The filters were washed 4 times at room temperature with 1X SSC/0.1% SDS for 15 minutes each. These washes were followed by two 10 minute washes with 0.2X SSC/0.1% SDS at 50°C. Washed filters were then exposed to a phosphor-imager screen for 16 hours and the image analyzed by a FUJIX Bio-Imaging Analyzer, BAS1000 (Fuji Photo Film Co., Japan).

Probe Preparation. The plasmid pHDR5A was kindly donated by M. Gottesman. This plasmid contains a cDNA insert of MDR*1* which when digested with *Eco* RI produces a 1.3 kbp MDR*1* cDNA fragment. This fragment was resolved by agarose gel electrophoresis and

purified by anion spin-column chromatography (Qiagen, Chatsworth, CA). One hundred nanograms of this purified fragment were radioactively labeled by the random-priming method (Stratagene or US Biochemical, Cleveland, OH) with deoxycytosine 5'-[a-^{32}P] triphosphate (3000 Ci/mmol, Amersham, Arlington Heights, IL). Labeled probe was purified using either a standard G-50 sephadex spin column procedure or a microconcentrator (Amicon, Beverely, MA). The activity of the probe, determined by liquid scintillation, was usually greater than 1 x10^8 cpm/mg. The plasmid pST1 was a kind gift of A. Godwin and contains a partial cDNA sequence of the human β-actin gene. *Pst* I digestion of this plasmid generates an 800 bp fragment that was purified and radioactively labeled as describe above for MDR1.

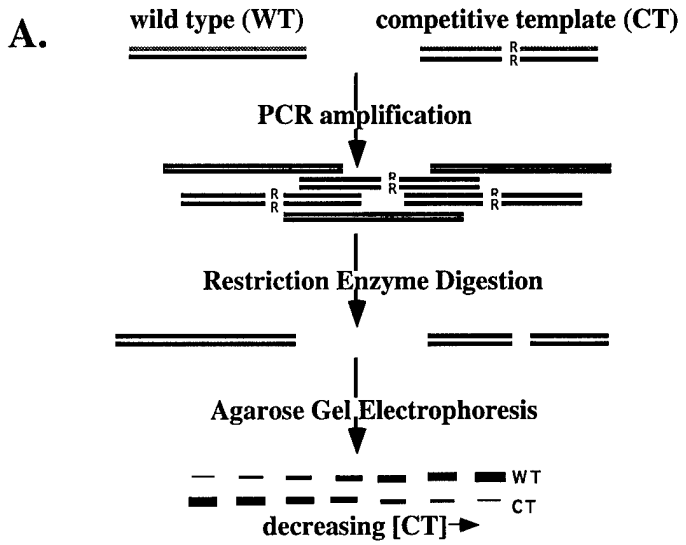
Image Anaylis and Quantification. A phosphor-image of the filter was quantitated using MacBAS software (Fuji Photo and Kohshin Graphic Systems, Inc.). The only image manipulation was an adjustment in brightness or contrast of the total image. Quantification of MDR1 expression was accomplished by comparing tumor signal to KB 8-5 signal. Quantitative analysis was performed by assigning an arbitrary value of 30 Units (U) to the KB 8-5 MDR1 signal. Tumor sample values were determined as a ratio of the tumor signal intensity to the arbitrary value for KB. To ensure reproducibility, signal intensities were also normalized for quantity of RNA on the slot blot filter as determined by b-actin signal intensity.RNA isolations, competitive RT-PCR, slot blot methodology as per previous reports.

C) Competitive RT-PCR

RT-PCR Conditions. First strand cDNA synthesis was performed on 50 -100 ng total RNA using the random priming extension method. All reagents were obtained from Gibco BRL (Bethesda, MD), unless stated otherwise, and master mixtures were routinely prepared. The reaction conditions were: 10 minutes at room temperature; 30 minutes at 42°C; five minutes at 99°C; five minutes at 5°C. The completed reaction was used directly in subsequent PCR assays.

Primers for MDR1, MRP, and β-actin were chosen using Primer Detective (Clonetech, Palo Alto, CA). The MDR1 primers (nucleotides 1325-1347, 1502-1523 of the cDNA sequence) are specific for MDR1. The MRP primers were chosen from the published human cDNA sequence (nucleotides 4208-4229, 4414-4435). β-actin primers were from nucleotides 237 - 258, 388-408 of the cDNA sequence. The predicted target size for each of the primer pairs was 198 bp (MDR1), 229 bp (MRP), and 171 bp (β-actin) and all spanned an intron to differentiate PCR products derived from RNA and DNA. PCR cycle conditions were 30 seconds at 94°C, 30 seconds at 60°C, 30 seconds at 72°C.

Competitive PCR. Competitive PCR was performed using the PATTY technique first described by Becker-Andre and Hahlbrock (1989) . Essentially this approach utilizes an exogenous RNA target sequence (competitive template, CT), which is identical to the endogenous target sequence except for the addition/deletion of a restriction enzyme site and is titrated in the presence of a constant amount of sample RNA and RT-PCR performed (Figure 1A). Absolute quantification of transcript is determined by using the equation: $\log(N_{n1}/N_{n2}) = \log(N_{01}/N_{02}) + n\log(eff_1/eff_2)$. By plotting the log input of CT versus the log ratio of CT product to wild-type product, a linear relationship between the two axis's can be generated and a curve drawn by least square's analysis (Figure 1B). This curve results in an equation that can be used to precisely determine the point at which the ratio of both products is equal to 1, the point where input CT is equal to endogenous target.



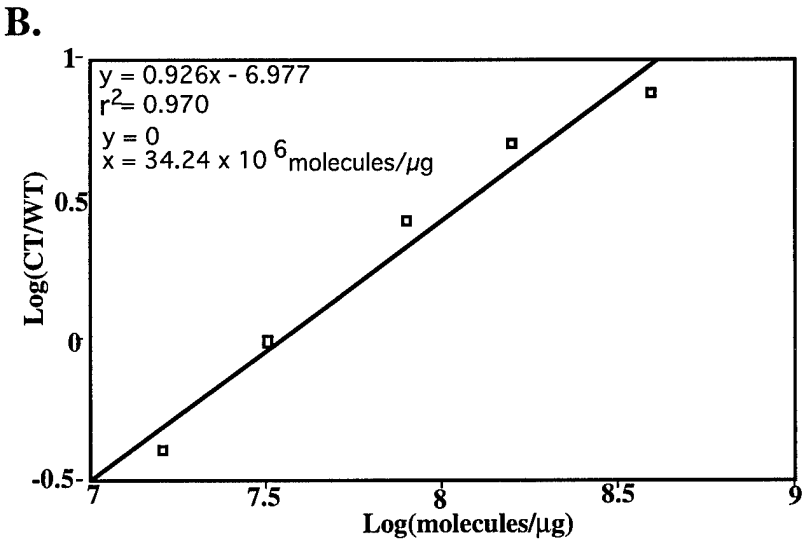


Figure 1. (A) Schematic of competitive RT-PCR reaction using a titration method to determine the point of equivalence. (B) Example of regression analysis to determine the exact equivalence point for competitive RT-PCR.

MDR1 and MRP competitive templates were constructed using PCR mutagenesis. MDR1 competitive template was constructed by inserting an *Eco* RI site at position #1425 of the cDNA sequence and MRP competitive template was constructed by inserting a *Sty* I site at nucleotide position #4319 of the published cDNA sequence. The resulting fragments were cloned into a TA cloning vector (Invitrogen, San Diego, CA). *In vitro* transcribed cRNA template was synthesized and subsequently used in the competitive PCR assay.

D) Immunohistochemistry

Immunohistochemical protocol was reevaluated because of concerns over appropriate controls and antibodies. Recently, an international workshop was held to standardize IHC protocols for P-170 detection in clinical samples (Beck, et al). While defined protocols were not put forth, general guidelines were recommended. Two important recommendations were the use of an appropriate 'gold standard' cell line which allowed accurate detection of clinically relevant levels of P-170 and the use of normal tissue, which expresses P-170, that is processed in a similar fashion as the clinical samples.

Per our previous report we had been using cytospins of KB 3-1 and KB 8-5 as our drug sensitive (P-170-) and drug resistant (P-170+) standard cell lines respectively. Based on the guidelines, it was suggested that the gold standards be handled in a similar fashion as the clinical samples under review. This recommendation was generally referring to the examination of paraffin embedded samples and using paraffin fixed pellets of the contols, but we felt this recommendation was also valid for frozen sections, since sectioned cells would give a different signal than whole cells. We developed a mock solid tissue model for KB 3-1 and KB 8-5 cell lines by resuspending the cell pellets in 3% low-melt agarose prepared with PBS. These agarose plugs were then snap frozen in OCT and processed according to standard procedures. Frozen sections of these solid cell plugs were then processed along with tissue samples.

The second recommendation suggested that known P-170 positive tissues be processed along with clinical samples and gold standard cell lines. The purpose of this tissue is to monitor variability in batch to batch IHC staining. Since the control tissue, such as kidney or liver, has a specific region of staining and the level of P-170 is high in this tissue the intensity and region of staining should be similar from batch to batch. This control is probably more important than the gold standard since it allows one to monitor the success (sensitivity and specificity) of that particular round of staining. We have now incorporated the staining of frozen kidney sections and liver into our protocols. The kidney has positive staining on the apical surface of the lining of the proximal tubules while the liver has intense staining of the bile canniculi. If either of these controls does not give the expected result, that batch of samples is not analyzed and new sections are analyzed.

Per the recommended guidelines and as in our previous report, we are using frozen sections of breast specimens and analyzing them with two antibodies which recognize two different epitopes of P-170, one internal and one external. In our previous report we presented data from four antibodies, C219, JSB1, UIC2, and MRK16. However, we also, as well as others, reported concerns about using C219. We are now using JSB1 and UIC2 for our current IHC work. JSB1 recognizes an internal epitope and is one of the most commonly used P-170 antibodies. UIC2 recognizes an external epitope and was chosen because it can be used on acetone fixed slides, were as MRK16's epitope is reportedly destroyed by acetone fixation. Thus, the use of acetone fixation on all slides, controls and clinical specimens, allows a direct comparison of antibody sensitivity and specificity without confounding factors such as fixation method to undermine data interpretation. Our current working protocol is outlined below.

Slide Preparation:. Five μM cyrosections were prepared using standard techniques. Cyrosections were air-dried overnight then fixed with either acetone at 4°C for 10 minutes. Acetone slides were air-dried for 1 hour. Slides were stored at -20°C until processing. Slides were processed in an identical manner. Isotype non-specific mouse antibodies were used as a negative control. Cyrosections of agarose embedded cell pellets of KB 3-1 and KB 8-5 were used as gold standards for positive and negative staining for PGP. Cyrosections of agarose embedded cell pellets of HL60S (MRP negative) and HL60R (MRP positive) were used to optimize MRP staining protocol. Normal liver and kidney sections were used for positive tissue staining controls. The staining protocol is outlined below. Except for the first 2 steps, the staining procedure is identical to that outlined by the manufacturer of the staining kit (DAKO).

Antibody	[µg/ml]	Isotype Control
PGP		
JSB1	10	IgG1
UIC2	1	IgG2a
MRP		
QCRL1	1:20	IgG1
MRPm6	1:20	IgG1

Staining Protocol. Antibodies were diluted in antibody dilution buffer (DAKO). Slides were allowed to come to room temperature, rehydrated with PBS for 5 minutes, blocked with 0.1% avidin followed by 0.01% biotin for 10 minutes each and rinsed with 0.2% Tween/PBS, followed by PBS between blocking steps. Slides were blocked with 3% non-fat dry milk/PBS (NFM) for 10 minutes. Excess NFM was removed and the samples covered with the appropriate antibody (approximately 10-20 µl). The primary antibody was allowed to incubate overnight at 4°C in a humidified chamber, then the slides were rinsed as before. The secondary antibody was added and the slide incubated at room temperature for 15 minutes. Slides were rinsed and strepavidin-peroxidase complex was added. After a 15 minute incubation, slides were rinsed and the chromagen-substrate was added (DAB). After a 5 minute incubation, slides were rinsed with ddH_20, counterstained with hematoxylin and a coverslip mounted. Stained slides were scored by 2 independent observers blinded to PCR results. The staining was scored on a weighted scale using a 1+, 2+, 3+ scale for intensity of staining, combined with a percentage positive score for each intensity level.

5. Results

Thus far through the Fox Chase Cancer Center Tumor Bank, we have obtained a total of 205 breast specimens. Of these, 122 are tumor samples, 83 of which have accompanying normal adjacent tissue. Approximately 130 of these 205 have been analyzed by QRT-PCR, 47 by IHC and RT-PCR, and 31 by slot blot, IHC, and RT-PCR. Because of our ongoing current reevaluation of IHC data, only QRT-PCR data is sufficient to allow statistical analysis to be performed.

A) Slot Blot

Slot Blot data were reported previously and are represented in Figure 2. Since this technique proved to be the most unreliable (note the number of specimens that are greater thatn KB 8-5) and required the most amount of labor and materials it is currently not being performed on clinical specimens. Furthermore, the reduction in biospy size precludes slot blot from being performed on a significant number of samples. For instance, almost 70% of the samples recieved from PBT-3 are core biospies or FNAs. Finally, it is clear that PCR detection has become the preferred method for examining transcript levels in clinical samples since it is highly sensitive, can be quantitative, and only small clinical samples are needed.

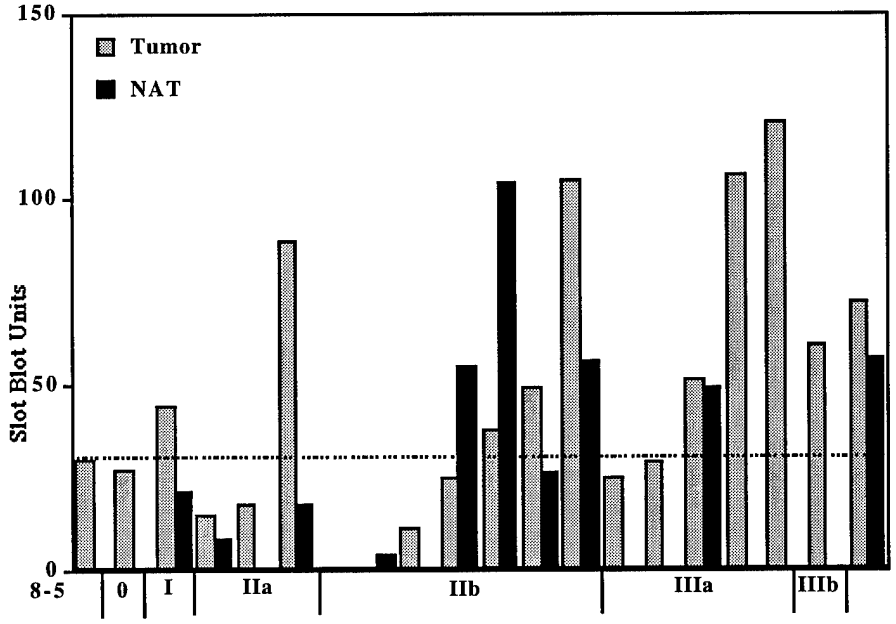


Figure 2. Slot blot determination of MDR1 expression in breast cancer and NAT (grouped by stage). Dotted line represents normalized level of expression of KB 8-5. Expression was detected in 23/26 (88%) of assessable samples with 15/26 having expression > KB 8-5.

