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FOREWORD

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Lawrence Bernstein
Principal Investigator's Signature

Date

8/24/94

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TABLE OF CONTENTS

	<u>PAGE #</u>
Front Cover	1
SF 29 8-Report Documentation Page	2
Foreword	3
Table of Contents	4
Introduction	5
Body	5
Conclusion	15
References	17
Figures & Tables	18-24
Appendix	25

INTRODUCTION

Drug resistance is a major obstacle in the treatment of cancer. The multidrug resistance gene (MDR1) encodes an energy dependent drug efflux pump, P170, 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, 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 recent data from the *in vivo* transgenic mouse model suggests that combining MDR modulators such as cyclosporine and quinine, may have an advantage over either alone. 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.

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, 1994:

1. Personnel

- a) Scientific Technician - Upon receiving the support from the DOD, advertisements were placed for a Scientific Technician both intramurally and extramurally. Interviews were conducted after selecting applicants from their submitted curriculum vitae and letters of recommendation; Ken Geles, BS was selected for this position on the basis of his academic achievements including an undergraduate Honors Thesis and his laboratory experience. Upon receiving his B.S. degree in May, 1993 and completion of his Honors Thesis in June, 1993, he began work in our laboratory at Fox Chase Cancer Center (FCCC) in July, 1993.
- b) Post-Doctoral Associate - Upon receiving support from the DOD, advertisements were placed in the key scientific journals to recruit a qualified Post-Doctoral Associate. Approximately 80 applications were initially received from which approximately 6 applicants met the skill requirements for this position. After interviewing these candidates, none were optimal for the position. Therefore another advertisement was placed in September, 1993 and another round of interviews resulted in an excellent candidate for this position, Dwayne Dexter, Ph.D. Dr. Dexter received his Ph.D. from Catholic University where he defended his thesis entitled "Identification and Characterization of a Novel Gene Affecting The Transcript Level of an ATP-Binding Cassette Transporter Gene, PDRS, in *Saccharomyces Cerevisiae*" in December, 1993 and began work in our laboratory in January, 1994. Dr. Dexter possesses the academic background and molecular skills necessary to accomplish our tasks.
- c) Research Fellow - Because of the unique setting of Fox Chase Cancer Center and its mission to promote clinical - laboratory studies we support a strong Medical Oncology Fellowship Program where medical trainees spend 18 months acquiring clinical oncology skills and 18 months doing laboratory research. We were fortunate to draw the interest of one of our brightest Fellows, Jack Leighton, M.D., who began work in our laboratory in January, 1994. Dr. Leighton's expertise nicely complements the scientific background of Dr. Dexter and Ken Geles.

2. Space and Facilities

With the assistance of the personnel above, our 500 sq. ft. laboratory space has been fully equipped over the past year to perform the molecular experiment described in our original proposal. Specifically, we have purchased a -70° freezer, DNA thermal cycles, hybridization chamber and a personal computer. In addition, we have supplied the laboratory with all appropriate centrifuges, pipettes, power sources, electrophoretic equipment, etc, all necessary to perform our experiments. We have utilized the FCCC core facilities including the instrumentation shop, tissue culture facility and oligonucleotide synthesis facility. All equipment and supplies have been checked for quality assurance and reproducibility.

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 are still in the process of establishing reproducible, quality controlled experiments with regard to cell culture, RNA isolation, quality control of our various cDNA probes, reverse transcription -PCR (RT-PCR) and our competitive template for the MDR gene to be used as a control for our RT-PCR experiments. In addition, the laboratory staff is still being evaluated for proper handling of tissue specimens and RNA isolation by doing experiments of tumor bank specimens looking for MDR1 gene expression.

4. Methods

- a) RNA Isolation - Cellular lysis and RNA extraction were accomplished using a modified one-step procedure of Chomczynski and Sacchi (1987). Approximately 100mg 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 1.5ml of lysis solution (2 M guanidinium thiocyanate, 12.5mM 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 2.0ml microcentrifuge tube and 0.4ml 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 1.5ml 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 the 1ml of 75% ethanol and then air dried. The pellet was resuspended in TE (10mM Tris, pH 6.8, 1mM 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 mM Tris, 890 mM boric acid, 20 mM EDTA, pH 8.0) containing 0.5 µg/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 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

1. Preparation of Slot Blot Filter. Slot blot filters were prepared using standard techniques (Sambrook, Fritsch, and Maniatis, 1989). 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. 1989). 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).

2. Hybridization of Slot Blot Filters. Slot blot filters were probed with ³²P-labeled DNA fragments for MDR1 and α -actin (See Probe Preparation). Before adding the probe, the filters were prehybridized for 18-24 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 μ g/ml of denatured salmon sperm DNA, and 50 mM 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 μ g/ml denatured salmon sperm DNA, and 20 mM sodium phosphate, pH 6.5) and denatured, radioactivity-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% SDA 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).

3. Probe Preparation. The plasmid pHDR5A was kindly donated by M. Gottesman and has been previously described (Ueda, et. al. 1987). This plasmid contains a cDNA insert of MDR1 which when digested with Eco RI produces a 1.3 kb MDR1 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 radioactivity labeled by the random priming method (Stratagene or US Biochemical, Cleveland, OH) with deoxycytosine 5'-[α -³²P] triphosphate (3000 Ci/mmol, Amersham, Arlington Heights, IL). Labeled probe was purified using either a standard G-50 sephadex spin column procedure (Sambrook, Fritsch, and Maniatis, 1989) or a microconcentrator (Amicon, Beverly, MA). The activity of the probe, determined by liquid scintillation, was usually greater than 1×10^5 cpm/ μ g.

Then 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 radioactivity labeled as described above for MDR1.

- c) Image Analysis and Quantitation - 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. Quantitation of MDR1 gene 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 β -actin signal intensity.
- d) Reverse Transcription - Polymerase Chain Reaction(RT-PCR) Quantitation
1. Competitive Template Construction. PCR quantitation of MDR1 gene transcript was determined through competitive template analysis. To minimize any discrepancies in efficiencies of reverse transcription or amplification of target sequences differing in size and/or sequence, the competitive template was designed to be exactly the same as the wild-type target except for one base change. A T to A alteration at position 1425 of the cDNA sequence introduces an Eco RI site at this position. The insertion of this site was accomplished by PCR *in vitro* mutagenesis and standard subcloning (See Figure 1A&B). The competitive template was subcloned in pGEM4 (Promega, Madison, WI) downstream from a SP6 RNA polymerase promoter site. The competitive template plasmid pLGCT was purified using standard techniques and subsequently used to generate *in vitro* transcribed RNA (cRNA).
 2. cRNA Synthesis. Purified pLGCT was linearized by Pvu II digestion and subsequently purified by agarose gel electrophoresis and anion spin-column chromatography (Qiagen). Approximately 20 μ g of linearized plasmid were reverse transcribed *in vitro* according to manufacturer's instructions utilizing the SP6 promoter region and SP6 RNA polymerase (Promega). The resulting 443 nucleotide cRNA was quantitated by UV spectral analysis and analyzed for purity by agarose gel electrophoresis. On the basis of the concentration determined by UV analysis, the cRNA was diluted to an appropriate range of concentrations for competitive template PCR assays and stored at -80°C . It is important to note that a dilution series was used only once and then discarded to avoid any discrepancy that may result from template degradation.

3. RT-PCR Amplification. Isolated tumor RNA was RT-PCR amplified using standard techniques (PCR Protocols). One-hundred nanograms of tumor RNA was reverse transcribed in the presence of competitive template (cRNA). A reaction was performed for each dilution of competitive template. A typical reaction consisted of: 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 2.5 μM random primers (Pharmacia, Piscataway, NJ), 200 μM of each dNTP, 100 units SuperScript II RNase H⁻ Reverse Transcriptase (Gibco-BRL, Bethesda, MD), 100 ng of tumor RNA, and appropriate concentration of competitive template. The reaction conditions were 10 minutes at room temperature, 30 minutes at 37°C, 5 minutes at 99°C, followed by 5 minutes at 5°C. Following the RT reaction, PCR was initiated by the addition of 80 μl of a PCR reagent master mix. The final concentration of PCR reagents was: 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 1 μM of each primer, 2.5 units Taq DNA Polymerase (Gibco-BRL). A master mix was prepared for each series of dilutions to minimize discrepancies that may have resulted from pipetting differences. The reaction conditions were 30 cycles of 94°C for 30 seconds, 38°C for 2 minutes, 72°C for 30 seconds. The amplification was followed by a 5 minute incubation at 4°C for 5 minutes.

The primers were also chosen such that they spanned an intron within the genomic DNA sequence. This positioning prevented the coamplification of the same size target sequence from DNA and RNA. Thus, DNA and RNA amplification could be easily discerned. The forward primer, 5'-ata aag aaa gct att aca-3', was from nucleotide 1289 to nucleotide 1306, and the reverse primer, 5'-att tcc ctt aat att atc-3', was from nucleotide 1597 to nucleotide 1580 of the cDNA sequence. Besides the tumor samples, the following controls were also included: a H₂O control to test for amplicon contamination of reagents; a positive RT control for MDR1 (KB 8-5 RNA), and a positive PCR control (p2000XS that contains a full length MDR1 cDNA). If any of the following controls gave an unexpected result the assay results were considered invalid and the assay was repeated.

4. Quantitation of MDR1 Transcript - Following PCR amplification, 8.5 μl of each reaction were digested with Eco RI and the digestion products resolved on a 2% Metaphor agarose gel (FMC, Rockland, ME) containing 0.5 μg/ml of ethidium bromide. The gel was photographed with Polaroid Type 55 film that generates a positive and negative image (Polaroid, Cambridge, MA).

Quantitation of the various PCR and Eco RI digestion products was accomplished by analyzing the negative by scanning laser densitometry (Ultrascan XL, Pharmacia LKB). The intensities of the two bands resulting from the competitive template amplification and subsequent digestion were compared to the intensity of the band resulting from the wild-type sequence. Since both amplification products are the same size they will intercalate approximately equal amounts of ethidium bromide/weight of target sequence. Thus, a direct comparison of ethidium bromide staining intensity can be used to quantitatively assess the amount of each product.

Absolute quantitation of MDR1 transcript was determined by using the equation: $\log(N_{01}/N_{n2}) = \log(N_{01}/N_{02}) + n \log(\text{eff}_1/\text{eff}_2)$ (Zachar, et. al. 1993). This equation calculates the yield of both PCR products in terms of the ratio of original target sequences and the efficiency at which they are amplified. This equation simplifies when both products are amplified with equal efficiency ($\text{eff}_1 = \text{eff}_2$); thus, the ratio of products during any cycle is solely dependent on the ratio of the initial target sequences at cycle 0. Therefore, when the ratio is equal to 1, the mass of each target is equal to one another. By plotting the log input of competitive template (cRNA) versus the log ratio of wild-type product to competitive template product, a linear relationship between the two ordinates can be generated and a curve drawn by least square's analysis. 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.

5. Results

- a) RNA Isolation - RNA was isolated using standard techniques (Chomczynski and Sacchi, 1987). The one-step procedure eliminates time-consuming steps that potentially may lead to RNase contamination and it also inactivates any endogenous RNase activity quickly. This procedure is also well suited to the extraction of RNA from small quantities of tissue. Two major concerns that must be considered when isolating RNA from such complex tissue like the breast is the complete dissociation of the tissue and the removal of impurities that co-purify with the total cellular RNA, i.e. stromal tissue. We currently employ the use of a mechanical dounce to further increase the homogenization of the tissue. The mechanical dounce also provides greater shear forces than a manual dounce. This shearing is important in the one-step procedure since sheared DNA will phase-separate better than intact DNA. Although we have not encountered any problems with impurities, we can add an additional precipitation step in the presence of 4 M LiCl, as recommended by Puissant and Houdebine (1990), that removes contaminating glycogen, as possibly other contaminants.

- b) Slot Blot Hybridization - Figure 2 is a representative example of a slot blot containing RNA from control cell lines drug sensitive, KB 3-1, and drug-resistant, KB 8-5, as well as two breast samples previously used in a large scale study of MDR expression in human cancer (Goldstein, et. al. 1989). The use of a phosphor-imager allows for a more precise quantitation of signal than assignment of arbitrary values by visual examination or scanning densitometry. However, since this technology is relatively new, we are confirming signal intensities by standard autoradiography. To date we have seen no differences between the two techniques; in fact, phosphor-imaging appears to be slightly more sensitive.

Interestingly, the two breast samples examined here and in the previous study produced quite different results in our hands. P27 had been previously reported to have 0 units of MDR1 gene expression, while P30 had 3 units of expression. Using the same scale, where KB 8-5's level of expression at 10 μ g mRNA is equal to 30 units, we determined P27 to have 111 units and P30 to express 19 units. Similar results have been shown in repeated experiments (data not shown). Furthermore, RT-PCR analysis indicates that both tumors have a substantial level of MDR1 gene expression (See Competitive PCR results).

- c) Competitive PCR Analysis - Figures 3-5 are representative examples of competitive RT-PCR analysis of the same samples analyzed by slot blot filter hybridization. Figure 3 shows the quantitation of KB 8-5 MDR1 RNA. The competitive template concentration and subsequent dilution series was based on UV spectral values. The cRNA concentration ranged from 20 pg to 0 pg (8×10^8 to 0 molecules) serially diluted 1:2. Lanes 8-10 are control samples that are performed with each assay. These include a positive PCR control, a water control, and a negative RNA control. As can be seen, the increasing concentration of exogenous target sequence (MDR1 cRNA) inhibits the production of endogenous target (KB 8-5 RNA). Furthermore, by plotting the densitometric values of the ratio of target/cRNA versus input of cRNA we can determine the concentration of MDR1 RNA by examining the equation generated by least square's analysis. Several independent experiments were performed on the same KB 8-5 RNA to determine the consistency of this assay. The same relative value was determined in each instance with a range of $3-5 \times 10^8$ molecules/ μ g of cellular RNA. Since both target sequences are alike, we do not have to be concerned with efficiency of linearity of amplification. Also, unlike other competitive assays which use cDNA as the competitor, the use of cRNA also accounts for differences in reverse-transcription. Figures 4 and 5 are similar experiments using RNA from different sources, including tumor samples. KB C-1 was analyzed twice, with a range of $2-4 \times 10^9$ molecules/ μ g of RNA for being determined. Because of the limited amount of RNA for the tumor samples, only one competitive PCR assay was performed. However, to ensure that the assay was functioning correctly, KB 8-5 RNA was also quantitated using the same reagents and conditions. This analysis done in concurrent environmental conditions is important since these variables may affect amplification efficiency (See Discussion).

Table 1 shows the numeric results of the competitive PCR assay and compares these results to the slot blot filter assay. As can be seen, PCR exhibits a greater fold difference in expression than slot blot. Furthermore, PCR is more sensitive than slot blot hybridization, thus it is not unexpected that different values from slot blot analysis be observed (Brophy, et. al. 1994).

6. Discussion

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. The sensitive nature of RNA isolation and RT-PCR require that strict protocols are followed. Thus, each technique has been scrutinized and potential pitfalls that may affect yield or introduce unwanted variability have been addressed.

RNA isolation, especially from heterogeneous tissue such as breast tumors, is extremely difficult. Furthermore, many biopsies may be from fine needle aspirates (FNAs) which can potentially contain a very finite amount of tumor. In order to assure consistent and reliable RNA extraction from human tumors as well as FNAs, we have obtained a variety of tissue biopsies (colon, liver, and breast) and perform extractions on these tissues. By extracting RNA from these samples we not only assure that our technique is consistent, we also assure the quality of our reagents.

These same samples are also analyzed by the various techniques used to assess MDR1 RNA levels. Slot blot analysis is performed using identical conditions for each blot. In order to minimize variability in hybridization several precautions are taken. Since the quality of the components of the hybridization buffers may differ from lot to lot, i.e. dextran sulfate, we routinely prepare, aliquots, and freeze large batches of these buffers. Each new lot is tested using standard controls before it is used for tumor work. Furthermore, fresh, unlabeled probe is prepared and quantitated for each blot. This prevents any variability in labelling due to hydrolysis of the DNA fragments. Finally, each probe is labelled to the same relative specific activity (CPM/ μ g).

RT-PCR is an extremely sensitive technique that requires a number of controls to be performed in order to assure the quality and validity of the reaction. In addition to the routine controls that are normally performed during RT-PCR, we also take extra precautions in terms of our competitive assay. The major concern of this assay is the loss or degradation of cRNA. In order to assure that each competitive assay receives the prescribed amount of cRNA. cRNA is quantitated by UV spectral analysis, serial dilutions are prepared, aliquoted, and stored at -80°C .

Serial dilutions are only used once to avoid numerous freeze/thaw alterations and the cRNA is diluted in 10 mM Tris, 1 mM EDTA, pH 6.8. Extended RNA storage in buffers with a pH greater than 7.0 can lead to a high rate of self-cleavage. Finally, each new synthesis of cRNA is assayed against KB 8-5 to assure that its UV spectral value is equivalent to the previous cRNA. One concern that we are currently addressing is the variability in reverse-transcription of the target and cRNA sequence. We currently perform all comparative PCR assays (sample 1 versus sample 2) under concurrent conditions. Each reaction is prepared from the same master mix, same primers, and the reaction performed at the same time. This procedure eliminates any variability introduced from lot differences in reagents, i.e. dNTPs, and differences in cycling parameters. At issue, though is the overall efficiency of the RT-PCR. In order to assess the general efficiency of RT-PCR we are currently developing an assay that measures the amplification of β -actin RNA. Since each tube is receiving equivalent amounts of target RNA (100 ng) the signal intensity of actin should be relatively equal for each tube. The protocol and conditions for this assay are currently being determined.

General comparisons of the various techniques will establish the relative reliability of each technique and the concordance between them. From our initial analysis (See Table 1) it would appear that slot blot analysis and competitive PCR identified a similar trend for some of the samples. However, the sensitivity of slot blot hybridization does not appear to be as great as PCR. Furthermore, in a recent study by Brophy, et. al. (1994), they showed that slot blot hybridization had a high false positive rate while PCR was extremely sensitive and specific. The authors further suggest that MDR1 expression levels be determined utilizing RT-PCR and immunohistochemistry.

We are currently developing immunohistochemical techniques to evaluate MDR1 expression. A panel of monoclonal antibodies, C219, JSB1, and MRK-16, are currently being considered for use in our analysis, however, we currently have delayed further development of our immunohistochemistry protocols, until standard approaches are published as a result of a recent meeting organized by William Beck, Ph.D.

7. Clinical Trials

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

- a) Philadelphia Bone Marrow Consortium - PBT3 (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 the four member institutions including Fox Chase Cancer Center (FCCC), University of Pennsylvania, Hahnemann University, and Temple University. The protocol,

FCCC IRB approval and consent forms are included in the appendix of this report. This study is now open for enrollment since the initial annual report, 4/7/94.

- b) 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 has subsequently lost its potency in mediating reversal of MDR we are in the process of discussing with Sandoz and the NCI the possibility of substituting CSA with its analogue PSC 388 which is a more potent MDR inhibitor. When this is accomplished this study will be open for accrual.
- 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. Now that the laboratory component of our effort has been established and we have acceptable control experiments as described above, this protocol will be open to accrual.
- d) Eastern Cooperative Oncology Group (ECOG) Registration Study of Induction with Adriamycin or Paclitaxel in Inoperable Locally Advanced and Inflammatory Breast Cancer to Evaluate for Multidrug Resistance. Since the 4/7/94 Annual Report this concept has been approved by the ECOG Breast Core Committee July 7, 1994, and a draft of the schema and eligibility are included in the appendix of this report. Both this study and PBT-3 will permit us to obtain sequential breast tumor samples before and after treatment with Adriamycin or Paclitaxel to support us in accomplishing the aims of this grant proposal i.e. to determine the clinical significance of MDR1 gene expression in breast cancer and to correlate expression with response and resistance to treatment with MDR substrate.

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, 1994 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 report. We are now in a position to accurately evaluate the breast cancer samples for each of the clinical trials included in this project.

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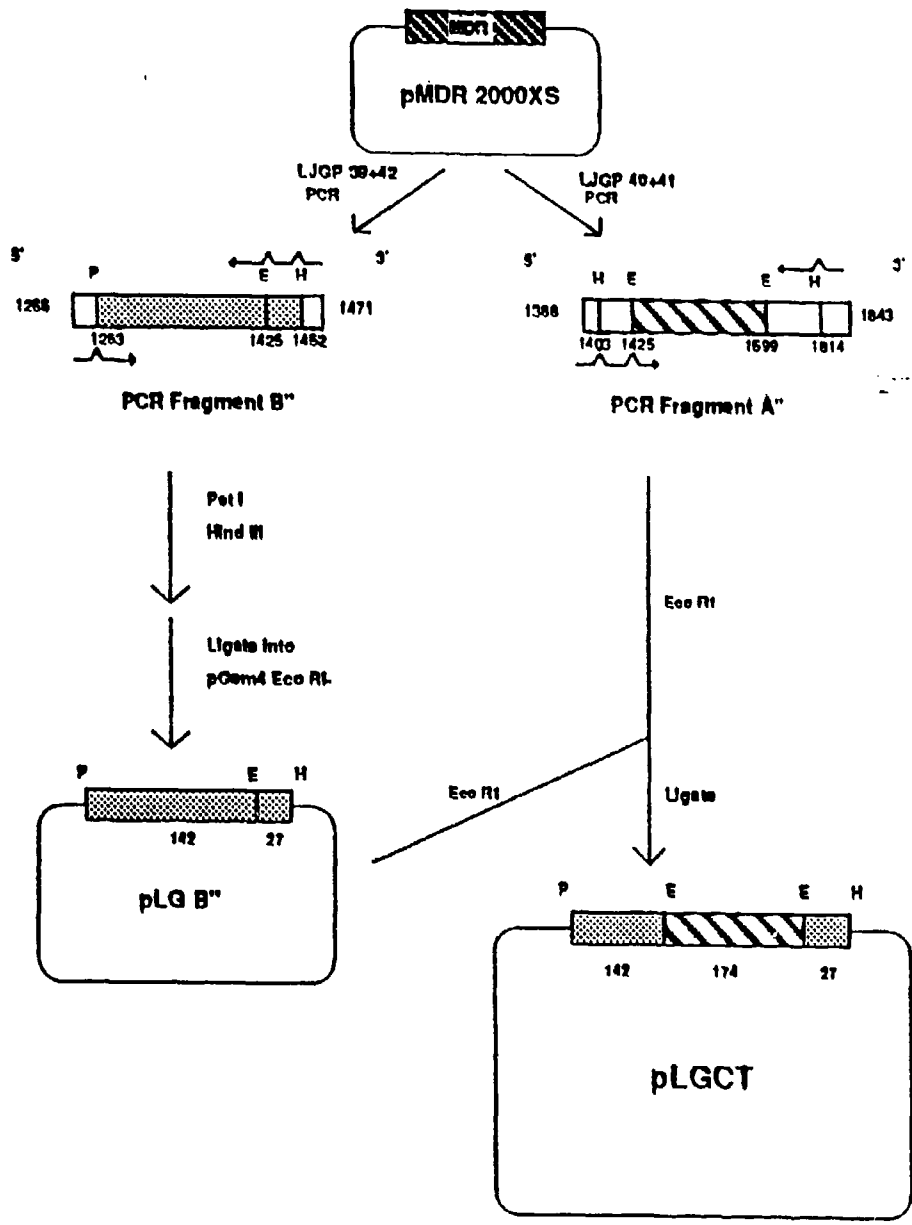


Figure 1A. Strategy for the construction of the competitive template, pLGCT. H = Hind III; E = EcoR1; P = Pst I.