B) Competitive RT-PCR.

RT-PCR Optimization and Assay Validation. Reaction conditions were optimized for each primer pair using cell line RNA as well as plasmids containing gene inserts (Figure 3A). These optimized conditions were subsequently employed in the competitive assay (Figure 3B). Initial sample analysis included a screen for MDR1, MRP expression and β-actin amplification was used to determine actual RT efficiency and RNA loading.

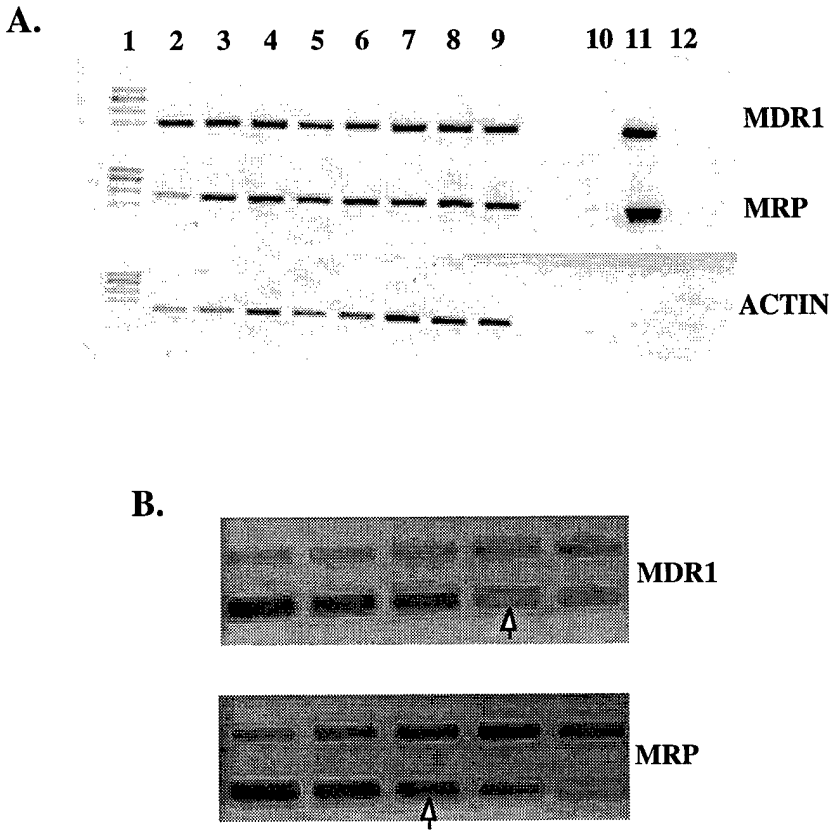


Figure 3. (A) Prescreen of tumor specimens for MDR1, MRP, and β-actin expression. Lane 1-MW marker; Lane 2-9-tumor specimens; Lane 10-RT water control; Lane 11-positive control (actin-PCR water control); Lane 12-PCR water control. (B) Typical competitive reaction for a tumor sample. Arrows mark visual point of equivalence.

The competitive assay was optimized by determining the efficiency of the PCR for each primer pair. Several reports have shown that accurate determinations can be made either in the linear range of amplification (LRA) where heteroduplex formation is minimal or in the plateau phase where heteroduplex formation can be maximized. We chose to perform the competitive assays in the LRA (data not shown), thus all reactions were done at 28 or 34 cycles depending on the titration range of CT used.

The statitistical limits of the assay were determined as follows. Since cell line RNA was readily available and replenishable, repeated measurements were made on RNA isolated from KB 8-5 for the expression of MDR1 and MRP. Competitive assays performed at different times using different RNA preparations were used to determine the statistical limits of the assay. The standard error of the mean (SEM) was approximately ± 25% for MDR1 and ± 20 for MRP. This error roughly translates into a two-fold difference in expression as being statitistically significant. Since many of the tumor samples had limited amounts of RNA, it was not possible to do repeated measurements on each sample, however several samples had sufficient amounts of RNA to do repeated measurements. The values determined in repeated QRT-PCR assays for these samples fell within the expected margin of error (data not shown).

Recommendations from our recent site visit suggested that we report tumor expression values in the context of established controls of drug resistant cell lines. From the outset of this project we have been using the cell lines KB 3-1 and KB 8-5 as our gold standards. We have previously reported all our results in the context of KB 8-5 expression. We have further examined other cell lines which are known to overexpress MDR1 or MRP to validate our PCR protocol. These values are shown in Table 1. As can be seen, the quantitative PCR technique is quite sensitive and is reproducible over different concentrations of RNA.

Table 1. MDR1 and MRP Expression in MDR Cell Lines
(as measured by QRT-PCR)

Cell line	Fold Resistance	[RNA ng]	MDR1 (molecules/μg ± 25%)	MRP (molecules/μg ± 25%)
KB 3-1	1		0	4×10^9
KB 8-5	3-4	50	-- *	11×10^9
		10	5.7×10^8	--
		5	5.9×10^8	--
MCF-7	1		0	5×10^9
MCF/ADR	100-200	50	-- *	19×10^9
		10	2.7×10^9	--
		5	3.2×10^9	--
MCF/VP	9-28	50	0	1.8×10^{11}
		10		1.9×10^{11}
		5		2.0×10^{11}

* These values could not be accurately determined since 50 ng of RNA out competed the highest concentrations of competetive template.

QRT-PCR results were obtained on approximately 130 of the 205 specimens. As reported previously, MDR1 and MRP expression was detected in 100% of the samples analyzed (tumor and NAT). Figure 4 illustrates the expression of MDR1 in matched sets of specimens. As can be seen, no clear pattern of expression presents itself. Pairwise analysis, however, of tumor and NAT expression indicated that the difference in MDR1 expression seen in tumor versus NAT was significantly different (Student t-test, p=0.0032). The mean expression data for both these genes is seen in Table 2. How the difference between 7.81×10^6 molecules/μg of RNA and 10.41×10^6 molecules/μg of RNA equates to differences in protein level is currently being examined by IHC. Like MDR1, no clear pattern of expression of MRP in these samples was seen (data not shown). Statistical analysis indicated no significant difference between mean expression in tumor and NAT (Table 1).

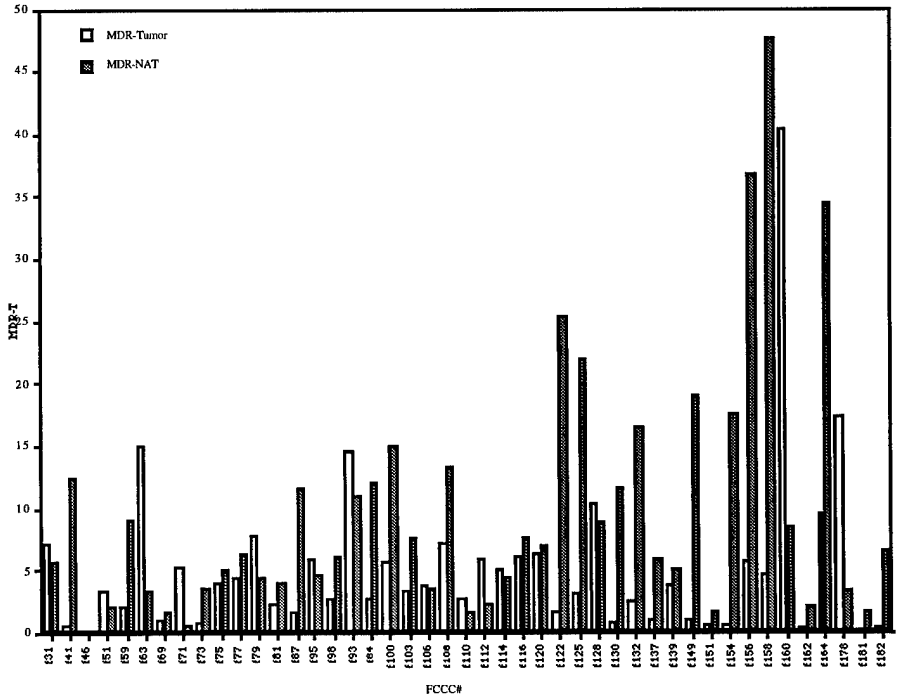


Figure 4. Level of Expression of MDR1 in Matched Breast Tumor and NAT.
(Determine by QRT-PCR)

TABLE 2. Mean Expression of MDR1 and MRP in Human Breast.

	MDR ($x10^6$ molecules/μg)	MRP ($x10^9$ molecules/μg)
TUMOR	7.81	11.42
NAT	10.41	8.85

Although a significant difference in expression of MRP between tumor and NAT was not observed, there was a significant correlation of the difference of expression of MDR1 and MRP between tumor and NAT. Thus, there was a significant correlation between the difference in MDR1 expression seen for tumor and NAT and the difference seen for MRP expression (p,0.05, r=.47). This result is represented in Figure 5 and suggests that these two genes may be coordinately regulated in breast tissue.

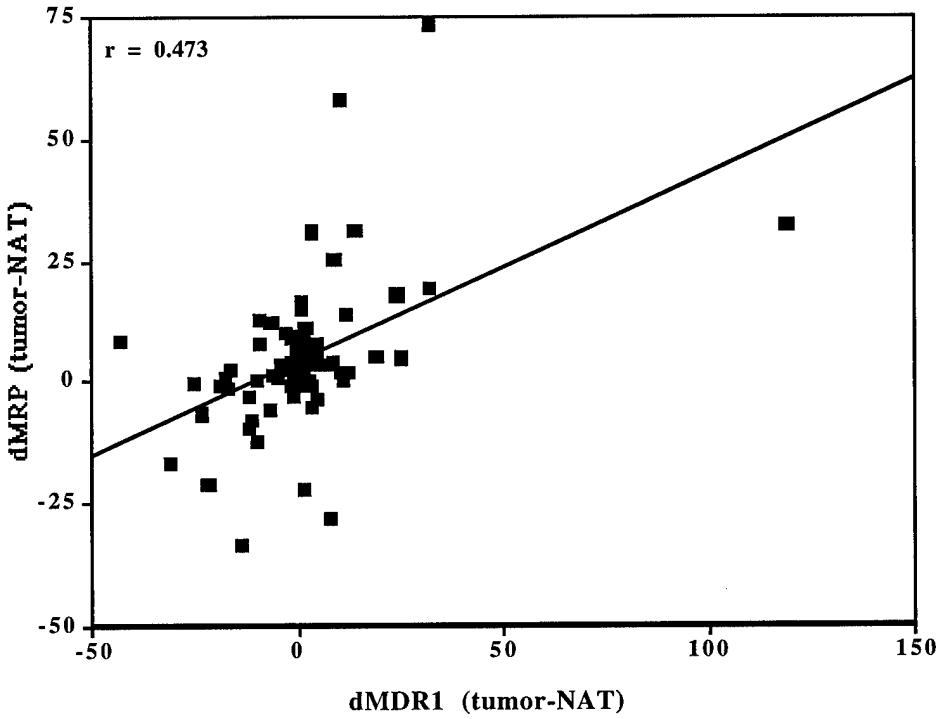


Figure 5. Correlation of differences in tumor versus NAT for MDR1 Expression and MRP Expression.

The correlation of expression of both MDR1 and MRP and clinical parameters was also explored. For example, Figure 6 represents a breakdown of MDR1 expression by stage. Also, MDR1 expression in KB8-5 is represented on this graph. As we reported previously, KB 8-5 expression is at least two logs higher than most of the specimens examined. Furthermore, there does not appear to be any correlation between stage and level of expression. Statistical correlation between the expression levels of MDR1 or MRP and a variety of clinical parameters were performed. A summary of this analysis can be seen in Table 3. A significant correlation between age and MDR1 expression was observed. Within the age subgroups, MDR1 expression in the <50 subgroup was approximately twice that of the >50 age group. This difference in expression was not seen for MRP or in NAT (data not shown). Although not significant, there also appeared to be a positive trend MRP expression levels and ER/PR status. Currently, no correlation between expression level and clinical outcome can be determined since a majority of these patients have no evaluable disease at this time. Long-term follow-up is needed to confirm any correlation.

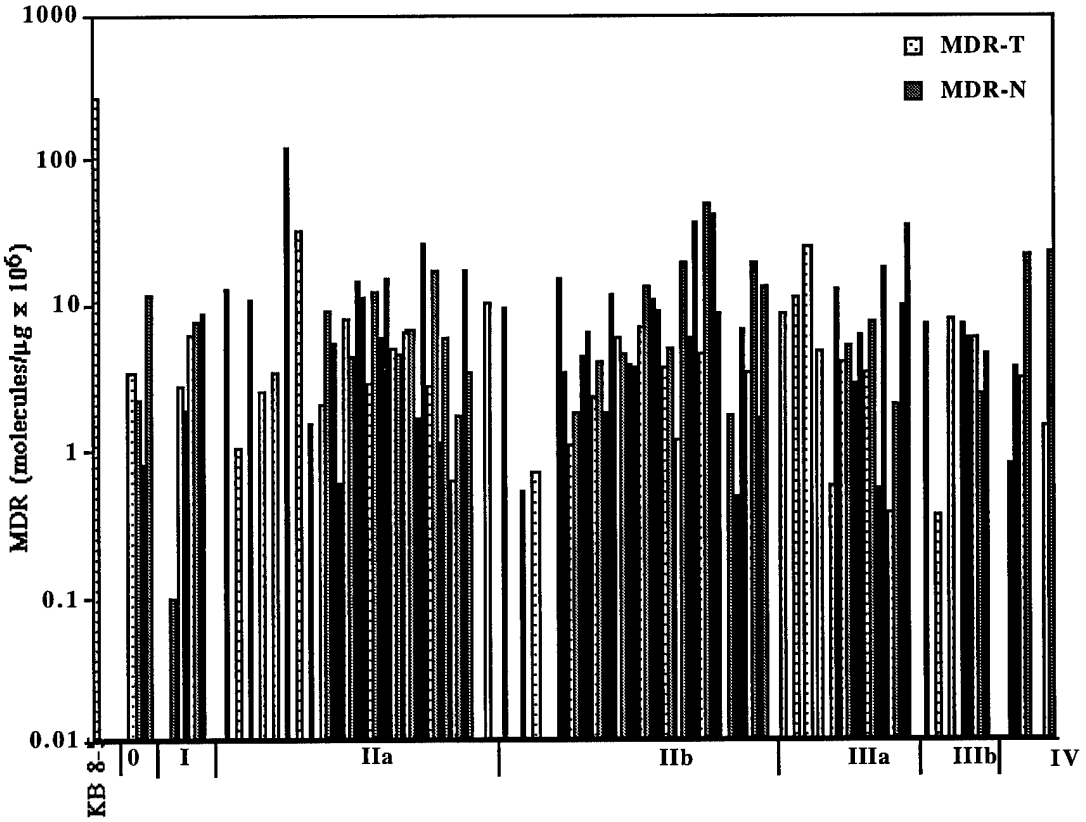


Figure 6. Expression of MDR1 based on Tumor Stage. Note average two log difference in expression for specimens versus KB 8-5