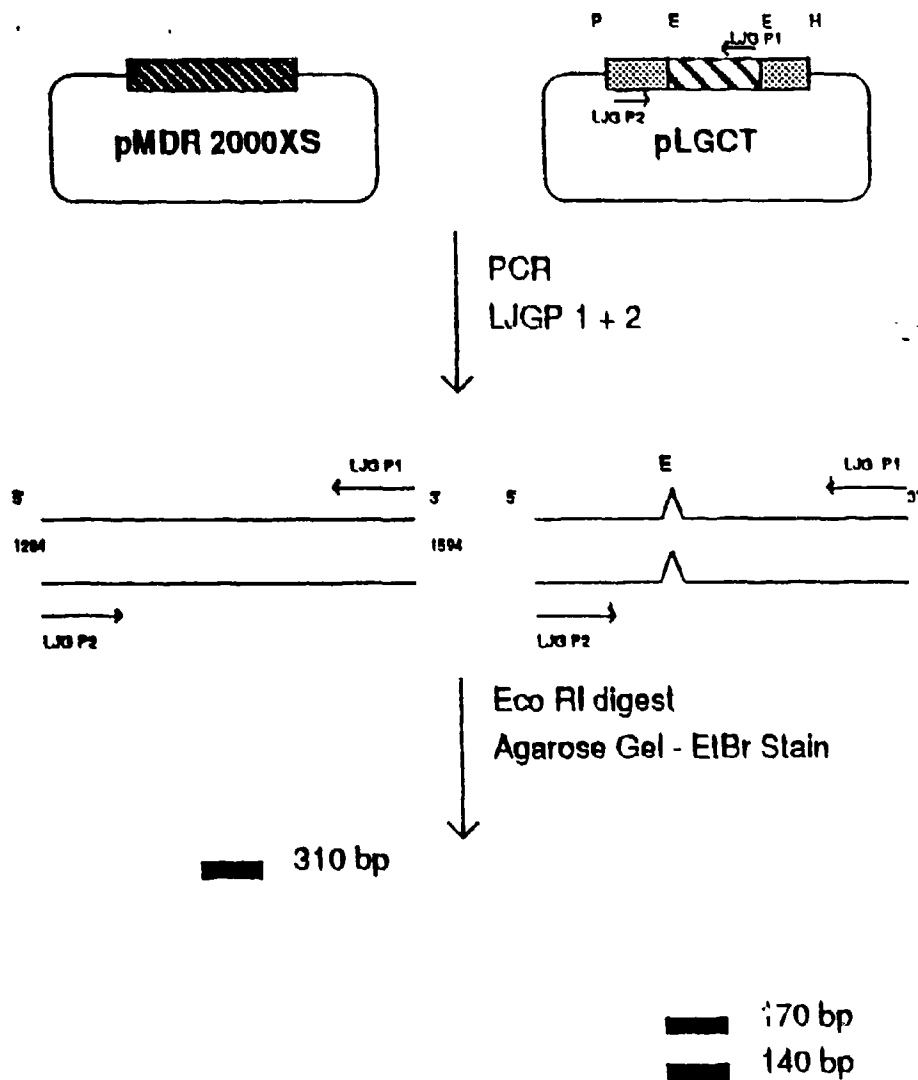
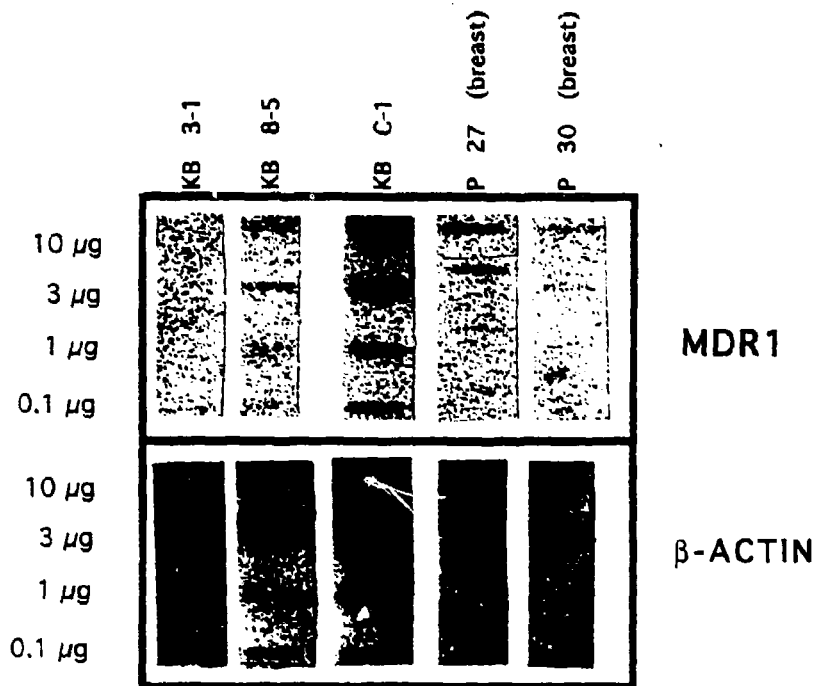


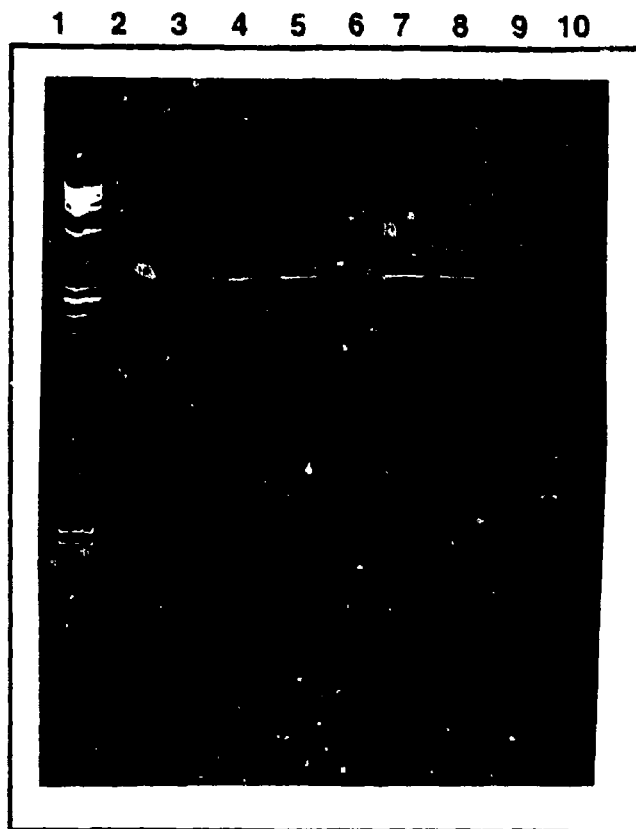
Figure 1B. Diagrammatic representation comparing the Eco RI digestion of the PCR products of pMDR2000xs and pLGCT. H = Hind III; E = Eco RI; P = Pst I.



MDR1 Levels
(adjusted for RNA loading differences)

	<u>KB 3-1</u>	<u>KB 8-5</u>	<u>KB C-1</u>	<u>P 27</u>	<u>P 30</u>
10 μ g -	0	30	284	111	19

Figure 2. Slot blot analysis of human breast cancers. Total RNA from two human breast cancers were analyzed as described in the Methods section. Serial dilutions of 10, 3, 1, and 0.1 μ g of total RNA were applied to the blot. For quantitative purposes, total RNA from drug-sensitive and drug resistant cell lines were also analyzed. In addition, blots were also hybridized with a β -actin probe to account for any variation in RNA concentration. KB 3-1: drug-sensitive; KB 8-5: drug-resistant (4-6-fold); KB C-1: drug-resistant (150-fold); P27: breast cancer, no chemotherapy; P30: breast cancer, no chemotherapy.



11 12 13 14 15 16 17

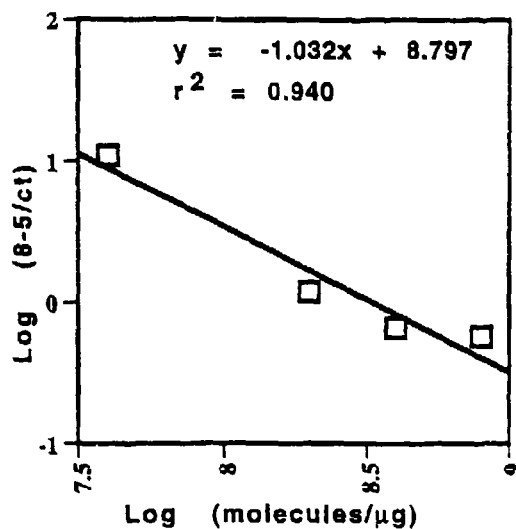


Figure 3. MDR1 transcript level for KB 8-5 determined by Competitive PCR analysis. The dilution series ranged from 8×10^8 to 0 molecules/ μg . Lane 1,11: phiX174; Lanes 2-7:KB 8-5 (*EcoRI* digested); Lane 8: positive control (pMDR2000XS); Lane 9: water control; Lane 10: KB 3-1; Lanes 12-17: corresponding undigested products for KB C-1. The plot of sample/ct (ratio of sample vs competitive template densitometric products) versus input of cRNA is linear and allows for the determination of where the ratio equals 1.

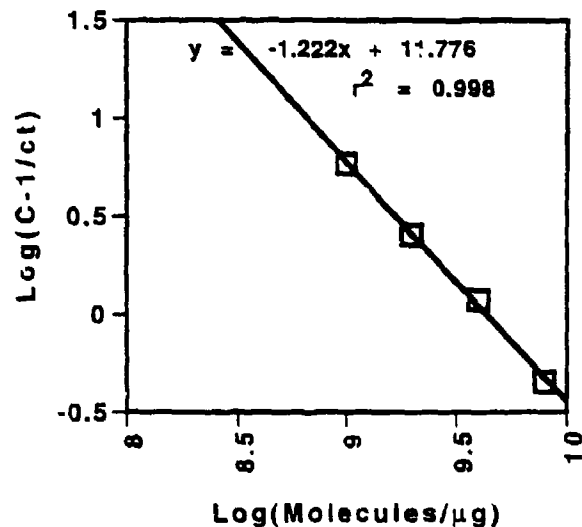
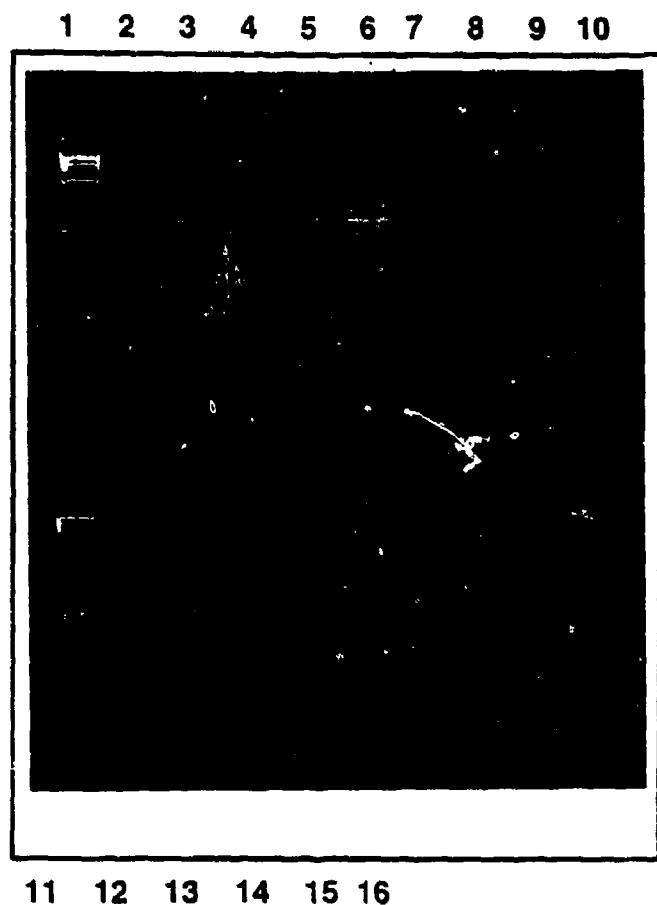


Figure 4. MDR1 transcript level for KB C-1. The dilution series ranged from 8×10^9 to 0 molecules/ μg . Lane 1,11: phiX174; Lanes 2-6: KB C-1(*Eco*RI digested); Lane 7: positive control (pMDR2000XS); Lane 8: water control; Lane 9: KB 8-5; Lane 10: KB 3-1; Lanes 12-16: corresponding undigested products for KB C-1. The plot of sample/ct (ratio of sample vs competitive template densitometric products) versus input of cRNA is linear and allows for the determination of where the ratio equals 1.

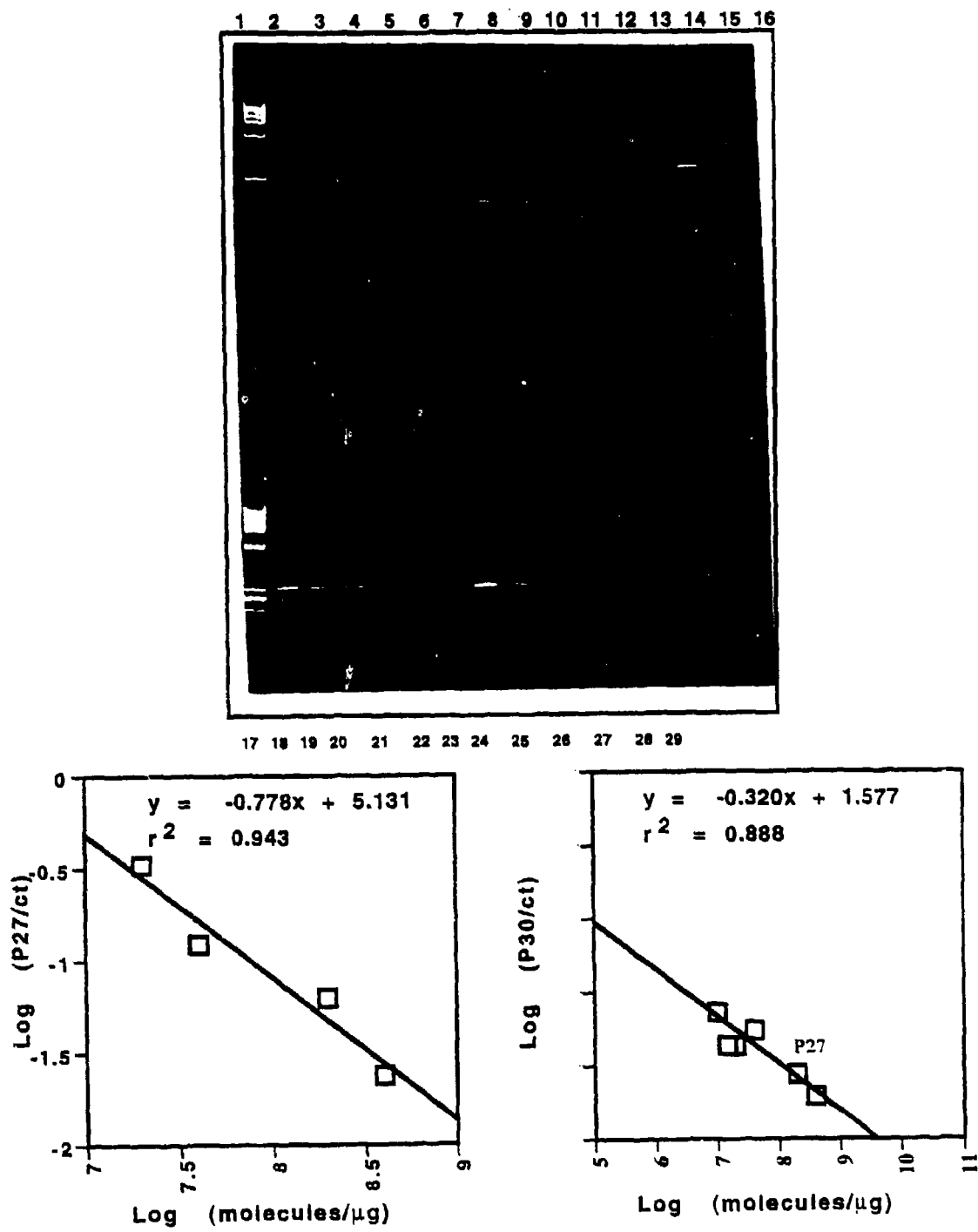


Figure 5. MDR1 transcript levels in two breast cancer cases, P27 and P30, that did not receive chemotherapy. The dilution series ranged from 4×10^8 to 0 molecules/ μg for both samples. Lane 1,17: phiX174; Lanes 2-7: P27 (*Eco*RI digested); Lanes 8-13: P30 (*Eco*RI digested); Lane 14: positive control (pMDR2000XS); Lane 15: water control; Lane 16: KB 3-1; Lanes 18-29: corresponding undigested products for P27 and P30. The plot of sample/ct (ratio of sample vs competitive template densitometric products) versus input of cRNA is linear and allows for the determination of where the ratio equals 1.

TABLE 1

Sample	MDR1 level (slot blot, arbitrary units)	Fold difference (expression)	MDR1 level (RT-PCR, molecules/ μ g)	Fold difference (expression)
KB 8-5	30 units	--	3.34×10^8	--
KB C-1	250	~8-fold greater	4.33×10^9	~13-fold greater
P27 (breast)	111	~4-fold greater	3.94×10^6	~83-fold less
P30 (breast)	17	~2-fold less	8.47×10^4	~4000-fold less

Table 1. Table 1 shows the relative levels of expression for each sample as determined by slot blot hybridization and competitive RT-PCR. In addition, the relative fold difference to KB 8-5 expression is shown. As can be seen RT-PCR displays a similar trend for KB C-1 when compared to slot blot analysis, but a completely different result for the two tumor samples. This difference may be the result of the greater sensitivity and specificity of RT-PCR. The comparison of a larger sample size may allow for a better comparison of the two techniques.

APPENDIX

I. PBT-3 Phase II Trial of High Dose Chemotherapy Cyclophosphamide, Thiotepa and carboplatin and Peripheral Blood Stem Cell Infusion in Women with Locally Advanced, Inoperable and Inflammatory Breast Cancer Who Achieve a Partial Response to Induction Chemotherapy.

II. ECOG Induction with Adriamycin or Paclitaxel in Inoperable Locally Advanced and Inflammatory Breast Cancer to Evaluate for Multidrug Resistance, A Registration Study. Draft 7/1/94.

Protection of Human Subjects Assurance Identification/Certification/Declaration (Common Federal Rule)

POLICY: Research activities involving human subjects may not be conducted or supported by the Department and Agencies adopting the Common Rule (56 FR 20002, June 18, 1991) unless the activities are exempt from or approved in accordance with the common rule. See Section 101(b) of the common rule for exemptions. Institutions submitting applications or proposals for support must submit certification of appropriate Institutional Review Board (IRB) review and approval to the Department or Agency in accordance with the common rule.

Institutions with an assurance of compliance that covers the research to be conducted on file with the Department, Agency, or the Department of Health and Human Services (HHS) should submit certification of IRB review and approval with each application or proposal unless otherwise advised by the Department or Agency. Institutions which do not have such an assurance must submit an assurance and certification of IRB review and approval within 30 days of a written request from the Department or Agency.

1. Request Type <input checked="" type="checkbox"/> ORIGINAL <input type="checkbox"/> FOLLOWUP <input type="checkbox"/> EXEMPTION	2. Type of Mechanism <input type="checkbox"/> GRANT <input type="checkbox"/> CONTRACT <input type="checkbox"/> FELLOWSHIP <input type="checkbox"/> COOPERATIVE AGREEMENT <input type="checkbox"/> OTHER: _____	3. Application or Proposal Identification No. (if known)
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4. Title of Application or Activity 94041 Phase II Trial of High Dose Chemotherapy with Peripheral Stem Cell Infusion in Women with Locally Advanced Breast Cancer (PBT-3)	5. Name of Principal Investigator, Program Director, Fellow, or Other Lori Goldstein, M.D.
--	--

6. Assurance Status of this Project (Respond to one of the following)

This Assurance, on file with the Department of Health and Human Services, covers this activity:
 Assurance Identification no. M- 1030 IRB identification no. 01

This Assurance, on file with (agency/dept.) _____ covers this activity.
 Assurance Identification no. _____ IRB identification no. _____ (if applicable)

No assurance has been filed for this project. This institution declares that it will provide an Assurance and Certification of IRB review and approval upon request.

Exemption Status: Human subjects are involved, but this activity qualifies for exemption under Section 101 (b), paragraph _____

7. Certification of IRB Review (Respond to one of the following IF you have an Assurance on file)

This activity has been reviewed and approved by the IRB in accordance with the common rule and any other governing regulations or subparts on (date) 6/28/94 by: Full IRB Review or Expedited Review.

This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by the common rule will be reviewed and approved before they are initiated and that appropriate further certification will be submitted.

8. Comments

9. The official signing below certifies that the information provided above is correct and that, as required, future reviews will be performed and certification will be provided.	10. Name and Address of Institution Fox Chase Cancer Center 7701 Burholme Avenue Philadelphia, PA 19111	
11. Phone No. (with area code) 15-728-6900	12. Fax No. (with area code) 215-728-3574	

13. Name of Official W. Thomas London, M.D.	14. Title Chairman, Institutional Review Board
---	--

15. Signature 	16. Date JUN 28 1994
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PBT Philadelphia Bone Marrow Transplant Group

File

HAHNEMANN UNIVERSITY CANCER PROGRAM TEMPLE UNIVERSITY CANCER CENTER FOX CHASE CANCER CENTER UNIVERSITY OF PENNSYLVANIA CANCER CENTER

Memorandum

To: Philadelphia Bone Marrow Working Group
From: Lori J. Goldstein, M.D.
Date: May 19, 1994
Subject: PBT-3 Amendment: Changes in Statistical Section 9.0



The Biostatistics Department of Fox Chase Cancer Center has revised the Statistical Consideration, Section 9.0 for PBT-3 as indicated below. Please put this through the IRB's at your respective institutions.

STATISTICAL CONSIDERATIONS

This is a trial of patients with locally advanced, inoperable and inflammatory breast cancer, and the principal outcome of interest is the time from treatment with intensive dose doxorubicin until death. The goal of the trial is to increase the 5-year survival rate among PR's from 40%, which is the expected rate under current therapies, to 60% under the intensive therapy. (This corresponds to an increase in median survival from 3.8 to 6.8 years.) Assuming 3 years of accrual and 2 years of follow-up and a 0.05 level likelihood-ratio test, the following table gives the powers of the test with two different PR rates:

Patients per year	PR Rate	PR's per year	Power
25	60%	15	73%
25	70%	18	81%

Thus, for example, suppose that 25 patients per year enter the study, and that 10% of them achieve a CR and 70% achieve a PR. of the total of 75 patients enrolled over three years, there would be 53 PR's, who would enter the intensive (transplant) phase of the study. The expected power of the test would then be 81%.

In the statistical analysis, the stratification of the patients based on the schema will be accounted for. In addition, a secondary endpoint, disease-free survival, will be analyzed.

cc: PBT Working Group Distribution:

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PROTOCOL PBT-3

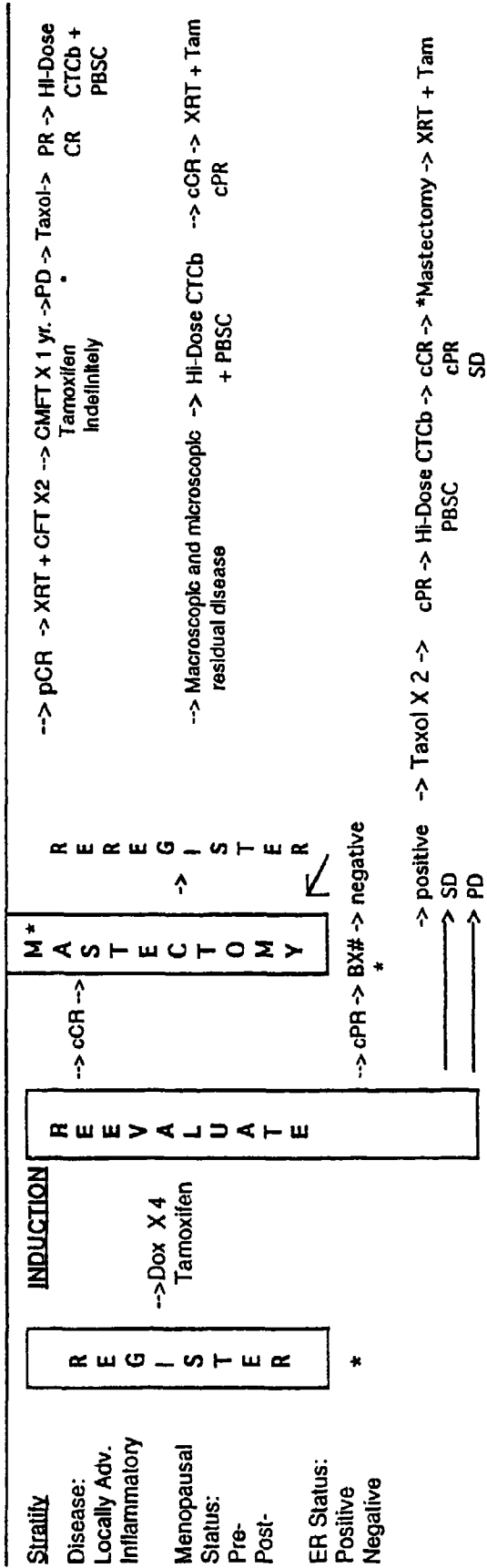
PHASE II TRIAL OF HIGH DOSE CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE,
THIOPETA AND CARBOPLATIN AND PERIPHERAL BLOOD STEM CELL INFUSION
IN WOMEN WITH LOCALLY ADVANCED, INOPERABLE AND INFLAMMATORY
BREAST CANCER WHO ACHIEVE A PARTIAL RESPONSE TO INDUCTION
CHEMOTHERAPY

Study Chairmen: Dr. Pamela Crilley - Hahnemann University Hospital
Dr. Lori Goldstein - Fox Chase Cancer Center

Participating Institutions:

Hahnemann University Hospital
Temple University Cancer Center
Fox Chase Cancer Center
University of Pennsylvania Cancer Center

SCHEMA FOR LOCALLY ADVANCED (T3B) AND INFLAMMATORY BREAST CANCER



* = Biopsy or mastectomy tissue analyzed for MDR1 and other possible studies.

= Fine needle aspirate X3, core bx or skin bx.

Doxorubicin 30mg/m² iv d 1-3 every 28 days X 4
 Taxol 175mg/m² iv 24h C1 q 3 wks (G or GM-CSF optional)
 Tamoxifen 20 mg po qd

C Cyclophosphamide 100mg/m² po d 1-14
 M Methotrexate 40mg/m² iv d1, 8
 F 5-Fluorouracil 600mg/m² iv d 1, 8
 T Tamoxifen 20mg po/day
 Repeat cycle every 28 days

G-CSF Stimulated Stem Cell Harvest
 10 ug/kg/d from 5 days prior to start of peripheral stem cell harvest maintained until the completion of harvest.

C Cyclophosphamide 6000mg/m²
 Continuous Infusion
 T ThioTepa 500mg.m²
 Continuous Infusion
 Cb Carboplatin 800mg/m²
 Continuous Infusion

cCR = Clinical Complete Response
 cPR = Clinical Partial Response
 SD = Stable Disease
 PD = Progressive disease

G-CSF Stimulated Marrow Recovery
 10 ug/kg/d peripheral stem cell infusion until ANC > 1000/mm³ has been maintained for two consecutive days.

INDEX

Schema

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Selection of Patients
- 4.0 Registration Procedures
- 5.0 Treatment Plan
- 6.0 Measurement of Effect
- 7.0 Study Parameters
- 8.0 Drug Information and Procurement
- 9.0 Statistical Considerations
- 10.0 Records to be Kept
- 11.0 Patient Consent and Peer Judgment
- 12.0 References

1.0 INTRODUCTION

1.1 Breast cancer represents the most common malignancy in females. It is estimated that in 1993, 182,000 new cases of invasive breast cancer will be diagnosed in U.S.A. Approximately 20% of breast cancers at the time of presentation are locally advanced without evidence of distant metastases and two-thirds of these are classified as locally advanced inoperable or inflammatory breast cancer (1). In general, locally advanced inoperable breast cancer is categorized by tumors of any size with chest wall fixation, edema, skin ulceration or satellite nodules, the presence of fixed ipsilateral axillary nodes, or arm edema. Previously, ipsilateral supraclavicular nodes were categorized in stage III, however, they are now classified as metastatic disease and therefore, constitute stage IV disease. The guidelines for operability were originally described by Haagensen and Stout (2). Inflammatory breast cancer represents a distinct clinical entity characterized by the presence of diffuse erythema, increased warmth of the involved skin, edema of the skin (peau d'orange) and induration of the underlying breast tissue with or without a palpable mass (3,4,5). The breast is usually diffusely enlarged and tender. The hallmark pathologically is the presence of dermal lymphatic invasion (3), however, pathologic confirmation of dermal lymphatic invasion is not required for a diagnosis of inflammatory breast cancer since multiple series (6,7,8,9,10) have demonstrated no difference in survival rates between patients with a clinical diagnosis than those with a clinical and pathologic diagnosis.

Historically, locally advanced inoperable breast cancer and inflammatory breast cancer were treated with radiotherapy alone. Employing doses of 6000 to 9000 cGy, local-regional recurrence rates ranged from 30-60% (11,12,13,14,15) and 5-year survival ranged from 5 to 40% (11,12,13,14,15,16). With local-regional treatment alone, 80 to 90% of patients developed distant metastases (11,12,13).

Induction or initial chemotherapy has become standard treatment in patients with locally advanced inoperable or inflammatory breast cancer in an attempt to decrease the risk of distant metastasis as well as to assess the response of the primary to treatment. Multiple regimens have been employed to treat these patients. The studies are difficult to compare and no direct randomized trial have evaluated Doxorubicin versus non-Doxorubicin based combinations. It does appear, however, that some conclusions can be reached. Using Doxorubicin-based combinations, response rates of over 80% have been consistently seen with complete responses ranging from 10 to 49% (17-26). For inflammatory breast cancer, similar complete response rates have been reported (27,28). Five-year survival employing induction chemotherapy with radiotherapy with or without mastectomy ranges from 40 to 60% (10,11,14,18,20,27,29,30); with a disease-free survival of 30-50% (10,11,18,20,27,30). Regimens employing estrogenic recruitment and hormonal synchronization have increased the complete response rate to 50% for stage IIIB and inflammatory breast cancer, however, 5-year survival remains in the range of 25 to 30% (10,26).

The University of Pennsylvania initiated a combined modality protocol for the treatment of inflammatory breast cancer in 1982. After metastatic evaluation and surgical biopsy, patients were treated with induction chemotherapy and tamoxifen. The rationale for Tamoxifen was previously documented response in local regional disease in 30 to 40% of patients with locally advanced breast cancer (27,28). Following maximum tumor regression, patients received local regional radiotherapy to the breast and ipsilateral, supraclavicular, and axillary lymph nodes. During radiotherapy patients continued to receive cyclophosphamide, 5-fluorouracil, and tamoxifen. Upon completion of radiotherapy, patients resumed CMFPT chemotherapy for 1 to 3 cycles before undergoing modified radical mastectomy. Following mastectomy, chemotherapy was resumed for a total of 10 cycles of CMFPT and 2 cycles of CFT. Tamoxifen was continued indefinitely. Between 1982 and 1988, a total of 56 patients with non-metastatic inflammatory breast cancer received this combined modality approach. Complete clinical response to induction chemotherapy occurred in 32 patients (65%), a partial response in 13. At the time of mastectomy, residual tumor was identified in the breast and/or axilla in 61% of the patients. The 5-year actuarial relapse-free survival is 45%.

The optimal induction regimen in either locally advanced breast cancer is not well defined. As previously stated, one can draw the conclusion that Doxorubicin-containing regimen may be superior to those not including the anthracycline. However, to give this drug in combination or alone and in what dose or schedule is also not clear. Recently, some reports have stressed that the use of high-dose or intensive Doxorubicin may be superior to standard dose regimen. Jones et al ²³ reported an 85% response rate with intensive dose Doxorubicin and a clinical CR of 38%. Based on these studies, ECOG and CALGB recently concluded a study of intensive Doxorubicin and the preliminary data indicates at least a 75% response rate and 19% complete response. We propose to employ this aggressive induction in our study.

- 1.2 PACLITAXEL (TAX-11-en-9-one, 5 beta, 20 epoxy-1,2 a, 4, 7 b, 13 a-hexahydroxy-4, 10-diacetate-2-benzoate-13-(a-phenylhippurate), is a diterpene plant produce derived from the Western yew *Taxus Brivifolia* (34).

- 1.21 Mechanism of Action

Paclitaxel represents the first of a group of compounds developed that have a unique mechanism of action. The cellular target appears to be the microtubular apparatus, but unlike vinca alkaloids or epipodophyllotoxin, Paclitaxel actually promotes microtubular assembly *in vitro* via direct high-affinity binding to polymerized tubulin (35-41). Paclitaxel also decreases the critical tubulin concentration required for polymerization. The resultant complex is unusually stable, and resistant to conditions known to favor depolymerization, including cold (4°C) or treatment with 4mM CaCl₂. Drug-treated cells are thus unable to depolymerize their microtubular cytoskeleton and this is the putative mechanism of

anti-tumor activity. Treated cells have replication blocked in the G₂ and M phases of the cell cycle.

1.22 Preclinical Studies

1.221 Activity

In animal studies, Paclitaxel has shown activity against a variety of transplantable tumors, with the most activity in intraperitoneally implanted B16 melanoma and subrenal capsular xenografts of the MX-1 human breast cancer cell line and less activity against L1210 and P388 leukemias, CX-1 colon, and LX-1 lung tumors. The drug showed no activity against Lewis lung carcinoma or CD8F1 murine mammary tumor. Xenografts from a variety of human tumors in nude mice have demonstrated responses to Paclitaxel (43).

1.222 Toxicity

Preclinical toxicologic studies were performed in intraperitoneally-treated rodents (CD8F1 mice and Sprague-Dawley rats) and beagle dogs (42). The Ld₁₀ in mice was 69.6 mg/M²/day X 5 days (350 mg/M²/course), and in dogs the TDL was 45 mg/M² for a single dose and 15 mg/M²/day when a 5-day schedule was used. Toxicities were similar for all species, and were most pronounced in the most kinetically-active tissues. Hematopoietic toxicity included anemia, leukopenia, thrombocytopenia, and lymphoid tissue depletion. Gastrointestinal toxicity in dogs included vomiting, diarrhea, and weight loss. Effects were also seen on the reproductive system of male rats, including spermatocytic necrosis, oligospermia, and giant cell formation in seminiferous tubules. Additionally, dogs appeared to be sensitive to the Cremophor vehicle used, with large volume single doses associated with anaphylaxis and death.

1.23 Clinical Studies

1.231 Adverse Effects

A hypersensitivity reaction manifesting as anaphylaxis has been seen in several trials, almost exclusively with shorter in shorter infusion times (1-3 hours). In the Memorial Hospital Phase I trial, 3 of 5 patients were treated at >190 mg/M² with a 3-hour infusion experienced this reaction, and 1 patient suffered a fatal anaphylactic reaction (44).

It is not clear whether the hypersensitivity reaction is directed against the drug itself or the Cremophor vehicle. All ongoing protocols now include premedication with an H₁ blocker, an H₂ blocker, and corticosteroids. With longer

infusion times and premedication, the occurrence of hypersensitivity reactions is much less frequent.

Myelosuppression has been seen with all schedules of administration, and has been dose-limiting in the majority of trials. This is manifested primarily as leukopenia, with nadir counts occurring between days 11 and 19. Thrombocytopenia has been seen much less frequently and has not been as severe.

Non-hematologic toxicities have been observed at higher doses and have not been dose-limiting. These include arthralgias, myalgias, alopecia, nausea, vomiting, stomatitis, transient rashes, pruritus, fatigue, mild and reversible SGOT and triglyceride elevation. Extravasation of the drug has occurred without serious consequence, although mild phlebitis has been reported.

Cardiotoxicity has been observed in Phase II trials (45). Transient asymptomatic bradycardia has been observed in as in Phase II trials (45). Transient asymptomatic bradycardia has been observed in as many as 29% of patients without known cardiac risk factors. More significant bradyarrhythmias has been seen in 2 patients, manifested as second-degree A-V block which appeared 5 hours after administration and persisted for 10 hours. One of these 2 patients experienced A-V block with 7 seconds of asystole, and although the patient remained asymptomatic, a pacemaker was inserted. Episodes of ventricular tachycardia have been observed in patients receiving both Paclitaxel and cisplatin concurrently, but have not been noted in patients receiving Paclitaxel alone.

The ECOG Toxicity Committee has recently conducted a review of Paclitaxel-related toxicity in patients with advanced prostatic and non-small cell bronchogenic carcinoma. This review suggested that patients with any cardiac ischemic or dysrhythmic conditions, patients with a history of thromboembolic events, and those being treated concurrently with cardiac-active agents may have exhibited an excess of cardiovascular toxicity, irrespective of whether patients were entered on study with a stated previous history of cardiac disease. The exact role that Paclitaxel played in the etiology of these adverse events is currently unclear, as is the relative increase of risk of these events in Paclitaxel-treated patients. Until a more extensive review of this potential toxicity is completed, it seems prudent to exclude such patients from ongoing or proposed studies.

1.232 Phase I Trials

Seven Phase I trials of Paclitaxel have been conducted at 6 institutions in the United States (44-51). Doses in these trials have ranged from 15mg/M² to 275 mg/M². A variety of dosing schedules have been used, including 1-hour, 3-hour,

6-hour and 24-hour infusions on one day, and 1-hour infusions daily for 5 days each course. Objective responses were seen in head and neck cancer, colon, gastric, ovarian, and melanoma.

1.233 Phase II Trials

Phase II trials in the United States have been performed in epithelial ovarian carcinoma, melanoma, and breast cancer. The most striking results to date have been in patients with previously treated ovarian cancer, including patients with cisplatin-refractory disease, as McGuire et al observed 11 partial responses, 1 pathologic complete response, and 7 minor responses in 40 evaluable patients (52), results which have been confirmed by the Gynecologic Oncology Group (53), and investigators at the Albert Einstein College of Medicine (54).

There have been 2 phase II trials conducted in patients with metastatic malignant melanoma, both with a 24-hour infusion of Paclitaxel at 250 mg/M². At the M.D. Anderson Hospital, 3 of 25 (12%) demonstrated a partial remission (55), while at Albert Einstein. 4 of 28 (14%) evaluable patients responded, including 3 complete remissions and 1 partial remission (56).

Recently, Holmes et al have reported their experience with Paclitaxel in patients receiving an initial dose of 250 mg/m² for breast cancer patients who had received one prior chemotherapeutic regimen (57). The objective response rate obtained in this study was 56% (12% CR, 44%PR). In this trial, granulocytopenia was dose-limiting; a chronic glove and stocking neuropathy developed in most patients. This trial suggests that Paclitaxel is an extremely active single agent in a setting in which most other single agents failed (i.e. an Adriamycin-refractory group of patients). A second trial was performed at MSKCC using Paclitaxel 250 mg/M² as 24-hour continuous infusion with G-CSF in a less heavily pretreated population (16 prior adjuvant chemotherapy, 1 only prior to chemotherapy). A response rate of 62% (12% completed and 50% pretrial) was noted. In 8 patients who had received a doxorubicin-based adjuvant regimen, one complete and four responses were observed (58). Patients who do not achieve CCR with Doxorubicin induction on this study, will receive 2 cycles of Paclitaxel to improve response.

- 1.3 Interest in autologous marrow transplantation in the treatment of breast cancer has increased over the last several years. While the marrow infusion is a rescue technique and not treatment itself, it allows the use of higher doses of chemotherapy with the potential to increase tumor cell kill and enhance tumor cyto-reduction. The rationale for intensive chemotherapy regimens is the dose response relationship which has been demonstrated both in metastatic breast cancer and in the adjuvant setting (59,60,61). At the present time,

prospective randomized trials evaluating the role of high dose chemotherapy and autologous bone marrow support are under way for women with metastatic breast cancer as well as those with non-metastatic breast cancer who have 10 or more positive axillary nodes. Several studies have combined high dose alkylating agents with marrow support for patients with metastatic breast cancer (62,63,64,65). Peters noted a 27% response rate even in patients with advanced refractory disease. Patients with disease responsive to an Adriamycin-containing induction regimen showed a higher response rate at 65% with a 25% freedom from progression at 24 months (66). A regimen of high dose carboplatin, cyclophosphamide, and thioTEPA for patients with metastatic breast cancer showed a high response rate with acceptable toxicity (67).

There is now mounting evidence that toxicity can further be reduced through the use of hematopoietic colony stimulating factors. Granulocyte-monocyte colony stimulating factor (GM-CSF) in particular has been used to reduce the duration of pancytopenia in bone marrow transplant patients (68-70). Peters' experience suggests that infections and importantly other complications of high-dose therapy may be reduced by the use of GM-CSF (65). Committed hematopoietic progenitor cells are present in human peripheral blood (71). Kessinger et al, reported in 1988 the successful engraftment in ten of ten patients with advanced malignant diseases involving the bone marrow using peripherally derived stem cells (72). Antman and others showed increased numbers of CFU-GM and BFU-E in peripheral blood in the majority of patients treated with GM-CSF (73). Peters and others collected GM-CSF primed peripheral stem cells following bone marrow collection and then infused both bone marrow and peripheral stem cells after intensive therapy and showed a reduction in the duration of leukopenia (74). The combination of GM-CSF augmented peripheral cells and bone marrow stem cell infusion with GM-CSF administration after reinfusion should maximally speed hematopoietic recovery and thereby minimize toxicity. The use of high dose chemotherapy with autologous marrow and stem cell support in patients with locally advanced breast cancer who achieve a partial clinical response to induction chemotherapy has the potential for enhancing tumor cyto-reduction, increasing the likelihood of obtaining a complete response, with the potential for an improved overall and disease-free survival.

In a pilot study performed at John Hopkins Oncology Center, 30 women with recurrent breast cancer whose disease was responding to outpatient therapy received high-dose consolidation with cyclophosphamide 6 g/m^2 and ThioTEPA 800 mg/m^2 given over 4 days by continuous infusion with subsequent autologous marrow reinfusion(75). Outpatient induction chemotherapy consisted of the multidrug dose intense 16-week regimen described by Abeloff et al and 24 of 30 patients responded with a complete response (CR) or partial response(PR). In all cases the marrow was purged with 60 ug/ml 4-hydroperoxycyclophosphamide and in all cases engraftment occurred. Two treated patients developed high grade IV nonhematologic toxicities. Specifically, no evidence of veno-occlusive disease occurred. Two of the original 29 patients on this study died; one of hemorrhagic cystitis and one of sepsis. The

overall CR rate following high-dose consolidation was 37%, and 13% of transplanted patients remain in remission without further therapy a median of 46 months after initiation of therapy (76). The median time to progression on this study was 13 months and median survival 22 months.

More recently, investigators from John Hopkins Oncology Center treated 51 patients with advanced breast cancer in a similar fashion with 2 exceptions, that being a variety of induction regimens and by using cyclosporine (CSA) to induce graft-versus-host disease (GVHD) which is associated with an anti-tumor effect (76). Results indicated that GVHD would safely be induced in a dose dependent fashion by CSA. Determination of GVHD efficacy requires further investigation.

Peters et al recently reported their experience in 102 women with Stage II and III breast involving 10 or more lymph nodes (77). Patients were treated with four cycles of standard CAF followed by dose-intense therapy with cyclophosphamide, cisplatin and carmustine with ABMT. The therapy-related mortality was 12% and 31% experienced pulmonary toxicity. At median follow-up of 2.5 years, the actuarial event-free survival is 72% compared with historical reports of 38% and 52%. These results warrant evaluation in a prospective randomized study such as ECOG 2190, described below, to fully evaluate the role of HDC/ABMT in the adjuvant treatment of breast cancer patients at high risk for recurrence.