Table 3. MDR and MRP Expression in breast carcinoma and clinical data

Clinical Data	All Patients (n=74)	MDR Expression (molecules/µg x 10^6)	P[a]	MRP Expression (molecules/µg x 10^9)	P[a]
Age (yrs)					
Median	58				
Range	28-89	7.81		11.42	
25-49	23	11.84	P<.05[b]	11.88	NS[c]
50-90	44	5.46		11.28	
Histological Type					
DCIS	3	1.40		5.89	
Ductal Carcinoma	57	5.73		11.72	
Lobular Carcinoma	5	29.38	p<.05[a]	13.34	NS[d]
Other	8	12.04		10.13	
Histological Grade					
GI-II	19	6.38		16.28	
GIII	43	5.02	NS[c]	9.20	NS[c]
Stage					
0-I	6	3.63		12.23	
IIA	22	12.10		12.53	
IIB	19	6.05	NS[b]	12.01	NS[b]
IIIA	17	6.44		9.85	
IIIB	6	5.57		11.36	
IV	3	1.84		4.82	
Tumor Size					
T0 and T1	10	7.81	NS[d]	12.71	NS[d]
T2	35	8.71		12.29	
T3	13	6.80		9.94	
T4	7	5.22		10.73	
Lymph Nodes					
N0	27	9.17		11.48	
N1	36	6.98	NS[d]	12.38	NS[d]
N2	2	3.18		3.46	
Metastasis					
M0	56	8.52		11.99	
M1	4	4.61	NS[b]	11.98	NS[c]
ER Status					
Positive	45	9.22	NS[b]	13.76	p=.07[b]
Negative	16	4.00		6.44	
PR Status					
Positive	34	9.98	NS[b]	14.11	p=.07[b]
Negative	27	5.16		8.01	

[a]Statistical Analysis of Difference in Expression (Instat Software)
[b]Welch's T Test (Log transformed)
[c]Student T Test
[d]Kruskal-Wallis Test

C) Immunohistochemistry

Currently, all clinical samples are being reevaluated using the revised staining procedure. Of the 205 specimens collected, 50 have been stained and are currently being scored by 2 independent pathologists blinded to PCR data. The reference pathologist at Fox Chase is Harry Cooper, M.D.. The scoring system includes % of cells staining positive and intensity of staining and was described previously. While this system is subjective, the use of more than one scorer allows a consensus score to be reached. The revised staining procedure has been thoroughly tested on the gold standard cell lines which are embedded in a agarose plug and sectioned under conditions similar to that of clinical specimens. Likewise, normal kidney and liver have been examined using this staining procedure. Both JSB1 and UIC2 stain these two tissues in the appropriate manner with UIC2 giving a slightly stronger signal.

We previously reported that UIC2 gave a very weak signal on control cells and breast tissue. Those data were generated from normal buffered formalin fixed slides using UIC2 from a different manufacturer. UIC2 has since become available from a second manufacturer with a long history in monoclonal antibody production, thus a better quality control system. Secondly, although it has not been reported, we found that UIC2 gave exceptional staining results on acetone fixed slides.

KB 8-5 staining is weak compared to the normal control tissues, but is very discernible from background staining using both antibodies. This result is in concordance with reported staining intensities using KB 8-5 and cell lines with similar levels of expression of P-170 (Beck, et al). Previously, we reported some background staining in KB 3-1, however, this new staining procedure does not produce any signal in KB 3-1. It should be noted that the blocking time with 3% non-fat dried milk was increased from 10 minutes to 30 minutes and this may have eliminated this background staining. Once we have evaluated these specimens using both antibodies we can address the issue of P-170 expression and its relationship to clinical parameters as well as its relationship to MRP expression. Furthermore, we can analyze the concordance between the use of IHC and PCR to detect and measure the expression of these two genes.

The question that arises when comparing these two techniques is what should be the expected frequency of positive cells in a tissue that has two logs less RNA than KB8-5. In order to derive some knowledge apriori about the expected result we have to make two assumptions. First, that RNA quantity is directly proportional to protein content. Thus, for a cell that has two logs less of MDR1 transcript, it is assusmed that it will have approximately two logs less P-170 protein. Secondly, the quantity of total RNA in one cell is approximately equal to 20 pg, thus for KB 8-5, 1 μg of RNA from KB 8-5 or any other cell line is equal to approximately 50,000 cells. Finally, quantitatively we can detect approximatly 10-100 cells of KB 8-5 in a background of 25, 000 cells of KB 3-1 (non-MDR1 expressing cell line). Based on our current PCR data, these assumptions predict that a cell that expresses the same level of MDR1 as KB 8-5 would be found at a concentration of 0.1-1 cell/25,000. This calculation predicts that this would be a rare event in a typical frozen section, thus suggesting a low positive rate for IHC. Converesly, if we assume that the low level of expression seen correlates to actual protein levels, the staining intensity of a population of cells that has two logs less protein than KB 8-5 would most likely be undetectable based on our staining protocol and KB 8-5 staining intensities. Table 4 is a preliminary summary of IHC data scored using the new staining procedure and comparing them to the appropriate controls. As can been seen, the majority of samples have scores ranging from 0 to low 1+ staining. This outcome is not unexpected based on the previous discussion concerning expected frequencies. The number of samples examined, however, preclude an conclusions concerning the level of expression of P-170 or MRP as determined by IHC and its relationship to PCR values or clinical parameters. We are currently examining a larger data set by this technique. **(Since our April report, we have been able to analyze approximatley 30 samples using the above protocol and these data Table 4 from our previous draft of April 1997).**

TABLE 4. Expression of PGP and MRP in Human Breast Carcinoma

Immunohistochemical Score	0	1	2	3
PGP	(No. of Samples in each group)			
JSB1-Tumor	29	2	0	0
JSB1-NAT	17	0	0	0
UIC2-Tumor	32	0	0	0
UIC2-NAT	17	0	0	0
MRP				
MRPm6-Tumor	0	13	8	4
MRPm6-NAT	0	7	1	4
QCRL1-Tumor	0	14	8	4
QCRL1-NAT	0	4	5	6

Table 4. Quantitative values of P-170 and MRP expression obtained by immunohistochemistry. Immunohistochemistry was scored on a combined scale of 1+ and % positive cells.

6. Discussion

The goal of this project is to test the hypothesis that drug resistance in breast cancer is mediated by the MDR1 gene. The specific aims to accomplish this goal include:

1) Evaluate and compare different detection assays used to determine MDR1/P-170 expression.
2) Identify the role of MDR1 in drug resistance in breast cancer.
3) Conduct clinical trials in the reversal of drug resistance and correlate with MDR1 expression.

1) Evaluate and compare different detection assays used to determine MDR1/P-170 expression.

One of the major goals of this project is to determine the correlative significance of MDR1 gene expression with treatment outcome. In order to achieve this goal, the measurement of MDR1 gene expression must be reliable and consistent. There are several techniques that are currently employed to determine MDR1 gene expression levels. At issue is the reproducibility, sensitivity, specificity and quantitative nature of these methods and the correlation between them. A major focus of this study is to determine the concordance between the most common techniques (immunohistochemistry, RNA slot blot hybridization, and RT-PCR) used to measure MDR1 expression.

A considerable amount of time has been devoted to establishing appropriate procedures for each technique and evaluating the lab's consistency in performing these techniques (see previous reports). The sensitive nature of RNA isolation and RT-PCR require that strict protocols be followed. Thus, each technique has been scrutinized and potential pitfalls that may affect yield or introduce unwanted variability have been addressed.

General comparisons of the various techniques have established the relative reliability of each technique and the concordance between them. In Figure 1, all the breast samples that express MDR1 do so at levels much less than KB-8-5. The sensitivity of slot blot hybridization does not appear to be as great as PCR and was not as reproducible as RT-PCR. We have found the limit of resolution for RT-PCR to be no less than a two-fold difference in expression for both MDR1 and MRP. A recent study by Brophy, et. al. (1994), demonstrated that slot blot

hybridization had a high false positive rate while PCR was extremely sensitive and specific supporting that MDR1 expression levels be determined utilizing RT-PCR and immunohistochemistry.

The immunohistochemical evaluation is now ongoing using our new controls and methodologies for MDR1 and MRP expression. We have discussed our data with the biostatisticians at Fox Chase Cancer Center who have determined that for adequate comparisons of detection methodologies and clinical parameters, 47 matched pairs will be required for proper analysis. Intial results indicate that P-170 expression is very low to undectable in most samples. This finding is not suprising based on the RT-PCR data which suggests that MDR1 expression is extremely low in these samples (at least 25 times less than KB 8-5). In summary, our data indicate that MDR1/P-170 expression is extremely low in primary breast carcinoma and that IHC corroborates RT-PCR data.

2) Identify the role of MDR1 in drug resistance in breast cancer.

MDR1 expression was detected in 100% of the samples examined by PCR, however, the level of expression was approximately 25 times less than KB 8-5. Of particular interest is our finding that MDR1 and MRP expression could be detected in NAT with an equal frequency to tumor. Although the biology of NAT cannot be considered to be equal to normal tissue, the finding that NAT had detectable levels of expression by both RT-PCR and IHC may have important biological implications. Namely, that the micro and macro-environment of the affected breast may influence not only tumor cell biology, but also normal epithelial cell growth. The finding of expression of both MDR and MRP in normal adjacent tissue using RT-PCR was unexpected since most previous studies have not reported expression of MDR1 in normal breast tissue . Wishart et al, however, has noted expression in stromal cells of breast cancer but not of normal breast.. If indeed adjacent stromal tissue stains for P-170 this might suggest up regulation of MDR1 in cells at risk for tumorigenesis. It might also indicate that since MDR is a transmembrane protein, cell-cell interaction might be important for function, however, investigation of these possibilities are not within the scope of this proposal. Recent work has demonstrated that insulin like growth factor II (IGF II) is primarily stromal in origin and possibly functions as a paracrine growth promotor in breast cancer suggesting that stromal epithelial interactions may be important in breast cancer.

MRP was detected in 100% of the samples by RT-PCR. MRP expression levels, in general, were equal to KB 3-1 or KB 8-5 and did not approach the levels seen in the MRP overexpressing cell line MCF-7/VP-16. MRP expression in tumors and NAT was not statistically different.

MDR1and MRP expression did not significantly correlate to any clinical parameter except for age and MDR1. Correlation of MDR1 expression with age with indicated that the <50 subgrouped having approximately 1-fold higher expression than the >50 population. This correlation, however, was not seen by IHC. There was also a positive trend seen between ER/PR status and MRP expression which may indicate that MRP expression may be lost during tumor progression. It should be noted that this association was also not seen by IHC. An analysis of a larger number of advanced stage breast carcinomas by PCR and IHC may resolve this discrepency.

Currently the prognostic and predictive significance of MDR1 and MRP expression in breast carcinoma is unknown. Our data indicate that MDR1/P-170 expression is extremely low in primary breast carcinoma and that IHC corroborates RT-PCR data. Furthermore, MRP/MRP expression is ubiquitous and varied. Correlations between RT-PCR determined expression levels and clinical parameters in this study indicate that only MDR1 expression and age may be related which may contribute to the worse prognosis of breast cancer in younger women stage for stage when compared to older women. For the most part, these samples were obtained prior to any chemotherapy and were from primary breast carcinomas, thus our data indicate that MDR1 probably does not play a significant role in intrinsic resistance of primary breast carcinoma. The role of MDR1 in acquired resistance awaits evaulation of specimens from ongoing clinical trials. The role of MRP may be more difficult to define since it is widely expressed in many tissues, including normal breast, and its level of expression is significantly higher that MDR1 making quantification difficult.

3) Conduct clinical trials in the reversal of drug resistance and correlate with MDR1 expression.

The clinical trials described in this project are at various levels of accrual, approval and development as outlined below:

a) Philadelphia Bone Marrow Consortium - PBT-3 (IRB94041). Phase II Trial of High Dose Chemotherapy with Cyclophosphamide, Thiotepa and Carboplatin and Peripheral Blood Stem Cell Infusion in Women with Inoperable Locally Advanced and Inflammatory Breast Cancer who achieved partial response to Induction Chemotherapy this protocol has been approved by the IRBs of the four member institutions including Fox Chase Cancer Center (FCCC), University of Pennsylvania, Hahnemann University and Temple University. This study was activated 6/94 and thus far, 29 patients have been enrolled, and specimens have been collected from 28 of these 29 with sequential samples from most patients.

b) Eastern Cooperative Oncology Group (ECOG) Registration Study of Induction with Adriamycin in Inoperable Locally Advanced and Inflammatory Breast Cancer to Evaluate for Multidrug Resistance. Since the Breast Biology Committee and the Breast Committee's Annual Report, this concept had been approved by the ECOG Breast Core Committee July 7, 1994, and a draft of the schema and eligibility were included in the appendix of the previous report. ECOG has decided not to proceed with the activation of this study.

c) Phase II Study of R-Verapamil (Dexverapamil) in Advanced Breast Cancer. This study has received FCCC IRB approval and consent forms have been approved by the DOD. R-Verapamil will likely be replaced by an alternative MDR modulator since it has not been shown to have significant clinical activity. The protocol has been written, and it has been submitted for appropriate review. The most recent draft of this protocol (E1195), a Phase II study of PSC 833 to modulate MDR mediated resistance is enclosed.

d) Phase I study of Cyclosporine and Quinine to Reverse MDR in Refractory Malignancy treated with Vinblastine. This study has been approved by the FCCC IRB and consent forms have already been approved by the DOD. Because Cyclosporine A (CSA) initially planned to be used in this trial has been reformulated to enhance its immunosuppressive activity and has subsequently lost its potency in mediating reversal of MDR this agent was not activated for this study. This agent has been replace by PSC833, a second generation cyclosporin which does not have potent immunosupressive activity. An activated phase II ECOG trial, E1195 (enclosed), is examining PSC833's modulating activity of paclitaxel reponse in metastatic breast cancer.

CONCLUSION

Drug resistance is a major obstacle in the treatment of malignancies. Although MDR1 mediated drug resistance has been well characterized in preclinical models, its role in clinical drug resistance is not as well characterized and requires further investigation. That is the aim of the studies proposed here. The ability to identify tumors with increased MDR1 gene expression has several potential applications, for example; the prediction of the response to chemotherapy or the design of studies of the reversal of resistance with agents that inhibit MDR1-mediated drug efflux. Prospective studies as described above are necessary to establish the role of MDR1 gene expression in clinical resistance. The initial goal of such trials is to demonstrate the ability to reverse MDR1 mediated drug resistance in appropriate advanced refractory malignancies. Ultimately, it will be important to incorporate these reversal strategies in the treatment of early stage disease at which time the tumor burden is smaller and fewer mechanisms of resistance may be present.