1.4 Peripheral Stem Cells

Early hemopoietic precursor cells are present in the peripheral blood (71). These cells can be identified by their differing expression of the MHC class II antigen HLA-DR and the CD34 antigen. Both antigens are lost during differentiation to a mature myeloid cell (HLA-DR-, CD34). The peripheral blood contains 50-100 CFU-GM/ml and 150 BFU/ml. Repeated leukopheresis can remove a sufficient concentration of peripheral stem cells to enable reconstitution of the bone marrow after myeloablative therapy. This was demonstrated in 48 pts. with CML given myeloablative chemotherapy +/- TBI followed by PBSCT. Forty-three pts engrafted (91.5%) with a mean of 11.5 d to achieve 500×10^6 granulocytes/L (76). Two studies of pts with solid tumors, including breast cancer, received myeloablative chemotherapy with hemopoietic rescue by PBSCT. All patients engrafted all hemopoietic lineages. No difference was found in the ability to produce engraftment when progenitor cells were obtained from the bone marrow or peripheral blood. (CD34+) (72,79).

1.5 Colony stimulating factors with high dose chemotherapy and stem cells.

Colony stimulating factors (CSFs) are glycoproteins that induce proliferation and differentiation of hematopoietic progenitor cells. Both G and GM-CSF have been shown to shorten the time to initiation of hematopoietic proliferation, shorten the mean cell cycle and sustain hematopoietic precursors through complete cell cycles. When applied in vivo, both G-CSF and GM-CSF can

substantially shorten the period of severe neutropenia that follows conventional and high dose chemotherapy with autologous bone marrow infusion (68,80). An important and unexpected finding during the early studies of growth factors was the high numbers of circulating progenitor cells induced in patients receiving G-CSF and GM-CSF.

When GM-CSF was given to patients before and after cytoreductive chemotherapy, an 18-fold increase in CFU-GM occurred in 75% of the patients (73). The greatest increase occurs when GM-CSF is given during a recovery period from chemotherapy. In a study from Milan, patients treated with high dose cyclophosphamide developed a five-fold increase in circulating CD34 cells, when GM-CSF was given (81). At Duke, GM-CSF was used to increase circulating CD34 positive progenitor cells which were obtained by leukopheresis and reinfused as bone marrow rescue following myeloblastic chemotherapy. GM-CSF was used after reinfusion to enhance myeloid recovery and resulted in adequate recovery of granulocytes and a reduced number of infections when compared to historical controls (82).

G-CSF has also been shown to be a powerful stem cell stimulant. Sheridan, treated 17 patients with non-myeloid malignant disorders with G-CSF, 12 mcg/kg daily x 6 days by subcutaneous infusion (83). The numbers of granulocyte macrophage progenitors in peripheral blood increased a median of 58-fold over pre-treatment values, and the numbers of erythroid progenitors increased a median of 24 fold. With 3 leukophereses, a mean total of 33×10^4 CFU-GM were collected per kg body weight. In this study, not only was there a rapid recovery of neutrophils, but platelet recovery was significantly faster in these patients than in controls who received the same treatment apart from the infusion of peripheral blood progenitors (83). In another study, peripheral blood stem cells mobilized by chemotherapy and G-CSF resulted in complete reconstitution after myeloablative therapy in seven children with neuroblastoma and non-Hodgkin's lymphoma (84).

- 1.6 This study will evaluate two different types of treatment in patients with locally advanced inoperable or inflammatory breast cancer who achieve either a complete (CCR) or partial (CPR) clinical response to four cycles of intensive Doxorubicin induction chemotherapy. Patients who achieve a clinical complete response (CCR) will go on to mastectomy. Further treatment will depend on pathologic findings at mastectomy. Patients with only pathologic CR's would go on to radiation therapy with 2 cycles of Cytosan and 5-FU followed by CMFT to a total of 1 year from registration, and Tamoxifen indefinitely. Patients with microscopic and macroscopic residual disease at mastectomy will subsequently be treated with high-dose chemotherapy with Cytosan, Thiotepe, and Carboplatin with peripheral blood stem cell infusion (PBSC). Transplant will be followed by radiation therapy and Tamoxifen. Those patients who achieved only a clinical partial response (cPR) will undergo tissue biopsy or fine needle aspirate. If the biopsy is negative for malignancy, then patients will undergo mastectomy and treated as above. If the biopsy is positive for malignancy the the patient will be treated with Paclitaxel. After 2 cycles of Paclitaxel,

patients who achieve a cCR will go on to mastectomy. Subsequent therapy will be dependent on residual disease as above. Patients who achieve a CPR after Paclitaxel will receive high-dose therapy with PBSC followed by mastectomy, radiation therapy and Tamoxifen. Patients with stable disease (SD) or progressive disease (PD) will come off study. Tamoxifen will be continued indefinitely in the three groups. Patients with stable or progressive disease will be removed from the study.

Local-regional treatment in this protocol will consist of both mastectomy and radiotherapy. The rationale for the combined treatment is related to optimizing local-regional control. Several prospective randomized trials have demonstrated that with chemotherapy and radiotherapy or chemotherapy and surgery for locally advanced breast cancer, local-regional recurrence rates range from 20 to 40% (21,85) with those undergoing chemotherapy and surgery doing somewhat better (21,85). Multiple retrospective studies have demonstrated enhanced local-regional control with the use of mastectomy and radiotherapy for the treatment of locally advanced and inflammatory breast cancer (13,14,86) and retrospective series have demonstrated a significant improvement in survival and disease-free survival with the combined modality approach (14,87,88,89). As well, it has been demonstrated that 50 to 60% of patients who achieve a complete clinical response to induction chemotherapy have residual tumor in the breast and/or axilla at the time of mastectomy (27,28,90,91,92,93). In patients with locally advanced or inflammatory breast who achieve a complete clinical response to induction chemotherapy and who undergo radiotherapy as the sole local regional treatment local-regional recurrence rates have ranged from 30 to 60% (85,87,94). Therefore, in the present study, local-regional treatment will consist of both mastectomy and radiotherapy. In patients who achieve a complete clinical response, mastectomy will precede radiotherapy.

This pilot study of women with locally advanced inoperable breast cancer who achieve a response to induction chemotherapy will evaluate the impact of subsequent autologous marrow transplant on disease-free and overall survival in patients who have macroscopic residual disease at mastectomy and those who have achieved a clinical PR to Doxorubicin. Due to the rigors of the marrow transplant, the age limit for the procedure will be 60 years of age. Patients will be followed for toxicity, time to progression, and overall survival.

1.4 Drug Resistance

1.41 Drug resistance is a major obstacle in the treatment of cancer. The multidrug resistance gene (MDR1) encodes an energy dependent drug efflux pump, P170, that confers cellular resistance to multiple therapeutic agents such as anthracyclines, vinca alkaloids, epipodophyllotoxins, paclitaxel, and actinomycin-D(95-98). MDR1 gene expression is tumor specific in both *de novo* resistant tumors and those that acquire drug resistance following chemotherapy(99). The central role of P-170 in this 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.

1.42 Multidrug Resistance in Breast Cancer

Although there is a significant response rate to doxorubicin in breast cancer, resistance and relapse are the usual outcome in advanced disease. The role of MDR in breast cancer has been studied by several investigators as seen in Table 1.

Table 1: MDR1 GENE EXPRESSION IN BREAST CANCER

STUDY	METHOD	UNTREATED	TREATED
Goldstein et al(99)	RNA Slot Blot, RNase Protection Assay	3/57(16%)	3/77(71%)
Wallner et al(100)	RNA Slot Blot	17/59(29%)low 10/59(17%)high	
Kaich et al(101)	RNA Dot Blot Northern Blot	35/19(61%)	
Merikal et al(102)	Southern, Northern or Western Blot	0/219	0/29
Gerlach et al(103)	Western Blot C219, C494	0/3	
Schneider et al(104)	Immunohistochemistry C219	2/12(16%)	3/4(→non-MDR- 2/4(→MDR drugs) 3/7(→MDR drugs)
Wishart et al(105)	Immunohistochemistry C219 ⁺ , MRK16	21/29 ⁺ (72%) 16/29 (55%)	
Ro et al(106)	Immunohistochemistry C219	0/8	20/40(50%)
Verelle et al(107)	Immunohistochemistry C494	17/20(85%)	
Sugawara et al(108)	Immunohistochemistry MRK16	1/9(11%)	
Sandilippo et al (109)	Immunohistochemistry C219	10/34(29%)	9/14(64%)

* Samples not obtained sequentially pre- and post-treatment in the same patients.

→ isolated tumor cells identified as MDR-1 positive
→ 20% tumor cells identified as MDR1 positive

The results of previously published studies of the role of the MDR1 gene in human malignancies are limited in several ways. A detailed analysis of the results from several studies of MDR1 gene expression in breast cancer from Table 1 is an illustration of these points. Many early studies were limited by small sample size which is illustrated by Ro, Sugawara and Gerlach whose studies demonstrate little to no MDR1 gene expression but the denominator is too small to reach any conclusion about the significance of the results(102,105,100). In addition, most of these studies except that of Wallner and Verelle are retrospective analyses done on archival frozen tissue without any clinical correlation(100,104). This may result in possible selection

bias. Verelle et al, used an anti P-170 monoclonal antibody C494 in a avidin-biotin-immunoperoxidase technique and detected P-170 in 17 of 20 breast cancer specimens(107). The authors used a semi-quantitative method of analysis by grading both the number of positive cells and the specific staining intensity. Though the numbers of patients in this study was limited, strong P-170 positive staining found in the majority of tumor cells significantly correlated with no initial response to chemotherapy ($p < .02$) and with a shorter progression free survival ($p < .02$). Further follow-up is needed to determine if Wallner's results from primary breast cancer specimens have any prognostic significance(100).

Another major limitation of these studies is the absence of sequential tumor sampling before and after treatment with a cytotoxic agent which is a substrate of P-170. Such sampling would enable us to delineate the role of MDR mediated intrinsic and acquired drug resistance in breast cancer.

Issues of method selection for detection of MDR1 gene expression (RNA vs. protein; quantitative vs. qualitative assays); selection of a definition of a positive control which would affect the sensitivity of the method chosen and the sensitivity and specificity of the given cDNA probes and monoclonal antibodies (MoAb) may all contribute to the disparate results noted in the untreated breast cancer studies.

Definition and significance of a positive result using immunohistochemistry may contribute to the inflated results seen in those studies using immunohistochemistry where most authors use any positive cells as a positive result. For example Schneider's result of 0 of 12 could be reinterpreted as 2 of 12 if any staining were considered positive as in the Wishart study(104,105). The major difference between immunohistochemical techniques and RNA analysis include measuring protein as opposed to RNA, however, neither method assesses protein function. Immunohistochemistry also has the advantage of analyzing individual cells such that tumor cell expression can be differentiated from adjacent normal cells and stroma whereas isolating RNA from a solid tumor lacks such discrimination. Of importance however is that Wishart recently reported that P-170 expression was noted in stromal cells in breast cancer but not those of normal breast tissue(110). Although immunohistochemistry is capable of detecting low levels of expression in individual cells, at the lower limit it may be incapable of distinguishing true expression from background and in that respect may not be as sensitive as detection of MDR1 RNA as PCR. In addition because of the heterogeneity of staining, immunohistochemistry is not as quantitative as RNA slot blots.

The other major factor is the sensitivity and specificity of the specific monoclonal antibody (MoAb) used. As noted,

Schneider found no expression of P-170(104). Verelle detected expression in 85% and found different results with different antibodies(107). The most commonly used MoAbs are C219 and MRK16. Others include C494, JSB-1 and HYB-241. C219, C494 and JSB-1 recognize internal epitopes while the others recognize external epitopes. Specificity is a significant issue in that C219 cross reacts with MDR2, which has not been demonstrated to confer drug resistance, and with myosin. MRK16 is specific for MDR1 but may have heterogeneous staining even in control cell lines. Differences in fixation techniques may also contribute to the variability in results even when the same antibody is used.

The aim of this proposal is to resolve these issues by prospectively analyzing breast cancer specimens before and after treatment with doxorubicin and Paclitaxel, and correlating response or resistance to MDR1 gene expression. We will also compare the various methods of measuring MDR1 gene expression with each other.

- 1.43 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 study 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. Breast cancer is an appropriate human tumor model for studying the role of the MDR1 gene 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. While Adriamycin is currently the most active drug in advanced breast cancer, the introduction of Paclitaxel, another substrate of P-170, into the clinical armamentarium of breast cancer treatment also requires a need for understanding its resistance. The subsequent initial goal of such studies 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.

2.0 OBJECTIVES

- 2.1 To assess the time to failure and overall survival in patients with locally advanced inoperable or inflammatory breast cancer who are stratified and subsequently treated depending on their response to intensive dose doxorubicin.

- 2.11 To assess the role of standard radiation therapy and maintenance combination chemotherapy in patients who achieve complete pathologic response to intensive doxorubicin.
- 2.12 To assess the role of high-dose chemotherapy with stem cell rescue in patients with partial pathologic responses to intensive doxorubicin by assessing time for relapse and overall survival.
- 2.13 To assess response to Paclitaxel after treatment with Adriamycin in patients who not achieve a clinical complete response.
- 2.2 To assess the toxicity of these treatment regimens.
- 2.3 To measure MDR1 gene expression and correlate with sensitivity and resistance to doxorubicin/Paclitaxel.

3.0 SELECTION OF PATIENTS

3.1 Eligibility Criteria: Induction Chemotherapy Phase

- 3.11 Patients must have histologically documented diagnosis of mammary adeno-carcinoma with inoperable locally advanced disease or inflammatory breast cancer.
- 3.12 T>5 cm with fixation to underlying pectoralis fascia.
- 3.13 T4: Tumor of any size with direct extension to chest wall or skin (excluding pectoral muscle). Grave signs allowed including fixation to chest wall, edema, including peau d'orange, ulceration of the skin of the breast or satellite skin lesions confined to same breast.
- 3.14 N₀, N₁, N₂, N₃ excluding supraclavicular nodes.
- 3.15 Patients must be age 18-60 inclusive.
- 3.16 Patients with ER positive, negative, or unknown are eligible.
- 3.17 Patients may be pre- or post-menopausal.
- 3.18 Patients must have ECOG performance status 0 or 1.
- 3.19 Patients must not have received prior chemotherapy or hormonal therapy for breast cancer.
 - 3.110 Patients must have measurable and/or evaluable disease. Initial tumor measurements must be obtained before beginning induction chemotherapy treatment and recorded on the flow sheets.
 - 3.111 Patients must have an absolute neutrophil count $\geq 1,500/\text{mm}^3$ and platelet count $\geq 100,000/\text{mm}^3$.

3.112 Patients must have adequate renal function (serum creatinine \leq 1.5 mg/dl and/or a creatinine clearance \geq 60 cc/min). These tests may be repeated after the patient is hydrated to demonstrate adequate function.

3.113 LVRF \geq 45% and no clinical cardiac arrhythmia or congestive heart failure.

3.2 Ineligibility Criteria

3.21 Failure to meet any of the criteria in Section 3.11.

3.22 Any radiographic, clinical or pathologic evidence of metastatic breast cancer.

3.23 Patients with a history of another malignant neoplasm are ineligible with the exception of curatively treated basal cell or squamous cell carcinoma of the skin, or carcinoma in situ of the cervix. Patients who are >10 years since curative treatment for another neoplasm will be eligible.

3.24 HIV positive or clinical AIDS.

3.25 Patients with severe medical or psychiatric illness are ineligible. These will include renal failure, active infections, active peptic ulcer disease, brittle insulin dependent diabetes, congestive heart failure, myocardial infarction within the past six months, cardiac arrhythmia requiring medication, poorly controlled hypertension, history of hospitalization for psychiatric illness including severe depression or psychosis, significant non-neoplastic pulmonary disease, current alcohol or drug abuse and patients within two weeks of major surgery.

3.26 Pregnant or lactating women.

3.3 Eligibility Criteria (Stem Cell Transplant or Maintenance Therapy)

3.31 Patients must be in documented CR or PR (see Section 6.0 for Response Criteria) following induction chemotherapy.

3.32 Patients must be within eight weeks of last dose of chemotherapy or within 4 weeks, maximum of eight weeks of mastectomy if PPR with macroscopic residual disease or if in CPR

3.33 Bilateral bone marrow biopsies and aspirates conducted at referral center must show normal $> 30\%$ cellularity and no neoplastic involvement by conventional methods. If hypocellular but otherwise normal marrow, biopsy may be repeated in two weeks to assess for eligible cellularity.

3.34 Patients must have an absolute neutrophil count \geq 1500/mm³ and a platelet count \geq 100,000/mm³.

- 3.35 Patients must have a gated pool cardiac nuclear scan with normal ejection fraction (not < 45%).
- 3.36 Patient must not have significant pulmonary symptoms and must have adequate pulmonary function including diffusing capacity > 50%.
- 3.37 Patients must have serum creatinine \leq 1.5mg/dl and/or creatinine clearance \geq 60cc/min.
- 3.38 Patients must have adequate hepatic function (bilirubin \leq 2.0 mg/dl, SGOT (AST) and alkaline phosphatase \leq 2 x the upper limit of normal). Patients with transaminase and alkaline phosphatase elevations clearly due to responding metastatic disease with values \leq 4 x upper limit of normal will also be eligible.

4.0 REGISTRATION PROCEDURES

To register a patient on study, contact Cheryl Sickles (X6394) to provide patient name, record number and review data for eligibility criteria.

5.0 TREATMENT PLAN

5.1 Induction Chemohormonal Therapy

The goal of induction therapy is rapid maximal cytoreduction prior to consolidation therapy or maintenance therapy.

5.11 Intensive Doxorubicin Plus Tamoxifen

5.111 Administration Schedule

Doxorubicin: 30 mg/m² IV bolus on days 1,2,3 given IV push through free flowing IV of NS) repeated every 28 days for 4 cycles
Tamoxifen: 10mg p.o. B.I.D.

5.112 Dose Modification

5.1121 Hematologic Toxicity

Neutrophils

Day 1 of cycle \leq 1,500 granulocytes/ul Hold 1 week
 \geq 1,500 granulocytes/ul give 100% dose

No adjustments are to be made based on day 2 or 3 counts. If counts are not adequate for treatment at week 5, perform bone marrow aspiration and biopsy and call study chair. G-CSF is permitted.

Nadir counts

If ≤ 500 granulocytes for more than 7 days (ie. two separate weeks apart) reduce dose by $5\text{mg}/\text{m}^2/\text{day}$ for all three days of all subsequent cycles.

If patients require admission for treatment of neutropenic fever, reduce dose by $5\text{mg}/\text{m}^2/\text{day}$ for all three days of all successive cycles.

Platelets

Day 1 of cycle $\geq 100,000/\text{ml}$ - give 100% dose
 $\leq 100,000/\text{ml}$ - hold therapy 1 week

No adjustments are to be made based on day 2 or 3 counts.

If counts are not adequate for treatment at week 5, perform bone marrow aspiration and biopsy and call study chair.

Nadir counts

If $\leq 50,000$ on two occasions one week apart reduce dose by $5\text{mg}/\text{m}^2/\text{d}$ for all days of all successive cycles.

If platelet transfusion is required at any time reduce dose by $5\text{mg}/\text{m}^2/\text{d}$ for all days of all successive cycles.

If bleeding requiring RBC transfusion is manifest at any time with platelet count $\leq 50,000$ reduce dose by $5\text{mg}/\text{m}^2/\text{d}$ for all days of all successful cycles.

Nausea and vomiting

Mild (≤ 4 episodes/day) give 100% dose. Moderate (5-10 episodes/day) or severe requiring IV fluids, reduce by $5\text{mg}/\text{m}^2/\text{day}$ next cycle only.

Mucositis

If unable to ingest any solid food, reduce by $5\text{mg}/\text{m}^2/\text{d}$ for all days in all successive cycles.

Diarrhea

If diarrhea occurs sufficient to cause clinical dehydration, reduce by $5\text{mg}/\text{m}^2/\text{day}$ for all days of all successive cycles.

Cardiac Toxicity

Any patient developing new symptomatic arrhythmias while receiving doxorubicin will be removed from therapy.

The maximum total dose of Doxorubicin is limited to < 500 mg/m².

Doxorubicin will be stopped if a significant absolute drop (>10%) in the LVEF has occurred; and/or LVEF drops into the abnormal range of if clear cut CHF or arrhythmias develop.

Hepatic toxicity

Adjust doses on day 1 of therapy as follows:

Bilirubin normal and SGOT \leq 1.5 x normal give full dose.

Bilirubin \leq 1.5 x normal or SGOT \leq 2.5 x normal reduce dose by 5mg/m²/day for all days of next cycle.

Bilirubin \leq 2.5 x normal or SGOT \leq 4 x normal reduce dose by 10mg/m²/d for all days of next cycle.

Bilirubin > 2.5 x normal or SGOT > 4 x normal discontinue drugs.

5.113 Duration of therapy

5.1131 Patients are to complete four cycles of induction chemotherapy with intensive doxorubicin. The goal is to achieve CR or PR as soon as possible.

5.1132 Development of new local, regional or distant recurrence is grounds for removal of a patient from study. This must be documented on flow sheets. Biopsy of recurrence is encouraged.

5.1133 Unacceptable toxicity from therapy after attempts to modify treatment so it is acceptable will constitute grounds for a patient's discontinuation of treatment but will not be grounds for removal from study. This must be documented on flow sheets and the registration office must be notified.

5.1134 Withdrawal of consent will automatically remove a patient from study; the reason must be documented on the flow sheets.

5.114 Assessment of Response to Induction Chemotherapy

Within four weeks following the completion of the induction chemotherapy, complete re-staging will be performed as defined in Section 7.0. Those patients who achieve a clinical complete remission (cCR) will undergo modified radical mastectomy. Based on pathologic response in modified radical mastectomy specimen, patients will either receive standard radiation therapy and chemotherapy or high-dose therapy with transplant. Patients who achieve a clinical

partial remission (CPR) will undergo 3-4 fine needle aspirate, core biopsy, or punch biopsy. If the biopsy is negative for malignancy, the patient will go on to mastectomy as above. If the biopsy is positive for malignancy, the patient will go on to receive Paclitaxel (5.2). After Paclitaxel patients who achieve CCR will go into mastectomy as above. Patients who achieve a CPR will go onto high-dose therapy with PBSC and followed by mastectomy and radiation. All patients with CCR or CPR should be seen at a transplant center prior to mastectomy. At that time, a discussion of subsequent treatment and explanation of both arms should be pursued. Patient will undergo bilateral marrow aspiration and biopsies to assess for the presence of tumor and cellularity.

5.2 Salvage Chemotherapy

Patients who do not achieve a CCR or are biopsy positive positive CPR after four cycles of Doxorubicin will receive 2 cycles of Paclitaxel.