Well designed phase I and II prospective clinical trials using reversing agents in conjunction with chemotherapy in malignancies that express the MDR1 gene are necessary prior to routine use of agents such as verapamil and quinidine which carry innate toxicities. Epithelial tumors such as colon and renal cell carcinoma express the MDR1 gene and are clinically resistant to most cytotoxic agents, many of which are not substrates of P-170. In this situation, MDR may be one of a complex array of drug resistance mechanisms. Breast cancer would be a more appropriate human tumor model since it is a tumor for which many active chemotherapeutic agents are handled by MDR. In such a setting an alteration in drug efflux may indeed have an impact on response and possibly improve survival for breast cancer patients. The transgenic mouse model may be used to assess novel MDR reversing agents, non-toxic analogues of known reversing agents and combinations of various MDR modifiers to be subsequently investigated in Phase I studies.

Over the period of March 15, 1993 to March 14, 1997, we have successfully outfitted our laboratory with staff, equipment, supplies and reagents to perform the necessary control experiments of MDR1 gene expression assays as described in the body of this and previous reports. Recent advances in biospy and diagnositic techniques have greatly reduced the amount of tissue available for study, making IHC analysis difficult. Thus, sensitive, quantitative methods for both genes may be critical in assessing the clinical significance of these resistance markers. The use of RT-PCR to assess gene expression while sensitive and specific, does not discriminate between non-malignant cells and tumor cells, yet it may be essential and useful in measuring small changes in gene expression if properly validated. We now have data on the expression of MDR1 and MRP in primary breast cancer specimens and normal adjacent breast tissue using properly validated RT-PCR and IHC assays. We will now follow these patients prospectively to determine the clinical significance of such expression as it relates to response and/or resistance to cytotoxins which are substrates for the proteins encoded by these genes. Furthermore, we also will begin to use these optimized assays to analyze specimens from clinical trials in which sequential samples (pre- and post-treatment) have been obtained.

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EASTERN COOPERATIVE ONCOLOGY GROUP

A Pilot Phase II Trial of PSC-833 Modulation of Multidrug Resistance to Paclitaxel in the Treatment of Metastatic Carcinoma of the Breast

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University of Pennsylvania
Stanford University
Fox Chase Cancer Center

ACTIVATION
June, 1997
Addendum #1, 6/97 - Added Fox Chase Cancer Center (Title Page).

REGISTER

Paclitaxel/ PSC-833

PSC-833	5 mg/kg, p.o. q 6 hours x 12 doses
Paclitaxel[1]	70 mg/m^2 IV by 3 hour infusion beginning 4 hours after 5th dose of PSC-833
	(Continue until progression)

* Repeat cycle every 3 weeks

1 Paclitaxel will be administered over 3 hours by IV. There will be no dose escalation. Approximately 14 hours and 7 hours prior to the Paclitaxel administration, the patient will be medicated with Dexamethasone 20 mg p.o. At 30-60 minutes prior to the Paclitaxel administration, the patient will medicated with Diphenhydramine 50 mg IV and Cimetidine (300 mg IV) or other H_2 receptor antagonist.

* Use patient's actual weight when calculating body surface area.

* At progression patient is off study.

1.0 INTRODUCTION

1.1 Background

Drug resistance, which may arise by somatic mutations during tumor growth or be present de novo, is an important cause of failure of cancer chemotherapy. Most cellular models of drug resistance selected *in vitro* by anthracyclines, vinca alkaloids, or epipodophyllotoxins display a broad cross-resistance mechanism termed multidrug resistance (MDR) at the molecular and cellular level (1, 2, 3). The MDR phenotype is related to the expression of *mdr* genes coding for P-glycoproteins. P-glycoprotein acts as an efflux pump with very broad specificity and actively transports antineoplastic drugs out of the cells, thereby reducing the intracellular concentration of antineoplastic drugs.

MDR 1 expression is strongly implicated in both intrinsic and acquired drug resistance in human cancers (4). It is likely that P-glycoprotein in normal tissues has an important role in the detoxification of certain anti-cancer agents, as well as in drug distribution as a component of the blood-brain and blood-testicular barriers (5, 6).

Several non-cytotoxic drugs, such as verapamil, phenothiazines and cyclosporines have been shown to modulate MDR, at least in part by competitive inhibition of P-glycoprotein function (3). However, verapamil and the phenothiazines modulate MDR at drug concentrations which produce unacceptable clinical toxicities (heart block and depression of the central nervous system) *in vivo.*

Cyclosporin A is a potent inhibitor of P-glycoprotein and may be safely administered in combination with etoposide, doxorubicin, or paclitaxel. Cyclosporin A does interfere with the elimination of etoposide, doxorubicin, or paclitaxel and so dose reductions are required to attain acceptable toxicities. Phase I/II trials have documented that cyclosporin A as a single agent has dose limiting toxicities of renal dysfunction and hyperbilirubinemia. Other toxicities included nausea, vomiting, increased myelosuppression, headache, hypertension, hypomagnesemia, and diarrhea.

PSC-833 is an analog of cyclosporin D, with the chemical formula [3'-keto-Bmt^1]-[Val^2]-cyclosporine. PSC-833 is highly effective at modulating multidrug resistance *in vitro* (3, 7, 8. 9). PSC-833 is superior in MDR modulation compared to amiodarone, verapamil, procaine, quinidine, quinacrine, lidocaine, and cyclosporine A. PSC-833 has produced a clear dose-dependent protective effect in mice inoculated with multidrug resistant tumors and treated with otherwise ineffective cytostatic chemotherapeutic drug doses.

A Phase I trial of PSC-833 alone followed by PSC-833 plus paclitaxel has been performed at Stanford University Medical Center. Patients were treated with paclitaxel 175 mg/m^2 by 24-hour intravenous infusion as a single agent until progression of disease. Patients were then treated with PSC-833 alone for one course. Subsequent courses included PSC-833 plus paclitaxel 52.5 mg/m^2 by 24-hour intravenous infusion. Six cohorts of patients were treated.

Cohort 1. PSC 4 mg/kg x 2 followed by 2 mg/kg x 5 q8h.	3 patients
Cohort 2. PSC 6 mg/kg x 2 followed by 4 mg/kg x 5 q8h	3 patients
Cohort 3. PSC 8 mg/kg x 2 followed by 6 mg/kg x 5 q8h	6 patients
Cohort 4. PSC 10 mg/kg x 2 followed by 8 mg/kg x 5 q8h	3 patients
Cohort 5. PSC 6 mg/kg x 10 q6h	2 patients
Cohort 6. PSC 5 mg/kg x 10 q6h	4 patients

The dose limiting toxicity of PSC-833 was reversible ataxia (Table 1). Other toxicities that occurred included hyperbilirubinemia (2/9 patients in cohorts 3 & 4), nausea and fatigue (2/6 patients in cohorts 5 & 6). No nausea, vomiting, myelosuppression, or renal toxicity was noted with PSC-833 alone. The myelosuppression of PSC-833 plus paclitaxel 52.5 mg/m^2 was comparable to that of paclitaxel alone at a dose of 175 mg/m^2.

TABLE 1					
DOSE LIMITING TOXICITY: ATAXIA WITH PSC-833 ALONE					
		Grade			
Cohort	N of Patients	0	1	2	3
1	3	3	0	0	0
2	3	3	0	0	0
3	6	3	1	1	1
4	3	0	0	2	1
5	2	0	0	0	2
6	4	2	1	1	0

A subsequent phase I trial performed jointly by Stanford University and Washington University administered PSC-833 at a dose of 5 mg/kg qid for 12 doses in combination with escalating paclitaxel as a 3-hour intravenous infusion. In this study, the recommended phase II dose of paclitaxel is 70 mg/m^2 by 3-hour continuous infusion.

The New York Gynecologic Oncology Group is currently performing phase II study of PSC-833 at a dose of 5 mg/kg qid for 12 doses plus paclitaxel 70 mg/m^2 by 3-hour continuous infusion in the treatment of ovarian cancer. Preliminary analysis of the toxicity experience in the 16 patients enrolled to date documents one episode of grade 3 ataxia, one episode of grade 4 neutropenia lasting eight days, and one episode of fever and neutropenia. Other adverse events that were felt to be disease related or otherwise unrelated to study drug included one each of: an upper gastrointestinal bleed secondary to peptic disease, abdominal distension with nausea and vomiting, nausea and vomiting secondary to bowel obstruction, urinary tract infection with fever, and a hypertensive episode immediately following treatment with paclitaxel (personal communication, Novartis Pharmaceuticals Corporation, October 8, 1995).

Recent phase II studies have demonstrated that paclitaxel has substantial antitumor activity in the treatment of metastatic carcinoma of the breast. In one trial of patients without prior chemotherapy for metastatic disease, a response rate of 62% was observed (10). In a trial of women with one prior chemotherapy regimen for metastatic disease a response rate of 56% was observed (11). Paclitaxel has been given by a number of schedules of administration. Nonrandomized comparisons of the duration of infusion suggest that paclitaxel by 3-hour infusion is safe and that anti-tumor efficacy is equivalent to longer durations of infusion (Table 2).

This phase II trial is designed to evaluate the antitumor activity of combination PSC-833 plus paclitaxel in the treatment of patients with anthracycline refractory carcinoma of the breast. The protocol should serve to address both biologic and therapeutic questions. These include an evaluation of the antitumor activity of combination PSC-833 plus paclitaxel in the treatment of breast cancer and also will provide an evaluation of the biologic activity of PSC-833 reversal of multidrug resistance.

TABLE 2
PHASE II STUDIES OF SINGLE AGENT PACLITAXEL
IN THE TREATMENT OF BREAST CANCER.

Series	Dose (mg/m^2)	Infusion Duration	Patients With Prior Treatment (%)	Partial Response Rate (%)	Complete Response Rate(%)	Overall Response Rate (%)
MD Anderson (Holmes, 1993)	250	24 hour	100	44	12	56
Memorial Sloan-Kettering (Seidman, 1993)	250 + G-CSF	24 hour	62	50	12	62
	200 + G-CSF	24 hour	100	0	22	22
National Cancer Institute (Wilson, 1994)	140	96 hour	100	48	0	48
Multi-institutional (Speilman, 1994)	135	3-hour	100			
	175	3-hour	100	24	3	27
Instituto Nazionale Tumori, Milan (Gianni, 1994)	175	3-hour	100	27	20	47

2.0 OBJECTIVES

2.1 To evaluate the antitumor activity, as measured by frequency of objective response and time to progression, of the multidrug resistance modulator PSC-833 in combination with paclitaxel in the treatment of women with anthracycline refractory, metastatic carcinoma of the breast.

2.2 To evaluate the toxicity of combination PSC-833 and paclitaxel in the treatment of anthracycline refractory, metastatic carcinoma of the breast.

3.0 SELECTION OF PATIENTS

3.1 Patients must have a histologically confirmed adenocarcinoma of the breast with bi-dimensionally measurable recurrent or metastatic carcinoma of the breast.

3.2 All patients must be female.

3.3 All patients must have either received prior anthracycline therapy or have a medical contraindication to anthracycline therapy.

3.4 Patients may, not have received prior paclitaxel, taxotere or more than one prior chemotherapy regimen in the treatment of their recurrent or metastatic carcinoma of the breast.

3.5 If patient has had prior adjuvant chemotherapy within 6 months of diagnosis of metastatic disease this will be considered to be chemotherapy for advanced disease. (Note: prior hormonal therapy in either a metastatic or adjuvant setting is allowed.)

3.6 Patients must not be receiving concurrent treatment with agents well substantiated to increase or decrease the blood concentrations of cyclosporin A.

Drugs Increasing the Serum Concentration of CSA	Drugs Decreasing the Serum Concentration of CSA
Calcium channel blockers: Diltiazem Nicardipine Verapamil Antifungals: Fluconazole Itraconazole Ketoconazole Antibiotics: Clarithromycin Erythromycin Glucocorticoids: Methylprednisolone Other drugs: Allopurinol Bromocriptine Danazol Metoclopramide	Antibiotics: Nafcillin Rifampin Anticonvulsants: Carbamazepine Phenobarbital Phenytoin Other drugs: Octreotide Ticlopidine

3.7 ECOG performance status of 0, 1, or 2.

3.8 Patients must have adequate bone marrow, hepatic, and renal function defined by the following: (Completed ≤ 2 weeks before registration)

3.81 Granulocytes ≥ 1500/mm^3

3.82 Platelets ≥ 100,000/mm^3

3.83 SGOT ≤ 2.5x upper limit of normal

3.84 Total bilirubin ≤ 1.5 mg/dl

3.85 Serum creatinine ≤ 2x upper limit of normal

3.9 No central nervous system metastasis.

3.10 No chemotherapy or hormonal therapy for at least 3 weeks prior to enrollment.

3.11 Not pregnant or lactating.

3.12 Women of childbearing potential are strongly advised to use an accepted and effective method of contraception.

3.13 Patients must not have had an active malignancy other than breast cancer, in situ carcinoma of the cervix, or non-melanomatous skin cancers in the past 5 years.

3.14 No active, unresolved infection.

3.15 No treatment with parenteral antibiotics for an established or presumed infection ≤7 days prior to study entry.

3.16 No prior history of allergic reactions to cyclosporine or drugs utilizing the vehicle Cremophor (some anesthetics and muscle relaxants).

3.17 All patients must give signed written informed consent.

4.0 REGISTRATION PROCEDURES

A signed HHS 310 Form, a copy of the institution's IRB-approved informed consent document, and written justification for any changes made to the informed consent for this protocol must be on file at the ECOG Coordinating Center before an ECOG institution may enter patients. The signed HHS 310, institution informed consent, and investigator's justification for changes will be submitted to the following address:

ECOG Coordinating Center
Frontier Science
ATTN: IRB
303 Boylston Street
Brookline, MA 02146-7648
FAX: (617) 632-2990

Patients must not start protocol treatment prior to registration.

Please refer to Section 8.18 for PSC-833 drug ordering procedures.

To register eligible patients on study, the investigator will telephone the Central Randomization Desk at the ECOG Coordinating Center at (617) 632-2022. The following information will be requested:

4.1 Protocol Number

4.2 Investigator Identification

- 4.21 Institution name and/or affiliate
- 4.22 Investigator's name

4.3 Patient Identification

- 4.31 Patient's name or initials and chart number
- 4.32 Patient's Social Security number
- 4.33 Patient Demographics
  - 4.331 Sex
  - 4.332 Birthdate (MM/YY)
  - 4.333 Race
  - 4.334 Nine-digit zip code
  - 4.335 Method of payment

4.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.0. An eligibility checklist has been appended to the protocol. The randomization specialist will verify eligibility by asking questions from the checklist. A confirmation of registration will be forwarded by the Coordinating Center.

4.5 Cancellation Guidelines

If a patient does not receive protocol therapy, the patient may be canceled. Reasons for cancellation should be submitted in writing to the ECOG Coordinating Center (ATTN: DATA) as soon as possible. Data will be collected on all canceled patients (see Section 10.0). Note: A patient may only be canceled if no protocol therapy is administered. Once a patient has been given protocol treatment, all forms should be submitted.

5.0 TREATMENT PLAN

5.1 Administration Schedule

Use patient's actual weight when calculating body surface area.

5.11 Paclitaxel/PSC-833

PSC 833 5 mg/kg, p.o. q 6 hours x 12 doses

Paclitaxel 70 mg/m^2 IV by 3 hour infusion beginning 4 hours after the 5th dose of PSC-833

Repeat cycles every 3 weeks.