5.21 Paclitaxel Administration Schedule

175 mg/m² over 24-hrs. continuous infusion
q 28d x 2 cycles

Paclitaxel must be filtered. In line filtration with a 0.2 filter is required. It may be diluted in 0.9% sodium chloride injection, USP or 5% dextrose injection, USP, 1000 ml, given over 24 hours (45 ml/hr). Paclitaxel must be prepared in glass bottles and administered with nitroglycerin administration sets (polyethylene line PVC tubing). Treatment will be repeated every 3 weeks.

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

5.22 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:

- 5.221 1 hour prior to the Paclitaxel administration, the patient will be medicated with Diphenhydramine 50 mg IV, Cimetidine 300 mg. IV, and Dexamethasone 20 mg. IV.
- 5.222 Epinephrine and Diphenhydramine will be immediately available during the infusion.
- 5.223 The patient's blood pressure and heart rate will be monitored during the infusion (every 15 minutes during the first hour and then every 4 hours during the remainder of the 24 hour infusion).
- 5.224 If, during the infusion, a patient develops chest pain, hypotension or a cardiac rhythm disturbance that is not accompanied by other signs of anaphylaxis, the infusion

should be stopped, but may be restarted at half the original infusion rate when symptoms resolve. To prevent allergic reaction when restarting at one-half the original infusion rate, retreat with Diphenhydramine and Cimetidine and increase the blood pressure monitoring interval to every 15 minutes x 1 hour.

5.3 Colony stimulating factors (G or GM-CSF) may be given at the discretion of the investigator during induction Doxorubicin and salvage Paclitaxel treatment.

5.4 Peripheral Stem Cell Transplant

5.41 Five days prior to the start of peripheral stem cell harvest, patients will receive G-CSF at a dose of 10 ug/kg subcutaneously daily to be continued until completion of the harvest. Should toxicity occur, dose modifications of G-CSF will be made as outlined in section 5.44. Peripheral blood mononuclear cells are harvested using a cell separator (such as Fenwall CS 3000) by 4 hour (10 liter) continuous flow leukapheresis. The pheresis procedure involves the placement of a 16 gauge needle in each antecubital vein or the use of large bore central venous catheters in patients with poor venous access to facilitate leukapheresis. Whole blood is drawn from one vein and processed in the apheresis device for the separation of mononuclear cells (stem cell fraction). The remainder of blood (white blood cells, platelets and red blood cells) are returned to the patient via the other vein. This procedure is usually well-tolerated and carries the minor risks associated with venipuncture. Immediately following the four hour collection, a cell count and differential are conducted on an aliquot of the total product to determine the number of mononuclear cells collected. Collections will continue on days 5, 6, and 7 until a minimum of 1.2×10^9 mononucleated cells/kg are obtained. The cells are pooled and a pre-cryopreserved sample is saved for further analysis. The remaining product is diluted with autologous plasma to a concentration of approximately 8×10^7 nucleated cells/ml and then diluted with an equal volume of freezing solution containing 10% irradiated autologous plasma, 20% DMSO and 70% RPMI solution. The cells are cryopreserved in approximately 100 ml aliquots using a programmable freezer and stored at 196°C . Samples for viability and culture will be obtained after processing.

5.42 High-Dose Chemotherapy Regimen
Transplantation Schema

	-6	-5	-4	-3	-2	-1	0	+17	>10,000
Cyclophosphamide 1500 mg/M ² /d in 3L DSNS contin. infusion	x	x	x	x					
ThioTEPA 125 mg/M ² /d in 1L DSNS continuous infusion	x	x	x	x					
Carboplatin 200mg/M ² /d in 1L DSNS continuous infusion	x	x	x	x					
Peripheral Stem Cell Reinfusion							x		
G-CSF X 10 ug/kg/d							x>	X
Acycloir (if indicated)				X>				X

Treatment is by continuous infusion with each agent through a separate Hickman Port. Bone Marrow is reinfused day +30 if there is no evidence of engraftment (ANC<100). G-CSF is continued until ANC>10,000/mm³ for 2 days.

5.421 Cyclophosphamide

Cyclophosphamide is given as a continuous intravenous infusion of 500 mg/M² in 1000 cc D₅NS every 8 hours on days -6 through -3. This should result in a total dose of 6 grams/M² of Cyclophosphamide administered as a continuous intravenous infusion in 12 liters of D⁵NS over 96 hours.

5.422 ThioTEPA

ThioTEPA is given as a continuous intravenous infusion of 125 mg/M² in 1000 cc D₅NS every 24 hours on days -6 through -3. This should result in a total dose of 500 mg/m² of thioTEPA administered as a continuous intravenous infusion in a 4 liters of D₅NS over 96 hours.

5.423 Carboplatin

Carboplatin is given as a continuous intravenous infusion of 200 mg/M² in 1000 cc D₅NS every 24 hours on days -6 through -3. This should result in a total dose of 800 mg/M² of Carboplatin administered as a continuous intravenous infusion in 4 liters of D⁵NS over 96 hours.

Note: The calculation of body surface area for cyclophosphamide, thioTEPA, and carboplatin is based on on ideal body weight or real weight, whichever is less.

5.424 Dose Modification

No dose reduction or escalation will be performed with cyclophosphamide, thioTEPA, or carboplatin.

5.43 Peripheral Stem Cell Reinfusion

Forty-eight hours after the completion of the protocol chemotherapy (Day 0), peripheral stem cell reinfusion occurs. Stem cell infusion was set for this day to exactly parallel the original regimen as reported by Antman (45). Peripheral stem cells are not washed before reinfusion and the osmolality of the cell suspension requires the use of a flowing central venous catheter. Twelve hours prior to reinfusion, hydration must be initiated with 0.5NS to ensure urine output of at least 3cc/kg/hr. NaHCO³ should be added to the hydration fluid to ensure an alkaline urine (ph<7). Emergency drugs: benadryl, epinephrine, and solumedrol, in appropriate doses must be at the bedside. Baseline vital signs are recorded. The patient is connected to a telemetry monitor during the infusion. Fifteen minutes prior to administration of thawed stem cells, the patient receives: 50ml of 25% mannitol solution IV, 50 mg benadryl IV, 250 mg solucortef IV. These drugs are to ensure

good urine flow and minimize the effects of DMSO-mediated histamine release. The bags of stem cells will be quickly thawed in a 37⁰ water bath in close proximity to the patient's room to minimize transit time and pulled into 50 ml syringes. The stem cells are then infused intravenously, without an in-line filter, over 3 to 5 minutes each. If the patient develops chest tightness or other symptoms, a short rest period may be required. Usually a 30-60 minute rest is required after 6-8 syringes. No additional pre-medication need be given after the rest period. Only one bag of stem cells should be thawed at a time and not until the preceding bag has been completely infused. A physician must be present during the stem cell infusion and for one hour afterwards. A nurse familiar with the adverse signs of blood transfusion should monitor signs between each stem cell infusion and every 15 minutes for one hour after the completion of the infusion. The number of infused peripheral mononuclear cells will be calculated and recorded on the Stem Cell Reinfusion Form 7.

5.44 Granulocyte Colony Stimulating Factor (G-CSF) Administration After Peripheral Stem Cell Reinfusion.

G-CSF will be administered at a dose of 10 ug/kg actual weight intravenously as a 30 min IV infusion or subcutaneous bolus from the day of peripheral stem cell reinfusion (Day 0) until an absolute neutrophil count > 10,000/mm³ has been maintained for 2 consecutive days. Once the ANC > 500/mm³, and antibiotics have been stopped, further doses of G-CSF may be given subcutaneously at the same dose at home.

5.441 Dose Modification for G-CSF

The investigator should seek to elicit any objective reaction(s) from patients being treated and determine the relationship to G-CSF. The duration of reaction(s) will be documented, i.e. hours, days, weeks; as well as whether the reaction(s) are intermittent or continuous. The reaction(s) will be graded according to the Common Toxicity Criteria (Appendix II) on the following scale:

Grade 1=mild
Grade 2=moderate
Grade 3=severe
Grade 4=life-threatening

5.442 Grade 1 or 2 Reaction

Patients who experience Grade 1 or 2 adverse reactions may continue treatment at the same dose as long as there is no progression to Grade 3.

5.443 Grade 3 or 4 Reaction

Any patient who experiences Grade 3 or 4 toxicity which is attributable to G-CSF should receive no further growth factor therapy until resolution of the adverse

reaction. PATIENTS WITH GRADE 4 TOXICITY MAY NOT BE RETREATED WITH G-CSF. Patients with Grade 3 toxicity may be retreated after resolution of the toxicity at 50% of the prior dose. Recurrence of the same toxicity (Grade 3 or 4) despite dose reduction will require permanent withdrawal of G-CSF. One exception to this is bone pain. Up to 20% of patients will experience Grade 3 or 4 bone pain that responds to non-narcotic analgesics. Patients who do not respond will have dose reduction as indicated above.

5.45 Supportive Measures

5.451 Venous Access

All patients are required to have triple-lumen indwelling silastic catheters placed prior to the initiation of therapy. Care and maintenance of these catheters will be at the discretion of the individual investigator.

5.452 Hydration

All patients should be well hydrated prior to the initiation of chemotherapy and at the time of bone marrow reinfusion. As the chemotherapy is administered in a volume of five liters per day, additional hydration during the period of chemotherapy administration will not be necessary in most instances. Supplemental hydration or Lasix will be administered as indicated at the discretion of the individual investigator.

5.453 Prophylaxis of Hemorrhagic Cystitis

High-dose Cyclophosphamide therapy has been associated with the development of hemorrhagic cystitis. To prevent this complication, patients will be well hydrated throughout the period of Cyclophosphamide administration. In addition, continuous bladder irrigation will be performed through a three-way Foley catheter, which will be inserted prior to the initiation of chemotherapy and will remain in place for at least twelve hours after the completion of chemotherapy. The management of the continuous bladder irrigation will be at the discretion of the individual investigator.

5.454 Mucosal Evaluation and Care

Mucositis is expected to be severe during the period of high-dose chemotherapy and autologous bone marrow transplantation, and patients will be encouraged to seek dental consultation prior to the initiation of therapy. Patients will be started on an intensive oral

hygiene regimen at the beginning of the high-dose chemotherapy and will continue throughout the period of hospitalization. The nature of the oral hygiene regimen will be at the discretion of the individual investigator.

5.455 Prophylactic Gut Decontamination

Prophylactic gut decontamination with oral anti-bacterial antibiotics is not allowed.

5.456 Anti-fungal Prophylaxis

Patients will receive Nystatin and/or Clotrimazole, and/or Diflucan in any topical or oral form four times daily and additionally as needed beginning prior to the initiation of high-dose chemotherapy and continuing until discharge as prophylaxis against oral thrush. The specific agent will be left to the discretion of the investigator.

5.457 Anti-viral Prophylaxis

Patients who have positive cytomegalovirus titers will receive Acyclovir 500 mg/M2 intravenously every eight hours beginning on the last day of high-dose chemotherapy (day -3) until seventeen days post-bone marrow reinfusion (day +17). Patients who are CMV negative but have positive herpes simplex virus titers will receive Acyclovir 250 mg/M2 intravenously beginning on the last day of high-dose chemotherapy (day -3) until seventeen days post-bone marrow reinfusion (day +17). Patients who have negative cytomegalovirus titers and negative herpes simplex virus titers will not receive prophylactic Acyclovir.

5.458 Anti-Pneumocystis carinii Prophylaxis

Prophylactic therapy against Pneumocystis carinii pneumonia with Trimethoprim-sulfamethoxazole, Pentamidine, or other antiparasitic agent is not allowed.

5.459 Anti-emetics

The use of anti-emetics during the high-dose chemotherapy and in subsequent management will be at the discretion of the individual investigator.

5.4510 Protective Isolation

Patients will be housed in protective (reverse) isolation rooms from the time immediately following bone marrow reinfusion until the end of neutropenia, defined as three consecutive days with an absolute

granulocyte count of $>500/\text{mm}^3$. Either laminar air-flow rooms or high-efficiency particulate air filtered (HEPA-filtered) rooms will be satisfactory for this purpose.

5.4511 Nutrition Evaluation and Therapy

All patients will be placed on a low bacterial, modified-protective (no fresh fruits or vegetables) diet at the initiation of the high-dose chemotherapy and will be continued on this throughout the remainder of the hospitalization. Intravenous hyperalimentation will be instituted at the discretion of the individual investigator when oral intake becomes inadequate.

5.4512 Blood Component Support Guidelines

5.45121 Packed red cell transfusions will be given for a hemoglobin < 8 grams %.

5.45122 Platelet transfusions will be given to maintain the platelet count $> 20,000/\text{mm}^3$.

5.45123 Patients who have negative cytomegalovirus titers should receive CMV-negative blood products.

5.45124 All blood products will be irradiated (2,000-3,000 rads) to prevent graft-versus-host disease and transfused through leukocyte filters.

5.4513 Guidelines for Management of Fever/Infection

5.45131 Patients with neutropenia (absolute neutrophil count $< 500/\text{mm}^3$) will be started on broad-spectrum empiric antibacterial antibiotics for fever ($> 101^\circ\text{F}$ or 38.2°C) after appropriate cultures (blood, urine, and any sites with signs or symptoms of infection) have been obtained. Patients with confirmed infections will be started on antibiotics regardless of ANC. Duration of antibiotic use will generally continue until the patient is afebrile with ANC > 500 for 2 consecutive days; this will also apply to patients with absence of confirmed infection. The selection of antibiotics will be left to the discretion of the individual investigator and should be based on the pattern of sensitivity of the organisms at the particular transplant center, but should probably include either a higher-generation semi-synthetic penicillin or higher-generation cephalosporin and gentamicin.

- 5.45132 Patients with a documented Herpes virus infection (including stomatitis and cutaneous zoster) will receive acyclovir 500 mg/M² intravenously every eight hours for 10 days.
- 5.45133 Patients with a documented fungal infection or with persistent unexplained fever that is unresponsive to at least 48 hours of broad-spectrum empiric antibiotics will receive intravenous Amphotericin B. The method of administration will be at the discretion of the individual investigator.
- 5.45134 Patients with fever and diffuse pulmonary infiltrates present a difficult diagnostic problem. Whenever possible, a tissue diagnosis should be attempted by bronchoscopy or open lung biopsy. In the absence of a tissue diagnosis, empiric antibiotic therapy should be expanded to include the potentially treatable etiologies.

- 5.46 Radiation Therapy (see Appendix I)
- 5.47 Tamoxifen 20 mg/day p.o. indefinitely
- 5.48 Adverse Reaction Reporting Requirements

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

5.481 Deaths

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 the protocol treatment has ended but which is felt to be treatment-related must be reported as follows:

- 5.4811 A telephone call must be made to the Central Registration Office (215-662-6394) and to the NCI (301-496-7957) within 24 hours of the event. An NCI Adverse Drug Reaction Form must be sent to the NCI and the Central Registration Office within 10 working days of the event.

NOTE: Mailing address for the NCI is:
Investigational Drug Branch
P.O. Box 30012
Bethesda, MD 20824

- 5.482 Unknown Reactions

5.4821 Grade 4 or 5 - any unknown life-threatening or lethal (Grade 4 or 5) reactions must be reported to the NCI (301-496-7957) and the Central Registration Office (215-662-6394) within 24 hours of the event. An NCI Adverse Drug Reaction Form must be sent to the NCI and the Central Registration Office within 10 working days of the event.

5.4822 Grade 2 or 3 - for any unknown Grade 2 or 3 toxicity an NCI Adverse Drug Reaction Form must be sent to the NCI and the Central Registration Office within 10 working days of the event.

5.483 Expected Grade 4 Toxicities

5.4831 Grade 4 expected toxicities must be reported by submitting an NCI Adverse Drug Reaction Form must be sent to the NCI and the Central Registration Office within 10 working days of the event.

NOTE: The exception to this is expected Grade 4 myelosuppression which need not be reported.

5.5 Conventional Therapy with Radiation, Chemotherapy, and Tamoxifen Response

5.51 Radiotherapy

Patients will receive radiotherapy (see Appendix I) with concurrent Cytosan (100 mg/m² po d1-14) and 5-FU (600 mg/m² IV d 1,8) for 2 cycles. Patients will continue to receive Tamoxifen.

5.52 CMF(T) Within four weeks of mastectomy, CMFT will be initiated.

Cyclophosphamide (CTX) 100mg/M² PC daily 1 through 14 as a single daily dose

Methotrexate (MTX) 40mg/M² IV Day 1 and 8

5-Fluorouracil (5-FU) 600mg/M² IV Day 1 and 8

Tamoxifen 20 mg po/day

Repeat cycles every 28 days for a total of one year. Tamoxifen will be continued indefinitely.

Day 1 and 8 Reductions

If absolute neutrophil count (ANC) <1,600 or platelets <100,000 on day 29, on any cycle, treatment is delayed for one week.

After 1 week delay, if absolute neutrophil count is <1,600 or platelets <100,000, day 1, treatment will be given as per table below. Also apply those guidelines on day 8 of the regimen if absolute neutrophil count is <1,600 or platelets <100,000.

Day 1 or 8 Count

	<u>ANC</u>	<u>Platelet</u>	<u>Percent Dose</u> <u>CTX, S-FU, MTX</u>
	1000-1600	75-100,000	75
	500-1000	50-75,000	50
	<500	<50,000	25

5.521 Renal Dysfunction

<u>Serum</u> <u>Creatinine</u>	<u>Percent</u> <u>MTX</u>
< 1.5	100
1.6-2.0	75
> 2.0	0

5.522 Hepatic Dysfunction

<u>Day 1</u>		<u>Percent Drug</u>
<u>SGOT</u>	<u>Bili</u>	<u>(CTX, MTX, S-FU)</u>
<300	<5	100
>300	>5	25

5.523 Infection

Clinical and/or laboratory evidence of a Grade 2 or worse infection is specifically a reason to reduce or omit therapy

5.524 Gastrointestinal Toxicity

5.5241 The appearance of severe anorexia, nausea, vomiting, diarrhea, stomatitis, dryness of the mouth, or epigastric pain will require that therapy with CMF be delayed until such symptoms improve. In the event of debilitating vomiting or diarrhea, Common Toxicity Criteria Grade 2 or worse, a 25% dosage reduction of CMF is recommended for the next dose with subsequent escalation to tolerance.

5.5242 If mucous membrane soreness or erythema occurs, Grade 2 or worse, the dosage of 5-FU and Methotrexate should be reduced by 50% during day 8 of that cycle. If mucous membrane soreness or erythema was present in the preceding cycle, the dosage of 5-FU and MTX should be reduced by 25% of the original dose. In the event of mucosal ulcerations,

Grade 3 or worse, hold 5-FU and MTX for the remainder of the cycle. If mucosal ulcerations occurred in the preceding cycle, 50% reduction of dosage of 5-FU and MTX should be made with escalation in subsequent cycles by 25% of original dose to tolerance.

6.0 MEASUREMENT OF EFFECT

6.1 Complete Remission (CR)

6.11 Clinical Complete Remission (CCR). The total disappearance of all reversible clinical evidence of disease maintained for minimum of 4 weeks.

6.12 Pathologic Complete Remission (PCR). This will be defined at the time of mastectomy and is the complete lack of histologic breast cancer throughout the surgical specimens.

6.2 Partial Response

6.21 Clinical Partial Response (CPR)

At least a 50% reduction in the size of all measurable tumor areas as measured by the product of the greatest length and the maximum width maintained for a minimum of 4 weeks. Where measurable lesions are non-existent, there must be a decrease of 50% or more in evaluable disease. These changes must be present in > 50% of the involved breast and overlying skin. No lesion may progress and no new lesion may appear.

6.22 Pathologic Partial Remission (PPR)

In patients who are found to be CCR who then undergo mastectomy, but are found to have microscopic histologic carcinoma in any section of the surgical specimen.

6.221 Microscopic residual disease. The presence of microscopic carcinoma in the histologic review of the breast or lymph node tissue, but is not felt to be in measurable quantities.

6.222 Macroscopic residual disease. Pathologically measurable disease in the breast or lymph node at the time of modified radical mastectomy

6.3 No Change (NC)

When a patient's status fails to qualify for either a response or progressive disease

6.4 Progressive Disease (PD)

Appearance within eight weeks of a new lesion, or an increase of 25% in the sum of areas of lesions within the breast or axillary lymph

nodes. Progressive disease occurring after eight weeks is called "stabilization with relapse".

6.5 Relapse

6.51 The appearance of new lesions.

6.52 The reappearance of old lesions in patients who achieved a complete remission. Relapse should be confirmed by tissue diagnosis (biopsy), whenever possible.

6.53 For patients in partial remission, an increase of 50% in the sum of the products of the diameters of all measured tumors over that which was obtained at the time of maximal regression.

7.0 STUDY PARAMETERS

7.1 General Considerations

7.11 All scans and X-rays will be conducted within 4-6 weeks prior to randomization or registration.

7.12 CBC, differential and platelets and all chemistries used to determine eligibility will be conducted within two weeks prior to registration or randomization and repeated within 48 hours prior to randomization or registration if abnormal.

7.2 Induction Chemotherapy Phase

<u>PARAMETERS</u>	<u>PRESTUDY</u>	<u>Day 1</u> <u>taxel</u>	<u>Day 8</u>	<u>Nadir</u>	<u>COMPLETION</u> <u>OF Induction</u>	
					<u>THERAPY</u>	<u>Postpacli</u>
History & Physical	X	X			X	x
WT & Performance Status	X	X			X	x
Tumor Measurements	X	X			X	x
CBC, Platelets, Diff	X	X	X	X*	X	x
BUN, Creatinine	X	X			X	x
SGOT, Alk Phos,	X	X			X	x
Bilirubin; Total & Direct						
Calcium, Phos,	X				X	x
Albumin, Glucose						
LDH	X				X	x
Chest X-ray PA & Lateral	X				X	x
Bone Scan	X				X	x
Bone Survey	Xa				Xa,d	xa,d
Liver CT	Xb				Xb,d,c	xb,d,c

* Between days 15 and 21

- a) If bone scan is negative; bone survey is optional. If bone scan is positive; x-1 positive areas and symptomatic areas
- b) May use MRI instead of CT
- c) Repeat if abnormal function or symptoms
- d) Repeat if abnormalities are present at pre-study

7.3 Peripheral Stem Cell Infusion

Parameters	Pre-infusion	PBSC Infusion		Monthly Until Day 100	Every* Three Months	At Prog. of Disease or Relapse
		Daily	TIW Weekly			
History & Physical	X	X		X	X	X
Weight & Performance Status	X	X		X	X	X
Tumor Measurements	X			X	X	X
CBC, Platelets, Diff	X	X		X	X	X
Electrolytes	X	X		X	X	X
BUN, Creatinine	X	X		X	X	X
SGOT, Alk Phos, Total & Direct Bilirubin, LDH	X		X	X	X	X
Calcium, Phosphorus, Glucose	X		X	X	X	X
Total Protein, Albumin	X			X	X	X
HIV, CMV, HSV Urinalysis	X X					X
Chest X-ray	X			X	X	X
EKG	X					Xa
Bone Scan	X				X	Xa
Bone Survey	Xb				Xb	Xb
Liver CT	X				Xa	Xa
Bilateral BM aspirate Biopsy			Xc			
Creatinine Clearance	X					
MUGA	X					
PFTs with DLCO	X					

-
- a) If abnormal pre-transplant
b) If bone scan negative, survey in optional; if positive, survey of symptomatic and positive areas
c) Must be performed at bone marrow transplant center
* Every 3 months x 2 years, then every 6 mont

7.31 Hematopoietic Laboratory Studies

7.311 CFU-GM Assay

The patient's bone marrow and peripheral blood will be assayed for CFU-GM prior to any therapy, and serially from the leukopheresis samples as they are obtained. This will permit comparison of the number of CFU-GM initially available in marrow and peripheral blood with the number obtained by peripheral harvesting before, during and at the end of G-CSF stimulation. Correlations between the number of CFU-GM available at reinfusion and the speed of granulocyte recovery will be examined.