Paclitaxel will be administered over 3 hours by intravenous infusion. There will be no dose escalation.

Paclitaxel must be filtered. In-line filtration with a 0.2 micron filter is required. It may be diluted in 0.9% sodium chloride injection, USP or 5% dextrose injection, USP. Paclitaxel must be prepared in glass, polypropylene, or polyolefin containers and infused via non-PVC containing (nitroglycerin) infusion sets. Treatment will be repeated every 3 weeks.

5.111 To Prevent Allergic Reactions

Due to the known toxicity of Paclitaxel and/or the Cremophor vehicle, the following precautions will be taken to decrease the possibility of anaphylaxis.

5.1111 Approximately 14 hours and 7 hours prior to the Paclitaxel administration, the patient will be medicated with Dexamethasone 20 mg PO.

5.1112 30-60 minutes prior to the Paclitaxel administration, the patient will be medicated with Diphenhydramine 50 mg IV and Cimetidine (300 mg IV) or other H_2 receptor antagonist.

5.1113 Epinephrine and Diphenhydramine will be immediately available during the infusion.

5.1114 The patient's blood pressure and heart rate will be obtained at 15 minutes during the first hour and then PRN.

5.1115 See Section 5.3 for the management of hypersensitivity reactions (5.34) and cardiovascular toxicity (5.35) if they occur.

5.2 Adverse Reaction Reporting Requirements

ADR Reporting should be based on Common Toxicity Criteria (see Appendix II).

5.21 The following adverse reactions must be reported to ECOG, the NCI and your local IRB in the manner described below.

Investigational Agents: PSC-833, Paclitaxel

	Gr 2-3 unusual[2]	Grade 4 & 5 unexpected[2,3]	Grade 3 cardiac	Grade 4 expected[3]	Death due to Rx or within 30 days of Rx[1]
Call to NCI within 24 hours		X			X
Call to ECOG within 24 hours		X			X
Call to Novartis within 24 hours		X			X
ECOG ADR Form to NCI within 10 days	X	X	X	X	X
ECOG ADR Form to ECOG Coordinating Center within 10 days	X	X	X	X	X
ECOG ADR Form to Novartis within 10 days		X	X	X	X
Notify local IRB within 10 days	X	X	X	X	X

1 Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after protocol treatment has ended but which is felt to be treatment related, must be reported.
2 Any unexpected toxicity not reported in the literature or the package insert must be reported.
3 Grade 4 expected myelosuppression need not be reported but should be documented on flow sheets.

ECOG requires ADRs to be reported on the Adverse Reaction (ADR) Form For Investigational Drugs (#391RF). The form must be signed by the treating investigator.

5.22 Reporting of All Second Primary Cancers

	NCI/CTEP Secondary AML/MDS Report Form[1]	ECOG Second Primary Form[2] (Form # 630)
AML/MDS	X	
All other secondary cancers		X

1 To be completed within 30 days of diagnosis of AML/MDS that has occurred during or after protocol treatment. A copy is to be sent to ECOG and to the NCI, accompanied by copies of the pathology report (and when available, a copy of the cytogenetic report).
2 To be submitted to ECOG within 30 days of diagnosis of a new primary cancer during or after protocol treatment, regardless of relationship to protocol treatment. Not for use for reporting recurrence or metastatic disease. A copy of pathology report should be sent, if available.

NCI Telephone Number: (301) 230-2330
NCI FAX Number: (301) 230-0159
NCI Mailing Address:
IDB
P.O. Box 30012
Bethesda, MD 20824

ECOG Telephone Number: (617) 632-3610
ECOG Fax Number: (617) 632-2990
ECOG Mailing Address:
ECOG Coordinating Center
ATTN: ADR
Frontier Science
303 Boylston Street
Brookline, MA 02146-7648

Manuel Litchman, M.D.
Novartis Pharmaceutical Corporation
Clinical Safety and Epidemiology (CS&E)
59 Route 10
East Hanover, NJ 07936-1080
Ph: (201) 503-5844
FAX: 1-888-299-4565
After hours/weekend Ph: (201) 503-8300

5.23 Non-Treatment Related Toxicities

If a toxicity is felt to be outside the definitions listed above and unrelated to the protocol treatment, this must be clearly documented on the ECOG Flow Sheets which are submitted to the ECOG Coordinating Center (ATTN: DATA) according to the Records To Be Kept Section (10.0). This does not in any way obviate the need for reporting the toxicities described above.

5.3 Dose Modifications

All toxicities **should be graded according to the Common Toxicity Criteria (see Appendix II).**

5.31 Hematology Toxicity

5.311 Day 1 of each course:

Granulocytes		Platelets	Dose Paclitaxel
$\geq 1500/mm^3$	and	$\geq 10^5/mm^3$	100%
$<1500/mm^3$	or	$<10^5/mm^3$	Hold therapy until granulocytes ≥ 1500 mm^3 and platelets $\geq 10^5/mm^3$

If treatment is held more than 3 weeks, the patient will be taken off study.

5.312 Any patient experiencing any of the following will have Paclitaxel reduced by 25% (permanently)

5.3121 Febrile neutropenic episode (≥ 38.5°C) between courses.

5.3122 An absolute granulocyte count < 500/µl for ≥ 5 days

5.3123 Bleeding episode with a platelet count $\leq 40,000/mm^3$

5.3124 Platelet count ≤ $20,000/mm^3$ with or without a bleeding episode.

If, on subsequent course, these toxicities recur, paclitaxel will be reduced by another 25%.

5.32 Gastrointestinal Toxicity

5.321 Nausea and/or vomiting should be controlled with standard antiemetics, with the exception that *orally* administered metoclopramide should *not* be administered.

5.322 If mucositis is present on day 1 of any cycle, the treatment should be withheld until the mucositis has cleared. If acute Grade 3 or 4 mucositis occurs, Paclitaxel should be given at dose reduced by 25% when mucositis is completely cleared for all subsequent courses.

5.323 Grade 3 or 4 diarrhea is sufficient reason to reduce the Paclitaxel dose by 25% (after resolution of diarrhea) for all subsequent courses. For grade 2, the dose should be held until the diarrhea clears, and then resumed at full dosage.

5.33 Hepatic Toxicity

SGOT		Bilirubin (mg/dl)	% Dose to give Paclitaxel
>2 x baseline	OR	>2 x baseline	Contact study chair.

5.34 Anaphylaxis/Hypersensitivity

5.341 Mild symptoms (e.g., mild flushing, rash, pruritus): Complete Paclitaxel infusion. Supervise at bedsidé. No treatment required.

5.342 Moderate symptoms (e.g., moderate rash, flushing, mild dyspnea, chest discomfort): Stop Paclitaxel infusion. Give intravenous diphenhydramine 20-25 mg and intravenous dexamethasone 10 mg. Resume Paclitaxel infusion after recovery of symptoms at a low rate, 20 ml/hr for 15 minutes, then 50 ml/hr for 15 minutes, then if no further symptoms, at full dose rate until infusion is complete. If symptoms recur, stop Paclitaxel infusion. The patient will go off study and it will be reported as an adverse event.

5.3421 **Caution: Patients who had a mild to moderate hypersensitivity reaction have been successfully re-challenged but careful attention to prophylaxis and bedside monitoring of vital signs is recommended.**

5.343 Severe or life-threatening symptoms (e.g., hypotension requiring pressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria): Stop Paclitaxel infusion. Give intravenous diphenhydramine and dexamethasone as above. Add epinephrine or bronchodilators if indicated. The patient will go off study and it will be reported as an adverse event.

5.35 Cardiovascular

5.351 Cardiac rhythm disturbances have occurred infrequently in patients in clinical trials; however, most patients were asymptomatic and cardiac monitoring is not required. Transient asymptomatic bradycardia has been noted in as many as 29% of patients. More significant AV block has rarely been noted. Cardiac events should be managed as follows:

5.3511 Asymptomatic bradycardia: No treatment required.

5.3512 Symptomatic arrhythmia: Stop Paclitaxel and manage arrhythmia according to standard practice. The patient will off study and it will be reported as an adverse event.

5.3513 Chest pain, and/or symptomatic hypotension (<90/60 mm Hg or requires fluid replacement): Stop Paclitaxel infusion. Perform an EKG. Give intravenous diphenhydramine and dexamethasone as in 5.34 if hypersensitivity is considered. Also, consider epinephrine or bronchodilators if chest pain is not thought to be cardiac. The patient will go off study and it will be reported as an adverse event.

5.36 Peripheral Neuropathy - Neurosensory and Neuromotor

5.361 Grade 3: If grade 3 toxicity occurs, protocol treatment should be withheld until the patient recovers to grade 1 toxicity. When treatment is resumed, the dose of Paclitaxel should be reduced by 25% and continued at this level without further reduction for neurologic toxicity.

If grade 3 toxicity persists, or if a second episode of grade 3 neurotoxicity occurs, patient should be removed from study.

5.362 Grade 4: Remove from study.

5.37 CNS Toxicities

5.371 PSC-833 may cause reversible cerebellar dysfunction (ataxia, dysmetria) or paresthesias. If these occur during PSC-833 administration and are of ≥ grade 3 in severity, the dose of PSC-833 should be reduced from 5.0 mg/kg to 3.75 mg/kg (25% reduction).

Cerebellar dysfunction is the dose limiting toxicity of PSC-833. Since the definitions used in the Common Toxicity Criteria 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 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 gain, mild dysmetria, difficulty walking heel-to-toe or difficulty with rapid alternating movements.

Grade 3: Unable to walk without assistance from another person or 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 toxicity occurs before paclitaxel is administered, treatment should be discontinued until the toxicity resolves. The patient can then be restarted at 3.75 mg/kg/dose of PSC-833 and should receive the complete cycle of therapy (12 doses of PSC-833/paclitaxel, 3 hr., d2) at this dose.

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

If ≥ grade 3 toxicity recurs after dose reduction, the patient should be discontinued from the study.

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.

5.372 If Grade 3 or 4 toxicity occurs at the reduced dose (3.75 mg/kg) of PSC-833, the patient will go off study.

5.38 Other Toxicities

For any Grade 3 or 4 toxicity not mentioned above, treatment should be held until patients recover completely or to Grade 1 status. The next dose of Paclitaxel should be at 50% and, if well-tolerated (i.e., only Grade 1 toxicity occurs), subsequent doses should be increased by 25% in an effort to regain 100% dosing. For Grade 1 or 2 toxicities, no dose reduction should be made.

5.4 Supportive Care

5.41 All supportive measures consistent with optimal patient care will be given throughout the study.

5.42 Local radiotherapy necessary for control of pain or for life-threatening situations during the period of treatment will be considered progressive disease.

5.43 The use of G-CSF or GM-CSF for patients is allowed at the discretion of the individual investigator under established FDA guidelines.

5.5 Duration of Therapy

5.51 Patients with progressive disease or intolerable toxicity will be removed from study treatment and will be followed until death. Stable or responding disease will continue on study.

5.52 Patients with stable or responding disease and who are tolerating therapy will continue on study treatment on an indefinite basis.

6.0 MEASUREMENT OF EFFECT

6.1 Response Criteria

6.11 Methods of Malignant Disease Evaluation

6.111 Measurable, Bidimensional

Malignant disease measurable (metric system) in two dimensions by ruler or calipers with surface area determined by multiplying the longest diameter by the greatest perpendicular diameter (i.e., metastatic pulmonary nodules, lymph nodes, and subcutaneous masses). Malignant disease with sharply defined borders visualized by ultrasonography or computerized axial tomography is considered measurable. Repeat studies should be performed at the same pretherapy site(s) of malignant disease.

6.112 Measurable, Unidimensional

Malignant disease measurable (metric system) in one dimension by ruler or calipers (i.e., mediastinal adenopathy).

6.1121 Mediastinal and hilar involvement may be measured, if a preinvolvement chest x-ray is available, by subtracting the normal mediastinal or hilar width on the preinvolvement x-ray from the on-study width containing malignant disease.

6.113 Nonmeasurable, Evaluable

Malignant disease evident on clinical (physical or radiographic) examination, but not measurable by ruler or calipers (i.e., pelvic and abdominal masses, lymphangitis or confluent multinodular lung metastases, skin metastases, ascites or pleural effusions known to be caused by peritoneal or pleural metastases and uninfluenced by diuretics, liver scans, deviated or obstructed gastrointestinal tract, and masses with poorly defined borders on ultrasonography or computerized axial tomography).

6.1131 Malignant ascites known to be caused by malignant involvement of the peritoneum and uninfluenced by diuretics may be followed by serial abdominal girths measured through a specified fixed point.

6.1132 Serial x-rays of lymphangitis or confluent multinodular lung metastases, pleural effusions, or bone metastases should be compared to evaluate response.

6.1133 Tumor scans can be used to evaluate response except bone response.

6.1134 Bone disease must be x-ray proven for the site to be evaluable.

6.1135 Chemical parameters and biologic markers may be measured during therapy, but will not be used to evaluate response.

6.2 Definitions of Response by Organ Site Involvement

6.21 Complete Response (CR)

Complete disappearance of all clinically detectable malignant disease for at least 4 weeks. A patient who has radiographic evidence of bony metastases prior to therapy has to have normalization of radiographs or complete sclerotic healing of lytic metastases in association with a normal bone scan. A patient with an abnormal bone scan and normal radiographs prior to therapy has to have normalization of the bone scan.

6.22 Partial Response (PR)

Greater than or equal to 50% decrease in tumor area for at least 4 weeks without increase in size of any area of known malignant disease of greater than 25% or appearance of new areas of malignant disease.

6.221 Measurable, Bidimensional

Greater than or equal to a 50% decrease in tumor area (multiplication of longest diameter by the greatest perpendicular diameter) or a 50% decrease in the sum of the products of the perpendicular diameters of multiple lesions in the same organ site for at least 4 weeks.

6.222 Measurable, Unidimensional

Greater than or equal to 30% decrease in linear tumor measurement for at least 4 weeks.

6.2221 Mediastinal and hilar width response may be determined by the formula:

A = On-study width
B = Normal width (Preinvolvement x-ray)
C = Width after treatment

$$\frac{- \quad - \quad -}{- \qquad} \geq$$

6.2222 Palpable masses that can be measured in only one dimension may be evaluated for response by using the formula:

A = On-study measurement
B = Measurement after treatment

$$\frac{-}{} \geq$$

6.223 Nonmeasurable, Evaluable

Definite improvement in evaluable malignant disease estimated to be in excess of 50% and **agreed upon by 2 independent investigators.**

6.2231 Serial evaluations of chest x-rays (i.e., confluent multinodular and lymphangitis metastases, malignant pleural effusions) and physical measurements (i.e., abdominal girth) should be documented in the records and by photograph when practical.

6.2232 The response should last for at least 4 weeks.

6.2233 A partial response of bony metastases occurs if there is a partial decrease in the size of lesions, blastic transformation of lytic lesions, or decreased density of blastic lesions, lasting for at least 4 weeks. This requires x-rays for documentation.

6.23 Stable (SD)

No significant change in measurable or evaluable disease for at least 4 weeks (greater than or equal to 12 weeks for bony metastases).