7.312 Flow Cytometry Studies for Stem Cells

Although precise identification of human pluripotent hematopoietic stem cells is not feasible, recent evidence suggests that these cells reside in the population of cells marked by CD 34 antigen and/or class II MHC (HLA-DR) antigens. This population of cells contains the precursors for early (CCFU-GEMM) and later (CFU-GM and BFU-E) colony forming cells. Moreover, isolated CD 34+ cells provide full marrow engraftment in lethally irradiated baboons and exhibit active proliferative responses to colony stimulating factors of multiple types including GM-CSF and Interleukin-3 (IL-3). Although B lymphocytes are CD 34-, HLA-DR+, this population also contains later hematopoietic precursor populations than CD 34+, HLA-DR+ cells. Therefore, analysis of both markers provides another means to quantitate the yield of peripheral stem cells and to characterize partially whether a shift between early and later precursors occurs with GM-CSF stimulation. The results of these determinations will be examined for correlation between the CFU-GM colony assays and the speed of multi-lineage marrow reconstitution. Flow cytometry will be performed on initial bone marrow, peripheral blood and serial leukopheresis samples.

7.4 Maintenance Chemotherapy Phase

	Prior To Each Cycle	CMF Day 8	Every 3 Months	Every 6 Months	At Pro- gression of Disease or Relapse
History & Physical	X				
Tumor Measurements	X				
WT & Performance Status	X				
CBC, Platelet, Diff	X	X			X
BUN, Creatinine	X	X	X		
SGOT, Alk Phos, Bilirubin; Total & Direct	X	X	X		
Calcium, Phos, Albumin	X	X	X		
Glucose					
LDH	X	X	X		
Chest X-ray PA & Lateral			Xc	X	X
Bone Scan			Xa	Xa	X
Bone Survey			Xb		X
Liver CT			Xc		Xc

-
- a) Obtain Bone Scan every 3 months for patient with bone mets; every 6 months for patient without bone mets
 - b) If bone scan is negative, bone survey is optional; if bone scan is positive, perform bone x-rays of symptomatic and positive area
 - c) If abnormality present in past or suspected

7.5 MDR1 Gene Expression Laboratory Study

- 7.51 We plan to sequentially evaluate MDR1 gene expression pre- and post-treatment with doxorubicin and paclitaxel. Tissue will be obtained at the time of diagnostic biopsy and at mastectomy for clinical CR's and at time of port-placement, a biopsy of residual disease will be obtained for clinical PR's.

Specimens will be obtained by incisional biopsy at diagnosis. If fresh frozen tumor from the primary breast cancer is available prior to registration, it will be analyzed. Optimally 100-500 mg of tissue will be required for analysis.

- 7.52 Biopsies of tumors from patients described in this study will be frozen in liquid nitrogen or dry ice and stored at -70°C and shipped on dry ice in a styrofoam container to:

Lori J. Goldstein, M.D.
Department of Medical Oncology
Fox Chase Cancer Center
7701 Burholme Avenue
Philadelphia, PA 19111

7.53 Analysis of MDR1 Gene Expression

MDR1 gene expression will be measured by 4 methods, including immunohistochemistry, RNA slot blot, RNase protection assay and RT-PCR. When adequate tissue is available all methods will be done on each specimen. Expression by each method will be compared to each other and to clinical drug resistance. Although large numbers of patient samples would be required to provide statistical significance, a detailed approach such as this should contribute to answering the question of what level of MDR1 expression is clinically significant as methods of detection become more sensitive. Differences in results between these methods may lead to future experiments concerning transcriptional and translational control.

- 7.531. A portion of each specimen will be pathologically analyzed and then evaluated for immunohistochemical stains.

- 7.532 Immunohistochemistry will be used to measure MDR-1 gene expression at the protein level. Immunohistochemistry has the advantage over RNA analysis by being a cell specific and therefore may overcome tissue heterogeneity. Immunohistochemical studies will use MDR-1 monoclonal antibodies, MRK16, C219 and JSB1 be done in collaboration with Jose Russo, M.D., Chairman, Department of Pathology, Fox Chase Cancer Center. Using a panel of MoAbs should help to overcome the differences in sensitivity and

specificity among them. Control cell lines, KB-3-1 and KB-8-5 will be used for MDR negative and positive controls. In addition, MDR1 gene expression and clinical outcome will be correlated with cathepsin D, erb B2 oncogene and nm23 expression, other molecular markers implicated in the biological behavior of breast cancer(111,112,113). These will be done in collaboration with Dr. Jose Russo. This analysis is qualitative whereas the RNA assay described below are quantitative.

7.533 Measurement of MDR-1 transcript levels

MDR-1 transcript levels will be measured by three methods including slot blot, RNase protection assay and RT-PCR. Each of these methods has advantages and disadvantages. We have the advantage of comparing these different techniques that vary in sensitivity, specificity and ability to quantify MDR-1 transcripts in a prospective fashion in a well defined group of patients. Such an analysis may help to explain the disparate results of Merkel et al who detected no expression of MDR1 gene expression in breast cancer specimens compared with many of the more recent studies which show a higher percentage of breast cancer samples express MDR1 RNA or protein(99-109).

Total cellular RNA will be extracted by homogenization in guanidium isothiocyanate followed by acid-phenol extraction(113). KB-3-1 is the drug-sensitive parental KB (HeLa) cell line. KB-8-5, which is four times as resistant to doxorubicin and six times as resistant to vinblastine, will be used as the positive drug resistant control cell line as we demonstrated in previous studies(99,114). The KB-8-5 cell line has increased levels of MDR-1 mRNA without gene amplification as is seen most commonly in clinical specimens(99,115). These cell lines will be used as controls in all of the proposed experiments.

a. Slot Blot Analysis

Slot blot analysis has the advantage over Northern blot analysis by being semi-quantitative. These assays in our hands are sensitive and reproducible. Serial dilutions of each sample of total RNA will be applied to each well of a Schleicher and Schuell slot blot apparatus and transferred to nitrocellulose filters. The filters will be prehybridized and then hybridized with the MDR-1 cDNA probe 5A(99). Hybridization with a nick-translated X-actin probe will be performed to control for RNA loading as in previous experiments(99). Probe 5A, which encodes about one-third of the coding region of a full-length MDR1 cDNA, will be labeled

by nick translation prior to the use in RNA slot blot analyses as used in my previous studies.

b. RNase Protection Assay

When adequate RNA is available we will perform RNA protection assays. This assay is extremely specific and allows for mapping of the starting sites of MDR1 transcription. An MDR1 genomic fragment of 785 base pairs (bp) will be used to make a riboprobe with SP6 polymerase for the RNase protection assays as we have previously demonstrated(99).

c. Reverse Transcription-Polymerization Chain Reaction (RT-PCR)

RT-PCR offers the advantage over the above methods of being extremely sensitive to detect low levels of MDR1 transcripts either when the tumor contains low levels of MDR1 RNA or when only a few cells express the MDR1 gene. Using the competitive template described below will enable us to quantitate the amount of MDR1 RNA present.

Total RNA will be reversed transcribed using random primers (commercially available), and Mo/MLV reverse transcriptase (commercially available). The reverse transcription product or control cDNA will be used as the template in the Polymerization Chain Reaction (PCR) using two MDR1 specific primers, LJG P1 and LJG P2 under the proper PCR conditions(116).

While other mechanisms of adriamycin resistance are not the focus of this proposal, we will have a unique data set of breast tumor RNA to measure glutathione-S-transferase pi (GST pi) and topoisomerase II(117,118,119). Resistance to topoisomerase II inhibitors (i.e. adriamycin and epipodophyllotoxin) can be due to changes of a number of parameters such as reduced topoisomerase II levels or mutant topoisomerase II. Less cleavable complexes are invariably detected in cells with reduced topoisomerase II levels. Although the best analysis of topoisomerase II is a functional assay of DNA topoisomerase II measuring decatenation, we will not have adequate tissue to accomplish this. Since RNA will have already been extracted we will use published cDNA probes and/or design PCR primers to measure GST pi and topoisomerase II RNA by slot blot analysis and RT-PCR.

8.0 DRUG FORMULATION AND PROCUREMENT

All study compounds are available commercially.

8.1 Cyclophosphamide (Cytoxan, CTX, CPM)

8.11 Classification: Alkylating agent

8.12 Mode of Action

Cyclophosphamide prevents cell division primarily by cross-linking DNA strands. The cell continues to synthesize other cell constituents (RNA and protein), an imbalance occurs and the cell dies. Cyclophosphamide is considered cell cycle, phase nonspecific.

8.13 Storage and Stability

Tablets are stable at room temperature for storage. It is recommended that the temperature for storage not exceed 90° F. Injectable forms are stable at room temperature for storage. It is recommended that temperature for storage not exceed 90°F. Reconstituted solution is stable 24 hours at room temperature or 6 days if refrigerated when paraben-preserved diluent is used for reconstitution. Unused diluted drug should be discarded. Shake vials vigorously and warm slightly to facilitate dissolving of Cyclophosphamide crystals. Vials may be immersed in lukewarm water to facilitate solution without risk of decomposition.

8.14 Route of Administration

The doses are given orally or injection - IV push or infusion. Check vials closely for evidence of undissolved crystals before injecting solution.

8.15 Incompatibilities

Barbiturates, phenytoin, and chloral hydrate may increase the rate of hepatic conversion of Cyclophosphamide to toxic metabolites.

8.16 Availability

Commercially available in 25 mg and 50 mg tablets, and also in 100, 200, 500 mg, and 1 gm vials. Dissolve 100 mg vials in 5 cc sterile water for injection; 200 mg vials in 10 cc sterile water for injection; 500 mg vials in 25 cc sterile water for injection.

8.17 Side Effects

8.171 Myelosuppression (nadir occurs approximately 8-14 days after administration of Cyclophosphamide).

8.172 Nausea and vomiting (occurs 6-10 hours after drug is administered).

8.173 Alopecia.

8.174 Hemorrhagic cystitis (onset of cystitis may be delayed from 24 hours to several weeks).

8.175 Some patients report a "metallic taste".

- 8.176 Nasal congestion and headache.
- 8.177 Potentiation of Adriamycin and Daunomycin cardiotoxicity.
- 8.178 Interstitial pulmonary fibrosis.
- 8.179 Amenorrhea.
- 8.1710 Liver dysfunction.
- 8.1711 Cardiac toxicity with high-dose Cyclophosphamide.

8.18 Nursing Implications

- 8.181 Check CBC and platelet count
- 8.182 Report and record any complaint of urinary urgency, burning on urination, urinary frequency, or hematuria.
- 8.183 May be helpful to premedicate patient with an antiemetic.
- 8.184 Instruct patient as to when oral Cyclophosphamide should be taken. Often best tolerated after meals. For this study a single daily dose after breakfast is recommended.
- 8.185 Patients must understand if doses are modified (especially on day 8) what their new dose is.

8.2 Methotrexate (Amethopterin, MTX, Methate, Folex)

8.21 Classification: Antimetabolite

8.22 Mode of Action

Binds to dihydrofolate reductase, thereby blocking the reduction of dihydrofolate to tetrahydrofolic acid, the active form. Thymidylc acid and purine synthesis are, therefore, stopped which, in turn, arrests DNA, RNA, and protein synthesis.

8.23 Storage and Stability

Store at room temperature, protected from light. Expiration date on drug.

8.24 Preparation

Lyophilized dosage forms may be reconstituted with sterile water, sodium chloride, DSW.

Examples: Mix 20 mg vial with 10.0 cc of suitable diluent = 1
cc = 2 mg
Mix 50 mg vial with 5.0 cc of N/S = 10 mg/cc
Mix 500 mg vial with 9.6 cc of sterile water = 50
mg/cc
Mix 1 gm vial with 19.4 cc of sterile water = 50
mg/cc

8.25 Route of Administration: IV

8.26 Incompatibilities

Actual incompatibilities not known, however, potentially toxic drug interactions can occur with other protein bound drugs such as, Salicylates, Coumadin, Sulfonamides, Phenytoin, nonsteroidal anti-inflammatory agents, and PABA.

8.27 Availability

Commercially available in 20 mg, 25 mg, 50 mg, 100 mg, 200 mg, and 250 mg vials (both as a dry powder and in preserved solution).

8.28 Side Effects

8.281 Nausea and vomiting, diarrhea.

8.282 Myelotoxicity.

8.283 Stomatitis, gastrointestinal ulcerations.

8.284 Malaise, chills, fever.

8.285 Nephrotoxicity.

8.286 Hepatotoxicity.

8.287 Photosensitivity.

8.288 Alopecia.

8.289 Pneumonitis.

8.2810 Rashes.

8.29 Nursing Implications

8.291 Administer anti-emetics as indicated.

8.292 Monitor for hematologic toxicity.

8.293 Observe for gastrointestinal toxicity (stomatitis, diarrhea); offer symptomatic care.

8.294 Instruct patient to use of suncreening lotion or cream when exposed to the sun.

8.295 In combination with Fluorouracil, should be given just prior to the 5-FU.

8.3 Adriamycin (Doxorubicin, Adriablastin)

8.31 Classification: Antitumor antibiotic

8.32 Mode of Action

The anthracycline portion of the molecule appears to intercalate between adjoining nucleotide pairs in the DNA helix structure. The drug also probably binds ionically around certain base pairs of DNA (adlineation). The overall effect then is interference with nucleic acid synthesis.

8.33 Storage and Stability

After reconstitution with NaCl (preferred by Adria Laboratories) or D5W, the solution is stable for 24 hours at room temperature, 48 hours under refrigeration. Recent studies show stability as long as 7 days at room temperature.

8.34 Preparation

Reconstitute the lyophilized drug with 0.9% NaCl for injection. Evidence indicates bacteriostatic diluents might worsen the reaction to extravasated drug. Add 5 cc to 10 mg vial and 25 mg to 50 mg vial resulting in conc. of 2 mg/ml. Shake vial to be sure drug is dissolved.

8.35 Route of Administration

IV push through the side arm. Large vein should be used for injection whenever possible. Although rate of administration will depend on the size of the vein, drug should not be administered in less than 3-5 minutes.

8.36 Incompatibilities

Forms a precipitate if mixed with Heparin or 5-FU. If administered through a Heparin lock, the lock should be flushed with NaCl injection both before and after administration of Adriamycin.

8.37 Availability

Commercially available in 10 mg vial and 50 mg vials.

8.38 Side Effects

- 8.381 Bone marrow - Dose-limiting toxicity. Increased myelosuppression in patients with previous radiation therapy.
 - 8.382 Gastrointestinal - Stomatitis (lowest incidence in high intermittent dose schedules).
 - 8.383 Nausea and vomiting - Usually controlled with antiemetics.
 - 8.384 Local hyperpigmentation of the nail beds and dermal creases especially in children.
 - 8.385 May have radiation recall with reactivation of radiation dermatitis in patients who have received prior irradiation or concurrent Cytosan. Once cumulative dose has been reached, no additional Adriamycin should be given.
 - 8.386 Alopecia.
 - 8.387 Irreversible cardiomyopathy.
 - 8.388 Fever, chills, urticaria.
 - 8.389 Hyperuricemia.
 - 8.3810 Local erythema streaking along vein and facial flushing with too rapid administration.
- 8.39 Nursing Implications
- 8.391 Check CBC and platelet count before administering. Adriamycin is a vesicant. IV should be checked frequently for patency. D/C immediately if any signs of local infiltration or symptoms of burning or stinging even if adequate blood return is obtained. Infusion should be avoided around joints where needle has a greater chance of dislodgement. Leg veins should not be utilized.
 - 8.392 Advise patients that their urine may turn red in color for about 24 hours after injection of the drug.
 - 8.393 Hair loss occurs 2-4 weeks after initial injection and is usually complete. There may be some growth during treatment but not a significant amount. In many instances, the use of ice caps has helped prevent or lessen the amount of hair loss.
 - 8.394 Stomatitis occurs within 7-10 days after injection. Viscous xylocaine can be used symptomatically. The ulcers generally last 3 days. During this period, adequate nutritional counseling is important.

- 8.395 Advise the patient that there is often a feeling of malaise and fatigue for 1-2 weeks after Adriamycin injection.
- 8.396 Be aware of the so-called "Adria" flare - most common reaction consists of an erythematous streak up the vein. It is associated with urticaria and pruritus. Occasionally the use of corticosteroids and/or antihistamines has been useful.
- 8.397 Extravasation policy would be useful in the event of an extravasation. At this time individual institutions should have a policy.

8.4 5-Fluorouracil (5-FU, Adrucil, Efudex)

8.41 Classification: Antimetabolite

8.42 Mode of Action

Pyrimidine antagonist that interferes with nucleic acid biosynthesis. Metabolized in liver, excreted in urine.

8.43 Storage and Stability

Stable for prolonged periods of time at room temperature if protected from light. Inspect for precipitate; if apparent, agitate vial vigorously or gently heat to not greater than 140o in a water bath.

8.44 Route of Administration

The drug may be given IV push.

8.45 Incompatibilities

8.451 Possibly incompatible with Methotrexate.

8.452 Definite incompatibility with Adriamycin and other anthracyclines. When giving Adriamycin IV push or through a running IV, flush line before giving 5-Fluorouracil.

8.46 Availability

Commercially available in 500 mg/10cc ampules.

8.47 Side Effects

8.471 Gastrointestinal: Nausea, vomiting, stomatitis, anorexia, diarrhea.

8.472 Dermatologic: Dermatitis, nail changes, hyperpigmentation.

- 8.473 Alopecia.
- 8.474 EENT: Eye irritation, nasal discharge, watering of eyes, blurred vision.
- 8.475 Blood: Leukopenia, thrombocytopenia, anemia.
- 8.476 Neurologic: Cerebellar syndrome (headache and cerebellar ataxia).
- 8.477 Other: Weakness and malaise.

8.48 Nursing Implications

- 8.481 Monitor blood counts.
- 8.482 Administer antiemetic and antidiarrheal drugs as needed.
- 8.483 Observe for neurological symptoms - discontinue drug if noted.
- 8.484 Inform patient of possible skin reactions.
- 8.485 In combination with Methotrexate, Methotrexate should be given first.

8.5 Thiotepa (N,N,N-Triethylenethiophosphoramidate)

8.51 Classification: Alkylating agent

8.52 Storage and Stability

Refrigerate at 2-8°C. When reconstituted, solution is stable for 5 days.

8.53 Preparation

Reconstitute 15 mg vial in sterile water to give an isotonic solution (since powder contains 80 mg NaCl and 50 mg NaHCO₃). A 15 mg vial may be diluted in 1.5 ml sterile water, and then added to other diluents as needed -e.g., NaCl, Dextrose or Dextrose-NaCl solutions.

8.54 Route of Administration

Continuous infusion, 125 mg/m² daily for 4 days. Not a vesicant.

8.55 Availability

Commercially available in 15 mg vials in powder form.

8.56 Side Effects

- 8.561 Bone marrow suppression.
- 8.562 Nausea and vomiting, anorexia.
- 8.563 Headache.
- 8.564 Mucositis (only at very high concentrations).
- 8.565 Allergic reactions.

8.6 Granulocyte-Colony Stimulating Factor (G-CSF-Neupogen) Amgen

8.61 Classification: Recombinant granulocyte colony stimulating factor.

8.62 Mode of Action:

Stimulates the production of CFU-G, enhances differentiation of granulocytes and increases the number of CFU-G in the circulation.

8.63 Storage and Stability

G-CSF (Neupogen) is a sterile, clear, colorless preservative-free liquid for parenteral administration. Each single-use vial of Neupogen contains 300 ug/ml of Filgrastim in a preservative-free solution. Single ML vials and 1.6 ml vials containing 480 ug are available. Neupogen should be stored in the refrigerator, and NOT frozen. Avoid shaking. Prior to injection, Neupogen may be allowed to reach room temperature for a maximum of 6 hours. Any vial left at room temperature for greater than 6 hours should be discarded.

8.64 Route of Administration of G-CSF

8.641 Intravenous: For use as an intravenous solution, Filgrastim is stable if after dilution the resultant concentration is > 15 mcg/ml in 5% dextrose in water (DSW). At dilutions from 2 to 15 mcg/ml in DSW, human serum albumin should be added to a final albumin concentration of 2 mg/ml to protect against absorption of the Filgrastim to the container walls (glass or plastic). The solutions described above are stable for up to 7 days at 2-8 C, or at controlled room temperature (15-30°C or 59-86°F). Filgrastim contains no preservatives. Therefore, to reduce the possibility of bacterial contamination, the diluted Filgrastim should be stored at 2-8°C and used within 24 hours of preparation. Filgrastim should not be diluted to concentrations less than 2 mcg/ml. In addition, Filgrastim should not be diluted in any solution containing saline. G-CSF will be given as a single 30 minute IV infusion if given by the IV route in this protocol.

8.642 Subcutaneous: Undiluted G-CSF is suitable for subcutaneous injections and is stable in a Bectin-Dickenson tuberculin syringe for up to 24 hours at controlled room temperature, or for up to 7 days in a refrigerator. G-CSF will be given preferentially as single subcutaneous bolus in this protocol

8.65 Availability

G-CSF will be supplied by Amgen-Thousand Oaks, CA

8.66 Side Effects

8.661 Exacerbation of pre-existing inflammatory conditions- e.g. psoriasis, vasculitis/

8.662 GI-elevated leukocyte alkaline phosphatase, uric acid, lactate dehydrogenase.

8.663 Musculoskeletal - muscle cramps, back and/or leg pain, bone pain.

8.664 Splenomegaly, thinning hair - with prolonged administration.

8.7 Carboplatin CBDCA (NSC 241240)

8.71 Classification: A second generation platinum analog.

8.72 Mode of Action

Though studies suggest that the mode of action of cis-Platinum was originally thought to be due to interaction with DNA, it may now include nuclear proteins. Mechanisms of action of CBDCA are undetermined. In vitro testing it produces a DNA-shortening effect.

8.73 Storage and Stability

Intact vials should be stored under refrigeration (2-8°C). Intact vials are stable for at least 24 months under refrigeration.

8.74 Dose Specifics

The doses used in ABMT protocols are significantly higher than in non-ABMT protocols, and should not be mistaken for a dose that could be used in a non-ABMT setting.

8.75 Preparation

When 150 mg vial is reconstituted with 9.8 ml of Sterile Water for Injection, USP, each ml will contain 15 mg of Carboplatin and 15 mg of Mannitol at Ph 4.5 to 7.0.

Further dilution to a concentration of approximately 0.5 mg/ml and 2 mg/ml in 5% Dextrose Injection, USP, results in solutions exhibiting no decomposition for at least 24 hours at room temperature.

8.76 Route of Administration

Continuous infusion, 6000 mg/m² over 4 days.

8.77 Incompatibilities

The use of 0.9% Sodium Chloride Injection, USP, as a reconstitution or diluting agent is not recommended because increased rates of decomposition have been observed.

8.78 Availability

Commercially available. Supplied as a white lyophilized powder, 150 mg CBDCA with 150 mg of Mannitol in a 20 ml amber vial.

8.79 Side Effects

8.791 Myelosuppression: Leukopenia, thrombocytopenia - usually more severe than leukopenia. Nadir occurs at approximately day 21. Delayed thrombocytopenia may occur between days 14 and 28.

8.792 Nausea and vomiting is mild to moderate. Anorexia.

8.793 Questionable renal toxicity, though this is not well documented. It is generally felt that diuretics and/or chloride/fluid therapy are unnecessary as in the case of Cisplatin.

8.794 Questionable musculoskeletal discomfort, though this is not well documented.

8.795 Profound anemia could indicate hemolysis. This has been reported to the NCI in a patient who received single-agent Carboplatin.

8.796 Pulmonary toxicity: Interstitial pneumonitis has been reported.

8.797 Ototoxicity

8.798 Hepatotoxicity: Elevated liver enzymes, hyperbilirubinemia.

8.799 Hyponatremia.

8.7910 Others: Neurotoxicity, hypocalcemia, hypomagnesium.

8.710 Nursing Implications

- 8.7101 Check CBC and platelet count prior to administration.
- 8.7102 Premedicate with anti-emetics, and maintain on schedule for 24 hours.
- 8.7103 Maintain saline infusion, and record stool volumes if diarrhea occurs or vomiting is a problem. Fluid rate and electrolyte content may need to be altered.
- 8.7104 Note any loss of hearing and/or ringing in ears (tinnitus).

8.8 Paclitaxel

- 8.81 How supplied: A concentrated sterile solution, 6 mg/ml in 5 ml vials (30 mg/vial) in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol, USP, 50%. The contents of the vial must be diluted just prior to infusion.
- 8.82 Reported Toxicities: Myelosuppression, N&V, diarrhea, stomatitis, mucositis, pharyngitis, sinus bradycardia, heart block, ventricular tachycardia, myocardial infarction (MI), hypotension, sensory (taste), peripheral neuropathy, seizures, mood alterations, anaphylactoid and urticarial reactions (acute), flushing, rash, pruritus, alopecia, fatigue, arthralgia, myalgia, light-headedness and myopathy.
- 8.83 Solution Preparation and Administration: The total calculated dose of Paclitaxel will be given as an intravenous infusion over 96 hours, as eight separate 12 hour infusions, each containing one eighth of the total dose diluted in 500 ml of 5% Dextrose injection, USP (D5W) or 0.9% Sodium Chloride for injection, USP (NSS). Paclitaxel must be prepared in glass or polyolefin containers due to leaching of diethylhexyl phthalate (DEHP) plasticizer from polyvinyl chloride (PVC) bags and intravenous tubing by the Cremophor vehicle in which Paclitaxel is solubilized. Paclitaxel will be administered via an infusion control device (pump). Nothing else is to be infused through the line where Paclitaxel is being administered.

NOTE: Formation of a small number of fibers in solution (within acceptable limits established by the USP Particular Matter Test for LVP's) has been observed after preparation of Paclitaxel. Therefore, in-line filtration is necessary for administration of Paclitaxel solutions. In-line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 microns (e.g.: IVEX-11, IVEC-HP or equivalent) into the IV fluid pathway distal to the infusion pump. Although particulate formation does not indicate loss of drug potency, solutions exhibiting excessive particulate matter formation should not be used.

- 8.84 Storage: The intact vial should be stored under refrigeration (2-8). Each bag/bottle should be prepared immediately before administration.
- 8.85 Stability: Shelf life surveillance of the vials is ongoing. All solutions of Paclitaxel exhibit a slight haziness directly proportional to the concentration of drug and the time elapsed after preparation, although when prepared as described above, solutions of Paclitaxel (0.3-1.2 mg/ml) are physically and chemically stable for 24 hours.
- 8.86 Toxicity
- 8.861 Hematologic-neutropenia is dose-limiting but does not appear to be cumulative. Anemia has been significant.
- 8.862 Gastrointestinal-stomatitis, nausea, vomiting and diarrhea.
- 8.863 Alopecia-partial; skin rash that may or may not be related to other allergic symptoms, pruritus.
- 8.864 Anaphylaxis(documented)-hypotension and flushing, chest tightness-resolved with discontinuation of infusion.
- 8.865 Cardiovascular-dysrhythmias (second-, third-degree heart block, bradycardia) may not be symptomatic.
- 8.866 Peripheral neuropathy, fatigue, arthralgias, myalgias, hepatic dysfunction.
- 8.867 Unknown vesicant properties. Cremophor is a vesicant, but is diluted 30-50:1 depending on the dose of Paclitaxel.

9.0 STATISTICAL CONSIDERATIONS

This is a trial of patients with locally advanced, inoperable and inflammatory breast cancer, and the principal outcome of interest is the time from treatment with intensive dose doxorubicin until death. The goal of the trial is to increase the 5-year survival rate among PR's from 40%, which is the expected rate under current therapies, to 60% under the intensive therapy. (This corresponds to an increase in median survival from 3.8 to 6.8 years.) Assuming 3 years of accrual and 2 years of follow-up and a 0.05 level likelihood-ratio test, the following table gives the powers of the test with two different PR rates:

Patients per year	PR Rate	PR's per year	Power
25	60%	15	73%
25	70%	18	81%

Thus, for example, suppose that 25 patients per year enter the study, and that 10% of them achieve a CR and 70% achieve a PR. of the total of 75 patients enrolled over three years, there would be 53 PR's, who would enter the intensive (transplant) phase of the study. The expected power of the test would then be 81%.

In the statistical analysis, the stratification of the patients based on the schema will be accounted for. In addition, a secondary endpoint, disease-free survival, will be analyzed.

10.0 RECORDS TO BE KEPT

The following forms must be submitted to the Central Registration Office at the University of Pennsylvania Cancer Center, 6 Penn Tower, 3400 Spruce Street, Philadelphia, PA 19104. A copy of the forms completed by the referring institution should also be sent to the transplant center once the patient is transferred.

<u>Form</u>	<u>To Be Submitted</u>
Breast Cancer Prestudy (Form 1) Measurement Form (Form 2)	Within 2 weeks of registration
Breast Common Toxicity Criteria Flow Sheets (Form 4)	At completion of induction chemotherapy.
Breast Measurement Form (Form 2) Metastatic Breast Cancer Interim Report Form (Form 5)	Every 12 weeks during maintenance chemotherapy or observation for patients randomized to transplant, until progression or relapse. Every 6 months if patient is 4 years from study entry.
Metastatic Breast Cancer Interim Report Form (Form 5)	After progression or relapse: every 3 months
ABMT On Study & Harvest Form (Form 6) ABMT Bone Marrow Reinfusion Form (Form 7)	Within two weeks of reinfusion
ABMT Daily Flow Sheet (Form 8) Breast Common Toxicity Criteria Flow Sheets (Form 4) Breast Measurement Form (Form 2) Metastatic Breast Cancer Interim Report Form (Form 5)	At day +30 and at hospital discharge if > 30 days
NCI Adverse Drug Reaction Form	Within 10 working days of reportable toxic event

11.0 PATIENT CONSENT AND PEER JUDGMENT

All institutional, NCI, State, and Federal regulations concerning informed consent and peer judgment will be fulfilled.

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APPENDIX I: RADIATION THERAPY

I. Radiation Therapy of the Intact Breast prior to Mastectomy

Whole Breast Irradiation

Treatment Planning

Treatment planning on a stimulator is required in all patients and for each field treated. The whole breast and underlying chest wall will be treated with opposed medial and lateral tangential fields. A half beam block, i.e., beam splitting or gantry angulation technique, will be used to achieve coplanarity, i.e., nondivergence of the posterior edges.

Patient Position

The patient will be treated in the supine position. A breast wedge may be employed. The ipsilateral arm is abducted 90 degrees or more. The head is turned to the contralateral side.

Field Margins

The medial border of the tangential field will be at midline. The lateral and inferior borders will be 2 cm around palpable breast tissue and the superior field margin will be at the matchline with the inferior margin of the supraclavicular field and generally be at the level of the inferior aspect of the sternoclavicular joint. These fields should not result in any more than 3 cm of lung at the center of the tangential field as measure on a stimulator film. For left sided primary tumors as much of the heart as possible should be excluded from the tangential fields. If more than 3 cm of lung is included with the tangential fields or a significant portion of the heart then a medial electron beam field should be employed to decrease the amount of lung and heart within the tangential fields.

Blocks

Blocking material with greater than or equal to five half value layers will be utilized in the half beam block technique to comply with the field margins and normal tissue dose requirements. In addition, blocking of the superior border of the tangential fields may be necessary to obtain a precise matchline with the nodal field.

Treatment Equipment

Megavoltage units with peak photon energies of less than or equal to 6 to 8 MV will be used. Large breasted patients may require higher energy, however, if greater than 8 MV photons are employed a beam spoiler must be used. The source axis distance or skin distance should be greater than or equal to 80 cm.

Dose

A total cumulative dose of 5000-5040 cGy will be delivered in 25 to 28 fractions at the rate of 180 to 200 cGy per fraction. The dose will be prescribed along the central plane at a point two-thirds the distance from the apex of breast to the deep margin at mid-separation of the beams. Both tangential fields will be treated daily, 5 days per week, for a total duration of 5 to 5-1/2 weeks. Treatment interruptions based on treatment related toxicity should be kept to a minimum and cumulatively must not exceed 10 treatment days. Wedge filters should be employed to increase homogeneity. The maximum inhomogeneity within the tangential fields should be no more than 15%. The intact breast should be bolused with 3/4 to 1 cm of tissue equivalent material every third day starting on day one.

II. Postmastectomy Chest Wall Irradiation

Treatment planning on a simulator is required in all patients and for each field treated.

Patient Position

The patient will be supine. The ipsilateral arm abducted greater than or equal to 90 degrees. A breast wedge may be employed and the head is turned to the contralateral side.

Field Margins

For the tangential fields the superior margin is at the level of the inferior aspect to the sternoclavicular joint. The medial margin is midline. The inferior margin is 2 cm below the contralateral inframammary fold and the lateral margin is the mid-axillary line. No more than 3 cm of lung will be included within the tangential fields as measured along the transverse plane of the central axis on the simulator film. For left sided primary tumors, care will be taken to exclude as much of the heart as possible from the tangential fields.

Blocks

Blocking material greater than or equal to five half value layers will be utilized in the 1/2 beam block technique to comply with the field margin and normal tissue dose requirements. In addition, blocking at the superior border of the photon tangential fields may be necessary to obtain a precise matchline with the nodal field.

Treatment Equipment

Megavoltage units with peak photon energies of less than or equal to 6 to 8 MV will be employed. For large patients, higher energy photon beams may be employed, although, for energies greater than 8 MV photons a beam spoiler should be used. The source axis distance or source skin distance of greater than or equal to 80 cm.

Doses

For the tangential photon fields a total cumulative doses of 5000-5040 cGy will be delivered in 25 to 28 fractions at the daily rate of 180 to 200 cGy. The dose will be prescribed along the central plane at a point two-

thirds the distance from the skin surface to the posterior edge of the beam of mid-separation. Both tangential fields will be treated daily, 5 days a week, for a total duration of 5 to 5-1/2 weeks. 3/4 to 1 cm bolus will be employed on the skin surface every other day beginning on day 1. Treatment interruptions based on treatment related toxicity should be kept to a minimum and cumulatively should not exceed 10 treatment days. The maximum inhomogeneity allowed will be 10-15%. Wedge filters or tissue compensating filters should be employed to achieve maximum dose homogeneity.

III. Regional Node Irradiation

Irradiation of the supraclavicular and apical axillary nodes will be given concurrent with whole breast or chest wall irradiation. No attempt will be made to include the internal mammary nodes within a separate treatment field. Treatment planning on a simulator is required.

Patient Position

The patient will be supine with the ipsilateral arm abducted greater than or equal to 90 degrees. The head will be deviated to the contralateral side and a breast wedge may be employed.

Technique

Treatment will be administered through a photon beam field angled 10 to 15 degrees to the ipsilateral side to avoid irradiation of the trachea and esophagus and spinal cord.

Field Margins

The superior margin of the supraclavicular and apical axillary field is at the level of the cricothyroid groove. The inferior margin corresponds to the superior border of the tangential field. A 1/2 beam block should be employed to avoid divergence into this field. The medial border is 1 cm to the contralateral side. The lateral border is at the level of the mid-humeral head. Blocking should be employed to shield the humeral head.

The posterior axillary field is employed to bring the midplane axillary dose to 4500 to 4600. The treatment is to be given at the rate of 100 cGy per fraction or less delivered concomitantly with the supraclavicular field over the same time of 4 1/2-5 wks. as the supraclavicular and apical axillary field. The superior border is at the coracoid process and usually is just superior to the clavicle or bisects it. The inferior border coincides with the inferior margin of the supraclavicular axillary field. The medial border is 1 cm within the rib cage and the lateral border is at the level to the midhumeral head.

Treatment Equipment

Megavoltage units with peak photon energies of less than or equal to 6 to 8 MV will be employed. A source skin distance of greater than or equal to 80 cm will be used.

Doses

The supraclavicular apical axillary field will be treated to a total dose of 4500 to 4600 cGy delivered at a depth of 3 cm at a rate of 180 to 200 cGy per fraction with 5 fractions per week. A separate calculation will be made to determine the dose to the midplane axilla from this. This dose will be calculated at the mid-separation between the anterior and posterior axillary surfaces.

Overall treatment time will be 4 1/2 to 5 weeks. The posterior axillary field may be required to supplement the dose from the anterior supraclavicular field.

IV Medial Electron Beam Field

For patients in whom greater than 3 cm of lung or a significant amount of heart is included with the tangential breast or chest wall fields, a separate medial electron beam field may be employed to decrease the amount of lung and heart in the tangential fields. The width of this field should be a minimum of 5 cm. The inferior border should correspond to the inferior border of the tangential fields. The superior border represents the inferior border of the supraclavicular field. The medial border is at midline and the lateral border is 5 cm to the ipsilateral side. The electron beam energy will be chosen depending upon the thickness of the chest wall or the thickness of the intact breast tissue in this region. 5000 cGy should be delivered to the 90% isodose curve at the appropriate depth at the rate of 180 to 200 cGy per fraction for a period of 5 to 5-1/2 weeks. No bolus will be employed on this field.

PATIENT CONSENT FORM

PHASE II TRIAL OF HIGH DOSE CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE, THIOTEPA, AND CARBOPLATIN AND PERIPHERAL BLOOD STEM CELL INFUSION IN WOMEN WITH LOCALLY ADVANCED INOPERABLE, AND INFLAMMATORY BREAST CANCER WHO ACHIEVE A PARTIAL RESPONSE TO INDUCTION CHEMOTHERAPY

(PHILADELPHIA BONE MARROW TRANSPLANT GROUP PROTOCOL PBT-3)

INDUCTION CHEMOTHERAPY-HORMONAL THERAPY PHASE

My doctors have informed me that I have **breast cancer**. Because the tumor is large and/or involves the **skin overlying the breast** my disease has been called "**Stage III, inoperable.**" This means that traditional surgery is not the best initial treatment for my disease as it is unlikely that the entire tumor mass can be removed with a mastectomy.

My doctors have also informed me that stage III breast cancer is usually treated with a combination of chemotherapy, hormone medicines, surgery, and radiation. A combination of treatments is used in an attempt to eliminate the tumor from the breast and to prevent recurrences of breast cancer in other vital organs (to prevent spread of the disease).

My doctors are offering me the opportunity of participating in a study to determine the best combination of treatments for stage III breast cancer. This study is being performed at Fox Chase Cancer Center, Temple University, Hahnemann University, and The University of Pennsylvania. This treatment plan is complex. How I will actually be treated depends on my tumor's response to each phase of treatment. If my tumor shrinks after the first treatment I may receive a second type of treatment. However, if my tumor does not shrink after the first treatment, a different second treatment may be offered. My doctors have explained to me the sequence of the various treatments outlined in this protocol and how I may be treated, based on my tumor's response to each of the previous treatments.

Purpose: The main purpose of this research study is to determine if giving high dose chemotherapy will improve my chances of controlling my breast cancer and preventing recurrence. An additional aspect of this study is to determine if Paclitaxel will help control my breast cancer.

In the first phase of this study, I will receive chemotherapy and hormone treatments.

Chemotherapy consists of a medicine to fight the cancer, called **adriamycin**. Adriamycin is one of the most potent anti-breast cancer medicines known.

If I agree to participate, my doctors will first confirm that I have breast cancer and that the cancer has not spread outside the breast. This will be done through the use of x-rays and other blood tests. Once my doctors have confirmed that my disease is localized to the breast, I will receive the cancer medicine adriamycin in my doctor's office by vein for three consecutive days. My doctors will repeat this adriamycin treatment every month for four consecutive months (total of 12 injections). In addition, I will receive an anti-hormone medication called **tamoxifen** twice per day by mouth which I will take every day for the four months. Tamoxifen also fights breast cancer.

The purposes of this chemotherapy and hormone treatments are to quickly shrink my breast cancer. This shrinkage will allow the surgeons to perform a mastectomy in hopes of completely removing the breast cancer.

This chemotherapy (adriamycin) is a commonly used anti-breast cancer medication. However, my doctors are giving a slightly higher dose of the medication in order to rapidly shrink my tumor. This medication may have side effects. These may include weakening of my heart, hair loss, nausea and vomiting, liver damage, and weakening of my immune system which may make me more susceptible to infection. Most women, however, do not experience all of these side effects. My doctor will carefully evaluate my health throughout the treatment and I may receive lower doses of the adriamycin or may be asked to stop treatment if the side effects become severe. Adriamycin has been used on many women with breast cancer and is considered to be among the best drugs for this disease. Even so, there is a small (less than 2%) chance that the adriamycin treatments may weaken my immune system or severely damage my organs leading to my death.

Hopefully, after the four cycles of adriamycin and tamoxifen, my breast cancer will no longer be able to be felt on examination or seen by the eye. When the tumor cannot be seen or felt on examination, this is known as a **complete clinical response**.

If, however, at the conclusion of the four monthly cycles of therapy, my disease can still be felt or seen, my doctors may wish to treat me with two additional months of chemotherapy. This chemotherapy will be different from the original medication and consist of a brand new medicine called **paclitaxel (Taxol)**. paclitaxel has already been shown to shrink a different cancer (ovarian cancer) that does not completely shrink to traditional medications. It is hoped that paclitaxel will also shrink breast cancer that does not shrink completely to adriamycin. paclitaxel is given by vein as a slow infusion over 24 hours. Therefore, I will need to be hospitalized for one day for administration of each of the two taxol treatments. Taxol also may have side effects. The most common and serious effect is an allergic reaction which may occur in up to 10% of all patients. To

prevent allergic reactions, prior to the taxol I will receive three medications (diphenhydramine, cimetidine, and dexamethasone). These three medications usually can prevent the allergic reaction. Other side effects noted from taxol therapy include heart damage, nausea and vomiting, liver disease, and numbness in the fingers and toes. There is also a small chance that this treatment could result in permanent or serious injury. However, most women receiving this medication have tolerated taxol well. It is hoped that these additional two cycles of taxol therapy will result in my tumor not being able to be seen or felt on examination (complete clinical response).

FUTURE THERAPY DEPENDS ON MY TUMORS RESPONSE TO THIS INDUCTION THERAPY:

A) If, after the adriamycin treatment and/or after the taxol treatment, my tumor can no longer be seen or felt (complete clinical response) my doctors will recommend that I have surgery to remove the breast. This surgery is called a mastectomy. The surgery will be performed in the hospital using standard surgical techniques. My doctors will have one of their surgical colleagues talk to me about the mastectomy and its risks at the appropriate time. However, generally this surgery is safe and carries only a small risk of potential complications. The most frequent complications include swelling of the arm on the side of the surgery, local skin infection at the area of the surgical cutting, and the risks associated with anesthesia. Additional treatment after the mastectomy will be recommended (See below)

B) If my tumor does not shrink during the adriamycin or taxol treatments (in other words, grows during the treatment), I will be taken off the study treatment. My doctors will then discuss with me other treatment options. I will have the opportunity of exploring other experimental treatments, other standard treatments, or seeking additional opinions by other doctors.

C) If, after the adriamycin treatment and the paclitaxel treatment, my tumor has shrunk but not gone away completely my doctors will not recommend that I undergo immediate surgery (mastectomy). Instead, if I have only a partial shrinkage of the tumor, to the first two chemotherapy medications, my doctors will recommend that I undergo an autologous peripheral blood stem cell transplant. My doctors will discuss the transplant, and the risks involved in a transplant, more completely with me at a later date. A copy of the informed consent form which explains transplantation has been given to me at this time so that I can understand about this treatment.