6.231 No increase in site of any known malignant disease.

6.232 No appearance of **new** areas of malignant disease.

6.233 This designation includes decrease in malignant disease of less than 50%, or decrease in unidimensional measurable disease of less than 30%, or increase in malignant disease of less than 25% in any site.

6.234 No deterioration in ECOG performance status of greater than or equal to 1 level related to malignant disease.

6.24 Progression (PD)

Significant increase in size of lesions present at the start of therapy or after a response, or appearance of new metastatic lesions **known not** to be present at the start of therapy or stable objective disease associated with a deterioration in ECOG performance status of greater than or equal to 1 level related to malignancy.

6.241 Measurable, Bidimensional, and Unidimensional

6.2411 Greater than or equal to 25% increase in the area of any malignant lesions greater than 2 cm^2 or in the sum of the products of the individual lesions in a given organ site (comparison of products of the longest diameter by the greatest perpendicular diameter).

6.2412 Greater than or equal to 50% increase in the size of the product of diameters if only one lesion is available for measurement and was less than or equal to 2 cm^2 in size at the initiation of therapy.

6.2413 Appearance of **new** malignant lesions.

6.242 Nonmeasurable, Evaluable

6.2421 Definite increase in the area of malignant lesions estimated to be greater than 25%.

6.2422 Appearance of **new** malignant lesions.

6.2423 Increase in size or number of bony metastases (pathologic fractures do not represent progression unless there is a documented increase in bony disease). During the first 12 weeks of treatment in patients with breast cancer there must be associated progression in another site and/or a worsening subjective status of the patient in order to qualify as progression.

6.243 Nonmeasurable, Nonevaluable

Definite evidence of **new** clinically detectable (physical or radiographic) malignant disease.

6.3 Evaluation of Patient's Total Response

6.31 Organ Site Evaluation

6.311 Record responses as complete (CR), partial (PR), stable (SD), progression (PD), or NED under appropriate methods of evaluation.

6.312 If more than one type of evaluation method exists for a given organ site, each must be recorded separately.

6.313 If there is more than one measurable lesion per organ site, an organ site PR occurs if there is a greater than 50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions.

6.314 Stabilization of evaluable disease will not detract from a PR of measurable disease by organ site, but will reduce a CR to a PR.

6.315 Progression in any classification of measurability or evaluability in an organ site shall prevail as the response for that organ site.

6.32 Objective Total Patient Response

6.321 Progression occurs if any previously measurable or evaluable malignant lesions fulfill progression criteria or new malignant lesions **not known** to be present at the start of therapy develop.

6.322 Organ site stabilizations will not detract from a total patient PR in the presence of other organ site PR's and CR's.

6.323 Stabilization of evaluable disease does not detract from CR's or PR's in measurable sites, but the patient's overall response should be a PR.

6.324 Patients with a deterioration in ECOG performance status of greater than or equal to 1 level related to malignant disease are considered to have progressed.

6.33 Onset of Response

The time between initiation of therapy and the onset of PR or CR.

6.34 Duration of Response

Time from onset of PR (even if patient later has a CR) until objective evidence of progression.

7.0 STUDY PARAMETERS

a. All pre-study scans and x-rays should be done ≤ 6 weeks before registration.

b. Scans or x-rays used to document measurable disease should be done within 2 weeks prior to registration.

c. Hgb, Hct, WBC, Plt but if abnormal, they must be repeated < 48 hours prior to registration. CBC with differential, should be done ≤ 2 weeks before registration.

d. All chemistries should be done ≤ 2 weeks before registration - unless specifically required on Day 1 as per protocol. If abnormal, they must be repeated within 48 hours prior to registration.

NOTE: When filling out these pre study results on the ECOG flow sheets, please make sure that ALL relevant dates are clearly given. Do NOT put all the results under the date for Day 1 of protocol treatment unless they were actually done that day. Record the actual dates.

For each cycle Hgb, Hct, WBC, Plt, these tests should be done within 48 hours of the day of treatment.

	Pre-treatment	Every Cycle	Every Other Cycle	Off treatment Every 2 months
Physical Examination	X	X		X
Tumor Measurements	X	X[6]		X
Performance Status	X	X		X
Height & Weight	X	X		X
WBC(diff), Hgb, Plt	X	X		X
Chest X-ray	X	X[1]		X
Serum creatinine	X	X		X
SGOT & Bilirubin	X	X		X
CT of Chest/Abdomen	X[2]		X[1]	X[1]
Bone Scan	X[4]			
X-rays of Bone Scan Lesions or Bone Survey	X[3]		X[3,5]	

1. If lesions or abnormalities persist.
2. If clinically indicated.
3. Required only if bone scan abnormal or known lesions are present.
4. Required only if no known osseous lesions are present, with follow-up scans after 3 cycles, then every 6 months.
5. After 3 cycles, then every 2 cycles if lesions present and only site of assessable disease. Every 3 cycles if lesions present but other sites of measurable disease available to assess response.
6. All areas of measurable disease will be measured monthly unless measurements require a CT scan. If the patient has only one site of measurable disease that is documented only by CT scan, then CT scans will be required after 3 cycles then every 2 cycles thereafter. If the patient has multiple sites of measurable disease including at least one site that is not measured by CT, then the sites not requiring CT imaging will be measured monthly and sites requiring CT imaging will be imaged by CT every 3 months.

8.0 DRUG FORMULATION AND PROCUREMENT

8.1 PSC-833, [3'-Keto-Bmt] - [Val2] - cycle sporin, IND #52,058, NSC # 648265.

8.11 Classification

Multidrug resistance (MDR) modulator, may also be referred to as an MDR reversing agent.

8.12 Dose Specifics

5 mg/kg, po every 6 hours X 12 doses

8.13 Mode of Action

PSC-833 has been shown to increase the anticancer drug concentration in cells that are defined as multidrug resistant. Cells defined as multidrug resistant characteristically overproduce a membrane glycoprotein called P-glycoprotein (P-gp) that appears to function as an energy-dependant drug-effiux pump. It is thought that MDR cells may be less susceptible to killing by anticancer drugs because of the ability of P-gp to result in intracellular drug concentrations below cytotoxic levels. If PSC-833 can inhibit the efflux of anticancer drugs by inhibiting P-gp it may result in increased cytotoxicity of cancer cells to drugs which are removed by P-gp.

8.14 Storage & Stability

PSC-833 oral solution is packaged in 50 ml bottles containing 5000 mg of PSC-833 at a concentration of 100 mg/ml. The manufacturer recommends that this product be stored at temperatures between 15-25° C.

8.15 Preparation

According to the Novartis Pharamaceutical Corporation, the preferred way for patients to administer the PSC-833 oral solution is to add the calculated dose to approximately 2 ounces of apple juice, Coca-Cola, orange juice, tomato juice or water and stir well. The diluted dose should be administered to the patient within 10 minutes of preparation. The medication should be taken on an empty stomach (1 hour before or two hours after meals). **Grapefruit juice should be avoided.**

8.16 Route of Administration

The oral solution should be administered on an empty stomach (1 hour before or 2 hours after a meal) within 10 minutes of dilution as described above.

8.17 Incompatibilities

The oral solution should not be diluted in grapefruit juice.

8.18 Availability

PSC-833 is being provided free of charge for this study by Novartis Pharmaceuticals Corporation and is available from Axion, Inc. PSC-833 is an INVESTIGATIONAL drug (IND #52,058). It is supplied as the Microemulsion Drink Solution in 50 ml bottles containing 5000 mg of PSC-833 at a concentration of 100 mg/ml.

Institutions may receive a starter supply of PSC-833 provided the appropriate paperwork has been received at the Coordinating Center. When a patient is randomized, the institution is responsible for reordering drug to maintain the starter supply. To obtain a starter supply Investigators must submit the following to the ECOG Coordinating Center, ATTN: DRUG ORDERS:

1. Signed and completed E1195 PSC-833 Drug Request Form (Appendix III).

2. The ECOG Coordinating Center will confirm a copy of the institution's current (<365 days) IRB approval letter for this protocol (HHS 310) is on file.

Any order received at the ECOG Coordinating Center between 8:00 am and 5:00 pm ET will be shipped that day provided the proper paperwork has been received. Orders received after 5:00 pm ET will be processed and shipped the following work day.

PSC-833 is labeled generically, there is no patient identification pre-printed on the label. Once a bottle is dispensed to a patient, the patient identification (patient's initials and E1195 sequence number) is written on the label. Dispensed medication cannot be returned to be re-dispensed to another patient. PSC-833 medication never dispensed can be used from one patient for another new patient randomized.

Note: There will be no weekend or holiday delivery of drugs.

After all patients at an institution have completed study treatment, unused drug must be returned to the following address:

Axion Healthcare, Inc.
1111 Bayhill
Suite 125
San Bruno, CA 94066

8.19 Side Effects

1. Neurological: reversible cerebellar-dysfunction has been the dose-limiting toxicity. Symptoms that have been described include ataxia, incoordination, unsteadiness, difficulty walking. These symptoms generally appear and are most intense within 1 to 3 hours after receiving the drug. Initial data suggests that these effects are completely reversible within 24 hours of discontinuing the drug. Other neurological side effects include dizziness, a "high" feeling, diplopia, and paresthesias affecting the lips, mouth and distal extremities, dysmetria, tingling, headache, hypoaesthesia.

2. Gastrointestinal: Nausea, vomiting and transient increases in AST, ALT and bilirubin have occurred frequently. These side effects have been reversible and not dose limiting. Constipation and esophagitis could also occur.

3. Cardiovascular: In one patient who developed the cerebellar side effects moderate hypertension was also documented. The hypertension recurred upon rechallenge but was reversible without any treatment.

4. Pulmonary: After intravenous (IV) administration a moderate feeling of suffocation associated with sternal pressure and an urge to cough has been reported. THESE EFFECTS HAVE NOT BEEN REPORTED TO OCCUR WITH ORAL FORMULATIONS.

5. Hematologic: Neutropenia could occur.

8.110 Nursing Implications

1. In order to assure optimal compliance it is important to carefully instruct the patient and/or family to correctly draw up, dilute and administer their dose according to protocol instructions.

2. Patients should be informed of the likelihood of the drug causing problems with balance and coordination. **Patients should be instructed not to drive or operate potentially dangerous machinery within 24 hours of the last PSC-833 dose. Patients should also be advised to avoid alcohol, sedatives or sleeping medications while taking PSC-833 as this could increase the chances of falling.**

3. PSC-833 is a derivative of the drug cyclosporin. Cyclosporin is known to interact with many other medications. Refer to Section 3.6 listing potentially significant drug interactions to assess the patient's risk for a drug interaction. While patients are taking PSC-833 they should be advised not to begin any additional medications without the knowledge of the study personnel.

8.111 References

1. SDZ PSC-833 Investigator's Brochure, September 1994.

8.2 Paclitaxel

8.21 Other Names

Taxol®, NSC 125973

8.22 Chemical Name

Tax-en-9-one, 5 beta, 20-epoxy-1,2 alpha, 4, 7 beta, 10-beta, 13-hexahydroxy-, 4, 10-diacetate-2-benzoate-13-(alpha-phenylhippurate), $C_{47}H_{51}NO_{14}$.

8.23 Classification

Antimicrotubule agent.

8.24 Mode of Action

Promotes microtubule assembly and stabilizes tubulin polymers by preventing their depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell function.

8.25 Storage and Stability

The intact ampules are stored under refrigeration. Freezing does not adversely affect the product. Solutions diluted to a concentration of 0.3 to 1.2 mg/ml in normal saline or 5% dextrose are stable for up to 27 hours when stored at room temperature and normal room light. Analyses of solutions filtered through IVEX-2 and IVEX-HP (Abbott) 0.2 micron filters showed no appreciable loss of potency.

8.26 Dose Specifics

Patients will receive 70 mg/m^2 IV by 3 hour infusion beginning 4 hours after the 5th dose of PSC-833.

8.27 Preparation

The concentrated solution must be diluted prior to use in normal saline, 5% dextrose, 5% dextrose and normal saline, or 5% dextrose in Ringer's solution to a concentration of 0.3 to 1.2 mg/ml. Solutions exhibit a slight haze, common to all products containing nonionic surfactants. Glass, polypropylene, or polyolefin containers and non-PVC-containing (nitroglycerin) infusion sets should be used. A small number of fibers (within acceptable limits established by the USP) have been observed after dilution. Therefore a hydrophilic 0.22 micron in-line filter shall be used. Analyses of solutions filtered through IVEX-2 and IVEX-HP (Abott) 0.2 micron filters showed no appreciable loss of potency. Solutions exhibiting excessive particulate formation should not be used.

8.28 Route of Administration

Administered as an intravenous infusion over 3 hours with a hydrophilic in-line 0.22 micron filter.

8.29 Incompatibilities

- Avoid the use of PVC bags and infusion sets, due to leaching of DEHP (plasticizer).

8.210 Availability

Paclitaxel is commercially available. A concentrated solution of 6mg/ml in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol 50% is commercially available in 5 ml ampules.

8.211 Side Effects

1. Hematologic: Myelosuppression (neutropenia, leukopenia, thrombocytopenia, anemia.

2. Hypersensitivity: Thought to be caused by the Cremophor vehicle. Minor symptoms include hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, and tachycardia. More severe reactions include hypotension requiring treatment, dyspnea with bronchospasm, generalized urticaria (acute), anaphylactoid reaction, and angioedema. The majority (53%) of the reported reactions occurred within 2-3 minutes of initiation of treatment and 78% occurred within the first 10 minutes. Reactions usually occurred with the first and second doses.

3. Cardiovascular: Arrhythmia, atrial arrhythmia (sinus bradycardia [usually transient and asymptomatic], sinus tachycardia, and premature beats); significant events include syncope, hypotension, other rhythm abnormalities (including ventricular tachycardia, bigeminy, and complete heart block requiring pacemaker placement), and myocardial infarction. Hypertension, possibly related to concomitant administration of dexamethasone, may also occur.

4. Neurologic: Sensory changes (taste changes); peripheral neuropathy; arthralgia and myalgia (dose-related, more common when colony-stimulating factors are also administered); seizures; mood alterations; neuroencephalopathy; hepatic encephalopathy; motor neuropathy; and autonomic neuropathy (paralytic ileus and symptomatic hypotension).

6. Dermatologic: Alopecia, universal, complete, and often sudden, between days 14-21; injection site reactions (erythema, induration, tenderness, skin discoloration); infiltration (phlebitis, cellulitis, ulceration, and necrosis, rare); radiation recall; and rash; nail changes (discoloration of fingernails, separation from nail bed).

7. Gastrointestinal: Nausea, vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis (neutropenic enterocolitis), ischemic colitis, and pancreatitis.

8. Hepatic: Increased SGOT (AST), SGPT (ALT), bilirubin, alkaline phosphatase; hepatic failure, and hepatic necrosis.

9. Other: Pneumonitis, fatigue, headaches, light-headedness, myopathy, elevated serum creatinine, elevated serum triglycerides, and visual abnormalities (sensation of flashing lights, blurred vision, scintillating scotomata).

8.212 Nursing Implications

1. Monitor CBC and platelet count prior to drug administration.

2. Symptom management of expected nausea, vomiting, and stomatitis.

3. Monitor for and evaluate abdominal pain occurring after paclitaxel administration (especially in severely neutropenic patients and in those receiving G-CSF) due to the risk of ischemic and neutropenic enterocolitis.

4. Advise patients of possible hair loss.

5. Cardiac monitoring for assessment of arrhythmias in patients with serious conduction abnormalities.

6. Monitor liver function tests.

7. Advise patient of possible arthralgias and myalgias which may occur several days after treatment. Monitor for symptoms of peripheral neuropathy.

8. Monitor for signs and symptoms of hypersensitivity reactions. Insure that the recommended premedications have been given. Premedications (diphenhydramine, steroids, and H2 blocker) appear to reduce the incidence and severity of hypersensitivity reactions but do not provide complete protection. Emergency agents (diphenhydramine and epinephrine) should be available.

9. Evaluate IV site regularly for signs of infiltration. It is not known if taxol is a vesicant; however, the Cremophor vehicle for this drug can cause tissue damage.

10. In-line filtration with a 0.22 micron filter should be used.

8.212 References

1. Rowinsky EK, Casenave LA, Donehower RC. Taxol: A novel investigational microtubule agent. J Natl Cancer Inst 82:1247-1259, 1990.

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4. Walker FE. Paclitaxel: Side effects and patient education issues. Semin Oncol Nurs 9(suppl 2):6-10, 1993.

9.0 STATISTICAL CONSIDERATIONS

This study seeks to assess the efficacy of paclitaxel plus PSC-833 in patients with anthracycline resistant metastatic breast cancer. Paclitaxel alone, as a single agent, is reported to be associated with a 56% objective response rate with patients who have received one prior chemotherapy for their breast cancer. Our hypothesis is that in patients with anthracycline resistant disease, the addition of PSC-833 can permit that same single agent response rate to be achieved in these anthracycline-resistant patients.

We propose a two stage design for this trial. In the first stage, 17 patients would be entered on study with the expectation that 15 would be eligible. If six or more objective responses are observed among these 15 patients, an additional 15 patients would be accrued to the study. The treatment would be considered worthy of further investigation if at least 14 of these 30 patients exhibit objective responses to therapy. If the true objective response rate for paclitaxel plus PSC-833 in this patient population is indeed 55%, the probability that the study will stop early is 0.08, and the overall probability of concluding that this regimen merits further study, or the power to detect a 55% response rate, is 0.84. If on the other hand, the true response rate to paclitaxel plus PSC-833 is 40%, the probability of stopping the study early is 0.40, and the probability of incorrectly concluding that the regimen should receive further study is 0.26.

We anticipate that a total of 34 patients could be accrued to this protocol by ECOG within 24 months.

10.0 RECORDS TO BE KEPT

The following forms must be submitted to the ECOG Coordinating Center, Frontier Science, 303 Boylston Street, Brookline, MA 02146 (ATTN: DATA).

Form	To Be Submitted
* BIG Form 2 Intergroup Advanced Breast Cancer Pre-Study	Within one week of registration
ECOG Breast CTC Flow Sheet (478RB)	Every month while on treatment
BIG Form 7 Breast Measurement Form	Every month while on treatment
ECOG Follow-Up Form (464R) Parts A, B, C, D, E * Parts A, B	Every month while on study treatment and at completion of treatment (Off Treatment): • every 3 months if patient is <2 years from study entry • every 6 months if patient is 2-5 years from study entry • every 12 months if patient is > 5 years from study
ECOG Metastatic Breast Supplement Response Form (#553B) (To be submitted for each disease site.)	Every month while on treatment
Adverse Reaction (ADR) Form for Investigational Drugs (#391RF)	Within 10 days of reportable event as defined in Section 5.2.
NCI/CTEP Secondary AML/MDS Report Form	Within 30 days of diagnosis of AML/MDS as described in Section 5.22.
ECOG Second Primary Cancer Form (#630)	Within 30 days of diagnosis of new primary cancer as described in Section 5.22.

* These forms are to be submitted for all canceled patients according to the above schedule.

11.0 PATIENT CONSENT AND PEER JUDGMENT

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

12.0 REFERENCES

1. Pastan I, Gottesman M. Multiple-drug resistance in human cancer. New England Journal of Medicine 1987;316:1388-1393.

2. Goldstein LJ; Pastan I; Gottesman MM. Multidrug resistance in human cancer. Critical Reviews in Oncology/Hematology, 1992, 12(3):243-53.

3. Sikic BI. Modulation of multidrug resistance: at the threshold [editorial; comment]. Journal of Clinical Oncology, 1993 Sep, 11(9):1629-35.
4. Goldstein LJ, Galski H, Fojo A, *et al.* Expression of a multidrug resistance gene in human cancers. Journal of the National Cancer Institute 1989;81:1116-1124.

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11. Holmes FA, Valero V, Walters RS, *et al.* The M.D. Anderson Cancer Center experience with Taxol in metastatic breast cancer. Monographs / National Cancer Institute 1993;15:161-169.

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APPENDIX I

Are you participating in any other research studies? ____yes ____no

EXPERIMENTAL SUBJECTS BILL OF RIGHTS

Persons who participate in a medical experiment are entitled to certain rights. These rights include but are not limited to the subject's right to:

- **be informed of the nature and purpose of the experiment;**
- **be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;**
- **be given a description of any attendant discomforts and risks reasonably to be expected;**
- **be given an explanation of any benefits to the subject reasonably to be expected, if applicable;**
- **be given a disclosure of any appropriate alternatives, drugs, or devices that might be advantageous to the subject, their relative risks and benefits;**
- **be informed of the avenues of medical treatment, if any, available to the subject after the experiment if complications should arise;**
- **be given an opportunity to ask questions concerning the experiment or the procedures involved;**
- **be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation without prejudice;**
- **be given a copy of the signed and dated consent form;**
- **and be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion, or undue influence on the subject's decision.**

Suggested Patient Consent Form

Research Study

I, _____, willingly agree to participate in the study which has been explained to you by Dr. _____. This research study is being conducted by the Eastern Cooperative Oncology Group and by _____.
(Institution)

Purpose of the Study

It has been explained to you that you have advanced breast cancer which is not controllable with surgery or radiation therapy, and your physician has decided that chemotherapy is the best treatment option available. You have been invited to participate in this research study. The study involves treatment with a chemotherapy drug (paclitaxel, also known as Taxol®), administered together with an experimental drug, PSC-833. Because the type of cancer you have can become resistant to chemotherapy, and PSC-833 is a drug designed to enhance the antitumor effects of chemotherapy, this study is to evaluate how well your disease responds to paclitaxel and PSC-833, and to learn what side effects and benefits are produced. If you decide to participate, Dr. _____ will discuss the study protocol, the rationale, and the plan of therapy with you in detail. Certain medications are not permitted to be used during the study, and we will discuss these before starting the study.

Description of Procedures

Prior to entering the study, you will undergo a screening examination that will include a complete medical history, and physical examination (including blood pressure, pulse, temperature, height and weight). A chest x-ray, a bone scan, laboratory blood tests, and a pregnancy test (if you are of childbearing potential) will also be performed. You will also undergo tumor measurement(s).

After you have been registered to the study, you and/or your family member(s) will be instructed on how to correctly dilute and administer each dose of PSC-833. (PSC-833 is a liquid taken every six hours by mouth, on an empty stomach for a total of 12 doses). After your 5th dose of PSC-833, you will begin chemotherapy treatment with paclitaxel.

Approximately 14 hours and 7 hours prior to the paclitaxel administration, you will take dexamethasone orally. To give the paclitaxel, an intravenous line (an "IV") will be placed in your vein. Thirty to sixty minutes prior to the paclitaxel administration, you will be medicated with diphenhydramine and cimetidine through your IV These three medications are used to reduce or prevent side effects which the chemotherapy can cause, including nausea, vomiting and possible allergic reactions to the chemotherapy. Thirty to sixty minutes later, you will receive Paclitaxel through the IV, administered over a three hour period. Blood pressure and heart rate will be monitored during the infusion.

Treatment will be repeated every three weeks as long as your response rates (determined by blood test, tumor measurements, physical exams [including height, weight, blood pressure, pulse and temperature], X-rays and/or bone scans) are within acceptable limits. If your tumor is shrinking, you will continue the treatments on an ongoing basis. If the tumor is growing despite the treatments, you will discontinue the study, as it is not helping you. Your doctor will discuss alternative treatments with you including other possible research treatments. If the tumor remains stable (unchanged) your doctor will discuss the risks and benefits of continuing treatment of the study, and you will decide together how to proceed.

Risks and Discomforts

Medications often have side effects. The drugs used in this treatment may cause all, some, or none of the side effects listed. In addition, there is always the risk of very uncommon or previously unknown side effects occurring. PSC-833 may cause reversible numbness of the lips, tongue and fingers, and some dizziness and lightheadedness may occur. You could experience some unsteadiness when walking. There can be the urge to cough or feeling of suffocation. Although this has not been reported with the oral form of this medication, this side effect disappears as soon as the PSC-833 is out of the system, approximately 6 to 12 hours after the last dose. A reversible, temporary rise in liver function tests, including the alkaline phosphatase, SGOT, SGPT, and bilirubin, may occur. Occasionally, patients have had a mild to moderate elevation in their blood pressure during the PSC-833 dose administration. This also returns to normal several hours after the last PSC833 dose. Your condition will be monitored closely and appropriate changes in therapy will occur, if necessary.

Your doctor has instructed you not to drive or operate potentially dangerous machinery within 24 hours of the last PSC-833 dose. You have also been advised to avoid alcohol, sedatives or sleeping medications while taking PSC-833 as this could increase your chances of falling. You and/or your family members have been instructed not to mix PSC-833 with grapefruit juice.

Paclitaxel may cause a lowering of your blood cell counts that could cause infection, bleeding or anemia. It can also cause a mild to severe allergic reaction characterized by a skin rash, itching, flushing, chest pain, a rapid heart rate, difficulty breathing, and low blood pressure. This allergic reaction is thought to be caused by Cremophor, a necessary part of the paclitaxel drug mixture, rather than by the paclitaxel itself. This reaction can be reversed with medication. Before treatment with paclitaxel begins, you will be given medication to try to prevent this type of reaction, and you will be closely watched for such a reaction. Occasionally, paclitaxel can cause a slowing of the heart rate, but this usually does not cause symptoms in patients. Rarely, paclitaxel can cause fainting, an irregular heart beat (arrhythmia), other rhythm changes (rapid heart rate, heart block, etc.), or heart attack. These can be life-threatening side effects; however, you will be given medication to prevent these and watched closely for such reactions. Patients being treated with paclitaxel may also experience neurologic symptoms such as confusion; mood changes; seizures (rare); paralysis of the bowel, leading to constipation (rare); and numbness and pain of the hands and feet that sometimes worsens with additional treatment and may not disappear after the drug is stopped. Redness, pain, tenderness, tissue firmness, skin discoloration, and a rash can develop at the place where the drug is put into your vein if the drug leaks into the surrounding skin; sores may also develop at the site, but this is rare. Rarely, a skin rash may also develop at areas where patients have received previous radiation treatments. Other possible side effects include a change in your sense of taste, nausea, vomiting, diarrhea, sores in the mouth or throat, sore throat, fatigue, muscle weakness, muscle and joint aches, hair loss, lightheadedness, headaches, high blood pressure, blurred vision, or a sensation of flashing lights. Paclitaxel can also cause changes in kidney function tests, an increase in triglyceride (blood lipid) levels, changes in liver enzymes, and, rarely, liver damage or liver failure. Other rare side effects include crampy stomach pain; inflammation, infection, or loss of blood supply to the intestines – which may require surgery; and inflammation of the pancreas.

Your physician will be checking you closely to see if any of these side effects are occurring. Routine blood and urine tests will be done to monitor the effects of treatment. Many side effects disappear after the treatment is stopped. In the meantime, your doctor may prescribe medication to keep these side effects under control. Schedules and dosages may be altered to reduce the side effects. The use of medications to control side effects could result in added costs. This institution is not financially responsible for the treatment of side effects caused by the study drugs.

Additional side effects of this treatment may include complications from drawing blood, such as pain, discomfort, and bleeding and/or bruising at the puncture site or complications from the intravenous catheter that may result in infection or bleeding. There is some potential health risk of radiation exposure from x-rays; however, the risk is considered small.

Contact Persons

In the event that physical injury occurs as a result of this research, facilities for treatment of injury will be available. You understand, however, you will not automatically be provided with reimbursement for medical care or receive other compensation. For more information concerning the research and research-related risks or injuries, you can contact Dr. _____, the investigator in charge, at _____.
(Telephone)

In addition, you may contact _____ at _____for
(Institution)

information regarding patients' rights in research studies.

Benefits

It is not possible to predict whether or not any personal benefit will result. Possible benefits are remission of the disease and prolonged survival. It is possible that the PSC-833 may prove to be less effective than the standard regimen. If you receive treatment with the experimental drug and do not show any benefit from the treatment, you will receive treatment that has previously showed to be effective. You have been told that, should your disease become worse, should side effects become very severe, should new scientific developments occur that indicate the treatment is not in your best interest, or should your physician feel that this treatment is no longer in your best interest, the treatment will be stopped. Further treatment will be discussed.

Alternatives

Alternative treatments which could be considered in your case include standard treatment appropriate for your breast cancer. Standard treatment might utilize chemotherapy drugs, hormonal therapy, or radiation therapy (in a few special circumstances) approved for the treatment of breast cancer. An additional alternative is no further therapy. Your doctor has provided detailed information about your disease and the benefits of the various treatments available. You have been told that you should feel free to discuss your disease and prognosis with the doctor.

The physician involved in your care will be available to answer any questions you have concerning this program. In addition, you will be advised of the procedures related solely to research which would not otherwise be necessary. Some of these procedures may result in added costs and some of these costs may be covered by your insurance.

Voluntary Participation

Participation in this study is voluntary. No compensation for participation will be given. You understand that you are free to withdraw your consent to participate in this treatment program any time without prejudice to your subsequent care. Refusal to participate will involve no penalty, or loss of benefits. You are free to seek care from a physician of your choice at any time. If you do not take part in or withdraw from the study, you will continue to receive care. In the event that you withdraw from the study, you will continue to be followed and clinical data will continue to be collected from your medical records.

Confidentiality

You understand that a record of your progress will be kept in a confidential form at _____
(Institution)

and also in a computer file at the statistical headquarters of the Eastern Cooperative Oncology Group. The confidentiality of the central computer record is carefully guarded. During the required reviews, representatives of the Food and Drug Administration (FDA), the National Cancer Institute (NCI), sponsoring

agencies and their designees may have access to medical records which contain your identity. However, no information by which you can be identified will be released or published. Histopathologic material, including slides, may be sent to a central office for review.

Compensation

No payment will be provided to you for participation in this study. The costs of treatments and exams will be charged to your regular medical insurance.

****

You have read all of the above, asked questions, received answers concerning areas you did not understand and you willingly give your consent to participate in this program. Upon signing this form, you will receive a copy.

(Patient Signature)	(Date)
(Witness Signature)	(Date)
(Physician Signature)	(Date)

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APPENDIX II

COMMON TOXICITY CRITERIA

		0	1	2	3	4
Leukopenia	WBC x 10^3 Granulocytes/Bands Lymphocytes	≥4.0 ≥2.0 ≥2.0	3.0 - 3.9 1.5 - 1.9 1.5 - 1.9	2.0 - 2.9 1.0 - 1.4 1.0 - 1.4	1.0 - 1.9 0.5 - 0.9 0.5 - 0.9	<1.0 <0.5 <0.5
Thrombocyto-penia	Plt x 10^3	WNL	75.0 - normal	50.0 - 74.9	25.0 - 49.9	<25.0
Anemia	Hgb	WNL	10.0 - normal	8.0 - 10.0	6.5 - 7.9	<6.5
Hemorrhage (Clinical)	-----	none	mild, no transfusion	gross, 1-2 units transfusion/episode	gross, 3-4 units transfusion/episode	massive, >4 units transfusion/episode
*Infection	-----	none	mild, no active Rx	Moderate, localized infection requires active Rx	severe, systemic infection requires active Rx, specify site	life-threatening, sepsis, specify site
Fever in absence of infection	-----	none	37.1° - 38.0° C 98.7 °- 100.4° F	38.1 ° - 40.0° C 100.5 ° - 104.0° F	>40.0° C (>104.0° F) for less than 24 hours	>40.0° C (104.0° F) for >24 hrs or fever with hypotension
	• Fever felt to be caused by drug allergy should be coded as allergy. • Fever due to infection is coded under infection only.					
GU	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 <0.3g% or <3g/l	2-3+ or 0.3 - 1.0g% or 3 - 10g/l	4+ or >1.0g% or >10g/l	nephrotic syndrome
	Hematuria	neg	micro only	gross, no clots	gross + clots	requires transfusion
	*BUN	<1.5 x N	1.5 - 2.5 x N	2.6 - 5 x N	5.1 - 10 x N	>10 x N
	• Urinary tract infection should be coded under infection, not GU. • Hematuria resulting from thrombocytopenia should be coded under hemorrhage, not GU.					
GI	Nausea	none	able to eat reasonable intake	intake significantly decreased but can eat	no significant intake	-----
	Vomiting	none	1 episode in 24 hours	2-5 episodes in 24 hours	6-10 episodes in 24 hours	>10 episodes in 24 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	requires 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
	• Viral Hepatitis should be coded as infection rather than liver toxicity.					
Pulmonary	-----	none or no change	asymptomatic, with abnormality in PFTs	dyspnea on significant exertion	dyspnea at normal level of activity	dyspnea at rest
	• Pneumonia is considered infection and not graded as pulmonary toxicity unless felt to be resultant from pulmonary changes directly induced by treatment.					
Cardiac	Cardiac dysrhythmias	none	asymptomatic, transient, requiring no therapy	recurrent or persistent, no therapy required	requires treatment	requires monitoring, or hypotension or ventricular tachycardia or fibrillation
	Cardiac function	none	asymptomatic, decline of resting ejection fraction by less than 20% of baseline value	asymptomatic, decline of resting ejection fraction by more than 20% of baseline value	mild CHF, responsive to therapy	severe or refractory CHF
	Cardiac–ischemia	none	non-specific T-wave flattening	asymptomatic, ST and T wave changes suggesting ischemia	angina without evidence for infarction	acute myocardial infarction
	Cardiac–pericardial	none	asymptomatic effusion, no intervention required	pericarditis (rub, chest pain, ECG changes)	symptomatic effusion; drainage required	tamponade; drainage urgently required

			0	1	2	3	4
Blood Pressure		Hypertension	none or no change	asymptomatic, transient increase by >20 mm Hg (D) or to >150/100 if previously WNL. No treatment required	recurrent or persistent increase by >20 mm Hg (D) or to >150/100 if previously WNL. No treatment required	requires therapy	hypertensive crisis
		Hypotension	none or no change	changes requiring no therapy (including transient orthostatic hypotension)	requires fluid replacement or other therapy but not hospitalization	requires therapy and hospitalization; resolves within 48 hours of stopping the agent	requires therapy and hospitalization for >48 hours after stopping the agent
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 <38° C, 100.4° F	urticaria, drug fever ≥ 38°C, 100.4°F, mild bronchospasm	serum sickness, bronchospasm, requires parenteral meds	anaphylaxis
*Phlebitis			none	arm	thrombophlebitis, leg	hospitalization	embolus
Local			none	pain	pain and swelling, with inflammation or phlebitis	ulceration	plastic surgery indicated
Alopecia		-----	no loss	mild hair loss	pronounced or total hair loss	-----	-----
Weight gain/loss		-----	<5.0%	5.0 - 9.9%	10.0 - 19.9%	≥20%	-----
NEUROLOGIC	Sensory	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	-----
		neuro – vision	none or no change	-----	-----	symptomatic subtotal loss of vision	blindness
		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
	Motor	neuro – motor	none or no change	subjective weakness; no objective findings	mild objective weakness without significant impairment of function	objective weakness with impairment of function	paralysis
		neuro – constipation	none or no change	mild	moderate	severe	ileus >96 hours
	Psych	neuro – mood	no change	mild anxiety or depression	moderate anxiety or depression	severe anxiety or depression	suicidal ideation
	Clinical	neuro – cortical	none	mild somnolence or agitation	moderate somnolence or agitation	severe somnolence, agitation, confusion, disorientation or hallucinations	coma, seizures, toxic psychosis
		neuro – cerebellar	none	slight incoordination, dysdiadockinesis	intention tremor, dysmetria, slurred speech, nystagmus	locomotor ataxia	cerebellar necrosis
		neuro – headache	none	mild	moderate or severe but transient	unrelenting and severe	-----
Metabolic		Hyperglycemia	<116	116 - 160	161 - 250	251 - 500	>500 or ketoacidosis
		Hypoglycemia	>64	55 - 64	40 - 54	30 - 39	<30
		Amylase	WNL	<1.5 x N	1.5 - 2.0 x N	2.1 - 5.0 x N	>5.1 x N
		Hypercalcemia	<10.6	10.6 - 11.5	11.6 - 12.5	12.6 - 13.5	≥13.5
		Hypocalcemia	>8.4	8.4 - 7.8	7.7 - 7.0	6.9 - 6.1	≤6.0
		Hypomagnesemia	>1.4	1.4 - 1.2	1.1 - 0.9	0.8 - 0.6	≤0.5
Coagulation		Fibrinogen	WNL	0.99 - 0.75 x N	0.74 - 0.50 x N	0.49 - 0.25 x N	≤0.24 x N
		Prothrombin time	WNL	1.01 - 1.25 x N	1.26 - 1.50 x N	1.51 - 2.00 x N	>2.00 x N
		Partial thromboplastin time	WNL	1.01 - 1.66 x N	1.67 - 2.33 x N	2.34 - 3.00 x N	>3.00 x N

* denotes ECOG specific criteria

A Pilot Phase II Trial of PSC-833 Modulation of Multidrug Resistance to Paclitaxel in the Treatment of Metastatic Carcinoma of the Breast

Appendix III

E1195 PSC-833 Drug Request Form

Requested By:

Dr. _____ Signature _____

Institution _____ Current IRB Approval Date _____

Principal Investigator _____ NCI Investigator # _____

Institution Contact _____

Telephone _____ Fax _____

Ship Drug To:

Name _____

Address:* _____

_____

_____

_____

*** Please do not use PO Box numbers**

ECOG Patient Seq. #	Starter Supply	Re-Order Supply	Date Drug Needed By

ECOG Coordinating Center use only:

_____

Authorized Signature Date

Return Completed, Signed and Dated form to:

ECOG Coordinating Center
Frontier Science
303 Boylston Street
Brookline, MA 02146-7648
Telephone: (617) 632-3610
FAX: (617) 632-2990

Reminder:

See protocol section 8.19 for required documentation that must be submitted to the ECOG Coordinating Center prior to requesting drug.

This eligibility check list should be completed by the institution prior to calling the ECOG Randomization Office to register a patient. All questions will be asked by the randomization specialist at the time of the call. Ineligible entries will not be allowed.

5. Enter patient's name.

enter last name: _____
enter first name: _____

8. NOTE: This study has limited institution participation. The following institutions can participate:

Breast Cancer Working Group
(Main Institutions and Affiliates)
Albert Einstein Cancer Center (NY)
Indiana University Medical Center
Fairfax Hospital
Fox Chase Cancer Center
University of Pennsylvania
Rush Presbyterian, St. Luke's Medical Center
University of Wisconsin
Stanford University

10. Does the patient have a histologically confirmed adenocarcinoma of the breast with bi-dimensionally measurable recurrent or metastatic carcinoma of the breast?

1. No [INELIGIBLE]
2. Yes [next question]
3. Unknown [INELIGIBLE]

20. Is the patient female?

1. No [INELIGIBLE]
2. Yes [next question]
3. Unknown [INELIGIBLE]

30. Has the patient received prior anthracycline therapy?

1. No [next question]
2. Yes [question 50]
3. Unknown [INELIGIBLE]

40. Does the patient have a medical contraindication to anthracycline therapy?

1. No [INELIGIBLE]
2. Yes [next question]
3. Unknown [INELIGIBLE]

50. Has the patient received prior paclitaxel or taxotere?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

55. Has the patient had more than one prior chemotherapy regimen in the treatment of their recurrent or metastatic carcinoma of the breast?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

60. Note: If patient has had prior adjuvant chemotherapy within 6 months of diagnosis of metastatic disease this will be considered to be chemotherapy for advanced disease. Prior hormonal therapy in either a metastatic or adjuvant setting is allowed.

61. Has the patient had prior adjuvant chemotherapy within 6 months of diagnosis of metastases?

1. No [question 70]
2. Yes [next question]
3. Unknown [INELIGIBLE]

62. In addition to this adjuvant chemotherapy, did patient have any other chemotherapy regimens for advanced disease?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

70. Drugs Increasing the Serum Concentration of CSA:

Calcium channel blockers: Diltiazem, Nicardipine, Verapamil Antifungals: Fluconazole, Itraconazole, Ketoconazole Antibiotics: Clarithromycin, Erythromycin Glucocorticoids: Methylprednisolone Other Drugs: Allopurinol, Bromocriptine, Danazol, Metoclopramide

Drugs Decreasing the Serum Concentration of CSA:

Antibiotics: Nafcillin, Rifampin Anticonvulsants: Carbamazepine, Phenobarbital, Phenytoin Other drugs: Octreotide, Ticlopidine

80. Is the patient receiving concurrent treatment with agents well substantiated to increase or decrease the blood concentrations of cyclosporin A?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

90. What is the patient's current ECOG performance status?

0: Patient fully active, able to carry on all pre-disease performance without normal functions restrictions.
1: Patient restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature.
2: Patient ambulatory and capable of all self care, but unable to work or carry any work activities, up and about more than 50% of waking hours.
3: Patient capable of only limited self care, or confined to bed or chair more than 50% of waking hours.
4: Patient completely disabled, unable to carry on any self care or totally confined to bed or chair.

enter value: _____

-equal to 0, next question.
-equal to 1, next question.
-equal to 2, next question.
-equal to 3, INELIGIBLE
-equal to 4, INELIGIBLE
-unknown , INELIGIBLE

100. Note all hematologic values and chemistries must have been obtained within the past 14 days.

What was the earliest date that any of the hematologic values were obtained?

enter date (mm/dd/yy): _____

105. Calculate: the number of days from the value of question 100 to TODAY

enter result: _____

-less than 0, INELIGIBLE
-less than or equal to 14, next question.
-greater than 14, INELIGIBLE
-unknown , INELIGIBLE

110. What is the patient's granulocyte count (mm3)?

enter value: _____

-less than 1500, INELIGIBLE
-greater than or equal to 1500, next question.
-unknown , INELIGIBLE

120. What is the patient's platelet count (mm3)?

enter value: _____

-less than 100000, INELIGIBLE
-greater than or equal to 100000, next question.
-unknown , INELIGIBLE

130. What was the earliest date that any of the serum chemistries were obtained?

enter date (mm/dd/yy): _____

135. Calculate: the number of days from the value of question 130 to TODAY

enter result: _____

-less than 0, INELIGIBLE
-less than or equal to 14, next question.
-greater than 14, INELIGIBLE
-unknown , INELIGIBLE

140. What is the institution's upper limit of normal value for SGOT?

enter value: _____

-greater than 0, next question.
-unknown , INELIGIBLE

150. What is the patient's current SGOT?
enter value: _____

-less than or equal to 0, INELIGIBLE
-less than or equal to the value of question 140 multiplied by 2.5, next question.
-greater than the value of question 140 multiplied by 2.5, INELIGIBLE
-unknown , INELIGIBLE

160. What is the patient's total bilirubin (mg/dl)?
enter value: _____

-less than 0, INELIGIBLE
-less than or equal to 1.5, next question.
-greater than 1.5, INELIGIBLE
-unknown , INELIGIBLE.

170. What is the institution's upper limit of normal for serum creatinine?
enter value: _____

-greater than 0, next question.
-unknown , INELIGIBLE

180. What is the patient's serum creatinine?
enter value: _____

-less than 0, INELIGIBLE
-less than or equal to the value of question 170 multiplied by 2, next question.
-greater than the value of question 170 multiplied by 2, INELIGIBLE
-unknown , INELIGIBLE

190. Does the patient have any central nervous system metastasis?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

200. Has the patient had chemotherapy or hormonal therapy?

1. No [question 220]
2. Yes [next question]
3. Unknown [INELIGIBLE]

210. What date was this therapy last adminstered?

enter date (mm/dd/yy): _____

215. Calculate: the number of days from the value of question 210 to TODAY

enter result: _____

-less than 21, INELIGIBLE
-greater than or equal to 21, next question.
-unknown , INELIGIBLE

220. Is the patient pregnant or lactating?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

230. Has the patient been advised that women of childbearing potential should use an accepted and effective method of contraception?

1. No [INELIGIBLE]
2. Yes [next question]
3. Not applicable [next question]
4. Unknown [INELIGIBLE]

240. Has the patient had an active malignancy other than breast cancer, in situ carcinoma of the cervix, or non-melanomatous skin cancers in the past 5 years?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

250. Does the patient have any active, unresolved infection?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

260. Has the patient had treatment with parenteral antibiotics for an established or presumed infection </= 7 days prior to study entry?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

270. Does the patient have a prior history of allergic reactions to cyclosporine or drugs utilizing the vehicle Cremophor (some anesthetics and muscle relaxants)?

1. No [next question]
2. Yes [INELIGIBLE]
3. Unknown [INELIGIBLE]

280. Has the patient given signed written informed consent?

1. No [INELIGIBLE]
2. Yes [ELIGIBLE]
3. Unknown [INELIGIBLE]

Demographic Data Required For Patient Registration:

Patient's Sex (m/f) ____ Birthdate (mm/yy) _____

Patient's Race _____ Zip Code _____

Patient's Hospital ID _____

Patient's Social Security Number _____

Method of Payment _____

Attending Physician _____

Institution Contact _____

Registrar _____

Additional Comments _____

_____

_____

signed_____ date_____
physician's signature (if required by the institution)