After the mastectomy, my doctors will carefully look at the tumor, obtained from the surgery, under the microscope. If my doctors are unable to find any tumor left in my breast at the time of the mastectomy, I will be told that I have had a pathologic

complete response. My doctors will then recommend to me additional treatments including radiation and one additional full year of chemotherapy. Tamoxifen (the antihormone medication) will also be continued indefinitely. My doctors will discuss this treatment approach with me more fully at a later date. A copy of the informed consent which explains this additional treatment has been given to me at this time so that I can be informed of these treatments. (TREATMENT A)

If, however, after the mastectomy, my doctors find small areas of tumor left in my breast, I will be recommended to have an autologous peripheral blood stem cell transplant. This treatment is extremely aggressive and consists of high doses of chemotherapy and requires a four to six-week hospitalization. Following the transplant, my doctors will recommend additional treatment, including radiation and continuation on the antihormone medication, tamoxifen. My doctors will discuss with me the transplant and the additional treatments with me at a later date. However, a copy of the informed consent explaining this treatment has been given to me at this time so that I can be informed of this therapy. (TREATMENT B)

If I had only a partial response to the adriamycin and taxol therapy and then underwent the autologous blood stem cell transplant, my doctors may recommend that I undergo a mastectomy and receive additional radiation and tamoxifen, depending on my response to the transplant. A copy of the informed consent explaining this treatment has been given to me. (TREATMENT C)

I UNDERSTAND THE COMPLEX NATURE OF THIS TREATMENT APPROACH. I UNDERSTAND THAT WHICH TREATMENT I EVENTUALLY RECEIVE (TREATMENT A, B OR C) DEPENDS ON HOW MY TUMOR SHRINKS TO THE INITIAL CHEMOTHERAPY AND HORMONE TREATMENTS. I UNDERSTAND THAT MY TOTAL TREATMENT MAY CONSIST OF CHEMOTHERAPY, HORMONE TREATMENTS, SURGERY, RADIOTHERAPY, AND AN AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANT. MY DOCTORS WILL INFORM ME OF MY PROGRESS THROUGHOUT THIS COMPLICATED TREATMENT, AND WILL DISCUSS WITH ME WHICH ADDITIONAL TREATMENT IS BEST.

RISKS

Risks From Induction Chemotherapy

Cancer therapy often has side effects. The drugs and procedures used in this program may cause all or some of the following side effects. In addition, there is also the risk that I might experience adverse reactions which other patients before me have not experienced. The induction chemotherapy may have side effects, as follows:

Adriamycin can cause nausea, vomiting, hair loss, darkening of the nail beds, skin rash,

mouth sores and lowering of the blood counts which can cause fatigue and increased risk of bleeding and infection. Rarely, treatment with Adriamycin can cause heart failure. Although this usually occurs after large cumulative doses of Adriamycin, it can rarely occur with small doses. If Adriamycin leaks outside of the veins into the surrounding tissues, it can cause severe skin damage.

Tamoxifen has been used to treat women with breast cancer for many years, and most side effects are well known. Side effects include hot flashes, vaginal discharge or bleeding, nausea and vomiting, dizziness, tremor, rashes, decrease in blood counts, bone pain, and a tendency to develop blood clots. Rarely, Tamoxifen can cause an increase in the calcium in the body causing thirst, nausea, vomiting, abdominal pain and confusion. Other rare side effects include mild reversible changes in vision, distaste for food, depression, light headedness, blood clots, abnormal PAP smears, ovarian cysts, increased lipids in the bloodstream, and minimally increased chance of developing liver & endometrial cancer (cancer of the uterus).

ALTERNATE TREATMENTS:

Stage III breast cancer can be treated in a variety of other ways. Treatments usually consist of a combination of surgery, chemotherapy, radiation, and anti-hormone medications. The use of bone marrow transplants is also being explored in stage III breast cancer. The exact mixture and order of these various treatments is unknown. My doctors will discuss with me other chemotherapy drugs, as well as other sequences of these various treatments. The use of new experimental medications can also be considered in patients with stage III breast cancer. Other research treatments are also available and my doctor will discuss these options with me.

CONFIDENTIALITY

I understand that the record of my progress while on the study will be kept in a confidential form at this hospital. The confidentiality of any central computer record will be carefully guarded and no information by which I can be identified will be released or published. Tissue samples and/or slides, may be sent to a central office for review. I understand that authorized representatives of The Food and Drug Administration (FDA), the National Cancer Institute (NCI) may inspect and copy the records.

CERTAIN COSTS

Procedures such as x-rays and laboratory tests purely related to the research will be explained. Some of the procedures or tests may result in added costs which may not be covered by insurance, and for which I may then be personally responsible. These possible additional costs will be discussed with me prior to the beginning of the study. In the event of physical injury resulting from this study, medical treatment to the extent

that it is available can be provided. The financial burden for this treatment may be my personal responsibility. No monetary compensation will be provided for wages lost or for any other reason because of injury resulting from this study.

QUESTIONS

I am free to ask questions at any time about these procedures and to ask for additional information from the doctor identified on this consent sheet or his designated representative or other doctors involved in my care. If I have questions, I can reach my doctor, Dr. _____ at this telephone number (215) 728-_____

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

TERMINATION

My participation in the project may be terminated by my doctor without my consent if I am not benefitting from the treatment or it develops that the treatment is not appropriate to my case or for other reasons at his/her discretion.

SIGNIFICANT FINDINGS

As the research progresses any significant new finding beneficial or otherwise, will be told to me and explained as related to my case.

If I have other questions about the research, or in the event of a research-related injury, I may contact the Institutional Review Board, which is concerned with protection of participants in research projects. I may reach the Board office by calling (215) 728-2931 9:00 am to 5:00 pm, Monday to Friday or by writing to the Institutional Review Board, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

By signing below, I indicate that I have read this form, received acceptable answers to any questions, and willingly consent to participate. I will receive a copy of this form.

SIGNATURE PARTICIPANT

DATE

SIGNATURE INFORMANT

DATE

SIGNATURE AUDITOR WITNESS

DATE

APPROVED BY THE
INSTITUTIONAL
REVIEW BOARD

JUN 28 1994

VOID ONE YEAR FROM ABOVE DATE
IRB No.

PATIENT CONSENT FORM

PHASE II TRIAL OF HIGH DOSE CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE, THIOTEPA, AND CARBOPLATIN AND PERIPHERAL BLOOD STEM CELL INFUSION IN WOMEN WITH LOCALLY ADVANCED INOPERABLE, AND INFLAMMATORY BREAST CANCER WHO ACHIEVE A PARTIAL RESPONSE TO INDUCTION CHEMOTHERAPY

(PHILADELPHIA BONE MARROW TRANSPLANT GROUP PROTOCOL PBT-3)

PROLONGED CHEMOTHERAPY, HORMONAL THERAPY, AND RADIATION
FOLLOWING PATHOLOGIC COMPLETE RESPONSE (TREATMENT A).

My doctors have informed me that I have stage III breast cancer. I have previously been treated on the Philadelphia Bone Marrow Transplantation Protocol PBT-3. Thus far, I have received induction chemotherapy with adriamycin and/or taxol. Because I had an **excellent response** to this initial treatment, I underwent a mastectomy to remove my diseased breast. After the mastectomy, my doctors carefully looked at the breast tissue and could not find any evidence of breast cancer remaining. They have informed me that this is called a "pathologic complete response."

PURPOSE

Although my doctors could not find any further evidence of breast cancer, because of the initial large size of the tumor and/or its involvement of the skin, there is a risk that the tumor will recur. Therefore, my doctors are recommending that I receive additional treatments to prevent the tumor from ever coming back.

PROCEDURES

I will first receive **radiation treatments** to my chest wall (the site of my prior breast) in hopes of preventing the disease from coming back in this area. Radiation is a very effective anti-breast cancer treatment. This radiotherapy will be performed as an outpatient over 25-28 sessions. This will take approximately five to five-and-one-half weeks for most patients. My doctors will have me speak with one of the Radiation Oncologists who will explain to me in greater detail the radiation treatments. However, these are generally well-tolerated and should not require hospitalization. Some patients may experience nausea and vomiting, a local skin reaction such as a sunburn, or mild shortness of breath during radiation treatment.

In addition to the radiation my doctors will recommend that I receive **chemotherapy** to prevent the spread of the breast cancer to other vital organs. Initially, during the

radiotherapy, I will receive two medications, **cyclophosphamide and 5-fluorouracil**. The cyclophosphamide is given by mouth for 14 days in a row and repeated every month for one full year. The 5-fluorouracil is given by vein as an outpatient two times per month (for example, on the first and eighth day of the month). This medication is also continued for one full year. During the radiotherapy, my doctors will also recommend that I continue on the hormone medication, **tamoxifen**.

Following completion of the radiation, I will continue to receive the cyclophosphamide, 5-fluorouracil, and tamoxifen. A fourth medication, **methotrexate**, will also be given to me by vein on the same days as the 5-fluorouracil (two times per month).

These chemotherapy medications (collectively known as CMF) are the most common cancer medications against breast cancer used today. This treatment will hopefully prevent the breast cancer from ever coming back.

At the conclusion of one full year of chemotherapy, my doctors will stop these medications. However, they will recommend that I continue on the antihormone medication, tamoxifen, indefinitely.

My doctors will continue to follow me as an outpatient on a routine basis every three to six months. Additionally, other studies such as x-rays, bone scans, CT scans, and blood tests may be requested to help my doctors determine whether or not my breast cancer has returned.

Hopefully, this aggressive combination of treatments will prevent my disease from ever returning. **Previously, this type of treatment has resulted in over 45% of women not having their breast cancer return over a five-year period.**

However, even with this aggressive approach, there is still a chance that the tumor could recur. If the breast cancer comes back, my doctors would offer me the opportunity to receive additional paclitaxel treatments. If my tumor shrinks to the paclitaxel therapy, an aggressive treatment such as peripheral blood stem cell transplantation may be recommended. My doctors will discuss with me these other treatment approaches should my cancer return.

RISKS

Risks from Standard Adjuvant Chemotherapy

Cancer therapy often has side effects. The drugs and procedures used in this program may cause all or some of the following side effects. In addition, there is also the risk that I might experience adverse reactions which other patients before me have not experienced. The standard adjuvant chemotherapy may have side effects, as follows:

Cyclophosphamide can cause nausea, vomiting, hair loss, watery eyes, metallic taste in the mouth, bladder irritation, which may produce burning or bleeding on urination, lowering of the blood counts, which can lead to fatigue, respiratory and liver abnormalities, and permanently impair my ability to have children. There is also a small chance the prolonged treatment with this drug can increase my risk of developing leukemia.

Methotrexate can cause nausea, vomiting, diarrhea, decrease in the blood counts, leading to fatigue, infection and bleeding, inflammation of the mouth and throat, mouth sores, chills, fever, liver injury, kidney damage, peculiar sensitivity to sunlight, hair loss, inflammation of the lung and rashes.

5-Fluorouracil can cause loss of appetite, diarrhea, nausea and vomiting, mouth sores, watery eyes, blurred vision, nasal discharge, hair loss, skin rash, and dryness, nail changes, difficulty in coordination, and lowering of the blood counts which can lead to fatigue, infection and bleeding.

Tamoxifen has been used to treat women with breast cancer for many years, and most side effects are well known. Side effects include hot flashes, vaginal discharge or bleeding, nausea and vomiting, dizziness, tremor, rashes, decrease in blood counts, bone pain, and a tendency to develop blood clots. Rarely, Tamoxifen can cause an increase in the calcium in the body causing thirst, nausea, vomiting, abdominal pain and confusion. Other rare side effects include mild reversible changes in vision, distaste for food, depression, light headedness, blood clots, abnormal PAP smears, ovarian cysts, increased lipids in the bloodstream, and minimally increased chance of developing liver & endometrial cancer (cancer of the uterus).

Paclitaxel (Taxol) may cause a decrease in the white blood cell count increasing my risk of infection and a decrease in my platelet count increasing my risk of bleeding or bruising. Also, paclitaxel may cause sores in my mouth nausea and vomiting, diarrhea, slowing of the heart rate, numbness in the fingers and toes, elevated liver function tests which might indicate liver damage, headache, altered taste, fatigue and allergic reaction. Total body hair loss occurs in all patients but is reversible. Side effects are generally reversible when taxol is stopped.

BENEFITS

It is unknown whether I will derive any personal benefit from participation in this study. A possible benefit from participation in this research study would be a reduced risk of recurrent breast cancer and possible cure. My life expectancy may increase for as long as my tumor does not come back. I understand that no result is guaranteed if I participate in this study.

ALTERNATIVE TREATMENTS

The treatment of stage III breast cancer is very complicated but usually involves the combination of chemotherapy, hormonal therapy, surgery, chemotherapy, radiation, and antihormone medications. The use of bone marrow transplants is also being explored in stage III breast cancer. The optimal mixture and order of these various treatments is unknown. My doctors will discuss with me other chemotherapy drugs, as well as other sequences of these various treatments. The use of new experimental medications can also be considered in patients with stage III breast cancer. Other research protocols are also available.

CONFIDENTIALITY

I understand that the record of my progress while on the study will be kept in a confidential form at this hospital. The confidentiality of any central computer record will be carefully guarded and no information by which I can be identified will be released or published. Tissue samples and/or slides, may be sent to a central office for review. I understand that authorized representatives of The Food and Drug Administration (FDA), the National Cancer Institute (NCI) may inspect and copy the records.

CERTAIN COSTS

Procedures such as x-rays and laboratory tests purely related to the research will be explained. Some of the procedures or tests may result in added costs which may not be covered by insurance, and for which I may then be personally responsible. These possible additional costs will be discussed with me prior to the beginning of the study. In the event of physical injury resulting from this study, medical treatment to the extent that it is available can be provided. The financial burden for this treatment may be my personal responsibility. No monetary compensation will be provided for wages lost or for any other reason because of injury resulting from this study.

QUESTIONS

I am free to ask questions at any time about these procedures and to ask for additional information from the doctor identified on this consent sheet or his designated representative or other doctors involved in my care. If I have questions, I can reach my doctor, Dr. _____ at this telephone number (215) 728-_____

WITHDRAWAL

Participation in this study is voluntary. No compensation for participation will be given. I understand that I am free to withdraw my consent to participate in this treatment program at any time without prejudice to my subsequent care. Refusing to participate will involve no penalty or loss of benefits. I am free to seek care from a physician of my choice at any time. If I do not take part in or withdraw from the study, I will continue to

receive care. In the event that I withdraw from the study, I will continue to be followed and clinical data will continue to be collected from my medical records.

TERMINATION

My participation in the project may be terminated by my doctor without my consent if I am not benefitting from the treatment or it develops that the treatment is not appropriate to my case or for other reasons at his discretion.

SIGNIFICANT FINDINGS

As the research progresses any significant new finding beneficial or otherwise, will be told to me and explained as related to my case.

If I have other questions about the research, or in the event of a research-related injury, I may contact the Institutional Review Board, which is concerned with protection of participants in research projects. I may reach the Board office by calling (215) 728-2931 9:00 am to 5:00 pm, Monday to Friday or by writing to the Institutional Review Board, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111. By signing below, I indicate that I have read this form, received acceptable answers to any questions, and willingly consent to participate. I will receive a copy of this form.

SIGNATURE PARTICIPANT

DATE

SIGNATURE INFORMANT

DATE

SIGNATURE AUDITOR WITNESS

DATE

APPROVED BY THE
INSTITUTIONAL
REVIEW BOARD

JUN 28 1994

VALID ONE YEAR FROM ABOVE DATE

PATIENT CONSENT FORM

**PHASE II TRIAL OF HIGH DOSE CHEMOTHERAPY
WITH CYCLOPHOSPHAMIDE, THIOTEPA, AND CARBOPLATIN
AND PERIPHERAL BLOOD STEM CELL INFUSION IN WOMEN WITH
LOCALLY ADVANCED INOPERABLE, AND INFLAMMATORY BREAST CANCER
WHO ACHIEVE A PARTIAL RESPONSE TO INDUCTION CHEMOTHERAPY**

(PHILADELPHIA BONE MARROW TRANSPLANT GROUP PROTOCOL PBT-3)

**PERIPHERAL BLOOD STEM CELL TRANSPLANTATION
FOR WOMEN ACHIEVING A PATHOLOGIC PARTIAL RESPONSE
TO INDUCTION CHEMOTHERAPY (TREATMENT B)**

My doctors have informed me that I have stage III breast cancer. I have previously been treated on the Philadelphia Bone Marrow Transplantation Protocol PBT-3. Thus far, I have received induction chemotherapy with adriamycin and/or taxol. Because I had an **excellent clinical response** to this initial treatment, I underwent a mastectomy to remove my diseased breast. After the mastectomy, my doctors carefully looked at the breast tissue and found small areas of breast cancer remaining. They have informed me that this is called a "pathologic partial response."

Because my doctors found small areas of breast cancer still present there is a chance that the tumor could come back in the chest wall or spread to other vital organs. Therefore, my doctors are recommending that I receive additional aggressive treatments to try to prevent the tumor from ever coming back.

My participation in this study is voluntary. Refusal to participate will involve no loss of benefits and will not influence further treatment.

INFORMED CONSENT

PURPOSE

In hopes of preventing the breast cancer from ever coming back my doctor has recommended that I receive very high doses of chemotherapy as part of a peripheral blood stem cell transplant (PBSCT).

PROCEDURE

I have been told that there are several different parts to this treatment plan.

A. First, I would have to undergo a number of **special tests** to make sure that my heart, lungs, kidneys, liver, and other organs are strong enough to allow me to undergo PBSCT. These tests include breathing into a special machine, having approximately eight tablespoons of blood removed for blood tests, having a small sample of bone marrow removed from my hip (numbed up with local anesthetic similar to what my dentist uses), and having a number of **special X-Rays**.

B. If my doctors decide that these tests show that my organs are strong enough to undergo bone marrow transplantation then the doctors will collect **stem cells** from me. Stem cells are the blood-making cells that will be needed to **re-grow new bone marrow** after I have received the high dose chemotherapy.

I would first have a **Central Line catheter** placed. This is a minor surgical procedure performed by a surgeon during which a special intravenous tube is placed under my skin, usually in the upper chest area near the shoulder. The Central Line is needed for all of the special procedures described below. The Central Line can be taken out after the transplantation is finished. The catheter can be placed by a surgeon as an outpatient (without hospitalization) under local anesthesia. Once the central line is working I can undergo the **Peripheral Blood Stem Cell Collections**. For these stem cell collections, I will have to come to the clinic approximately three to ten times. During these visits, my central line would be connected to a special machine called an apheresis machine. This is somewhat similar to a kidney dialysis machine. It takes special types of blood cells, called "stem cells," out of my blood. Each of the collection procedures takes 4-6 hours. The stem cell harvest is inconvenient because of the time involved. There is usually no discomfort from the stem cell harvest. Serious side effects from the stem cell harvest itself are very rare but I may notice a slight numbness of my lips or hands during the collections or I may notice weakness following the collection. A nurse will be with me the entire time I am attached to the collection machine. After each collection the doctors will count the number of stem cells obtained and decide if there are enough to re-grow my marrow or whether I need to have additional collections. The doctors will also give me medicines, called growth factors, in an attempt to increase the number of stem cells in my blood. This is called "mobilization" and is often helpful in making the collections better.

(If not enough healthy stem cells are obtained by this collection method, my transplant doctors may wish to obtain stem cells directly from the bone marrow in my hips. If this treatment is needed, I would come into the hospital for three days for a **Central Line Placement** and the **Bone Marrow Harvest**. Both of these procedures would be done after I am put to sleep in the operating room. The Central Line Placement is a minor

surgical procedure performed by a surgeon during which a special intravenous tube is placed under my skin, usually in the upper chest area near the shoulder. The Central Line is needed for all of the special procedures described below. The Central Line can be taken out after the bone marrow transplantation is finished. During the Bone Marrow Harvest bone marrow will be taken from both hips, under general anesthesia, for about 1-3 hours. Bone marrow is required to re-grow my marrow which will be eliminated by high doses of chemotherapy. About 1 to 3 quarts of bone marrow mixed with blood, representing less than 3% of my total marrow, will be collected. The hips are usually somewhat tender for a few days after the procedure, but most patients are able to go home the next day.)

C. After the stem cell collections are completed, I would then have to come into the hospital for approximately **four to six weeks for Peripheral Blood Stem Cell Transplant** itself. During the entire hospital stay it is necessary to draw approximately three tablespoons of blood per day to check the function of my bone marrow, kidneys, liver, and other organs. The blood can be drawn through the central line, which does not hurt. The first thing which will happen to me in the hospital is that I will receive my **high-dose chemotherapy**. The actual names of the high dose chemotherapy drugs are "cyclophosphamide", "thiotepa" and "carboplatin." Each of the medications are given intravenously through the central line by continuous infusion for four days.

Then, there will be two days to recover from the effects of the chemotherapy and to allow the medications to get out of my body. After two days, I will receive the actual **stem cell transplantation**. This is a simple procedure performed right in my hospital room in which my doctors give me my stem cells through the central line. These were the cells that were collected from me prior to the transplant from the catheter (during the multiple collections). These healthy cells, which were not exposed to the effects of the high dose chemotherapy, are the cells that will rescue my bone marrow.

The next step is **waiting for my bone marrow to grow back**. This usually takes 2-3 weeks. During this period of time I will have to stay in the hospital in a special isolation room to prevent any germs from getting in my body. The special isolation room is a little bit smaller than a regular hospital room. Everyone who comes to see me including nurses, doctors, and family must wear a mask and gown and sterile gloves. I will probably have a fever because my immune system will be weak due to the high dose chemotherapy. My doctors will treat the fever with special, powerful antibiotics. While I am waiting for my bone marrow to grow back I will probably feel weak and tired. I may have a poor appetite, a sore mouth, or diarrhea. My doctors have many special medicines and liquid nutrition which can often help patients feel better during this waiting period. While I am waiting for my new bone marrow to grow back my blood counts will fall dramatically and I will require many blood and platelet transfusions. I understand that occasionally, patients become so sick that they need to go to the intensive care unit during this time.

Another important aspect of my treatment is that I will be offered the opportunity to receive special bone marrow hormones called "growth factors" during my stem cell transplantation. These growth factors are virtually identical to growth factors which my own body makes. They are used to make the stem cells grow faster after they are replaced into my body.

Children under the age of 16 are not allowed to visit the bone marrow transplantation ward because of the risk of the chicken pox virus and other childhood viruses for patients with very weak immune systems.

D. After the bone marrow grows back and I am feeling better I will be discharged from the hospital. However I will need to stay in close contact with the transplant doctors. The doctors will need to monitor my blood. The recovery period usually lasts 3 to 6 months, but many patients are able to go back to work during the recovery period. During the recovery period, I will probably still feel somewhat weak and tired. I will need to come to the clinic for frequent checkups. I may need blood transfusions from time to time. My doctor may recommend special antibiotics or other treatments to help my immune system recover. My immune system will probably be back to normal after twelve months. During the recovery period my doctor will ask me to avoid crowds, public swimming areas, public restrooms, exposure to farm animals, gardening, and excessive exposure to dirt and dust. My skin may remain sensitive for a short period, and my doctors may advise me to use special sunscreens or avoid excessive sun exposure.

E. After I have recovered from the transplant I will be offered **additional radiation treatments** to my chest wall (the site of my prior breast) in hopes of preventing the disease from coming back in this area. Radiation is a very effective anti-breast cancer treatment. This radiotherapy will be performed as an outpatient over 25-28 sessions. This will take approximately five to five-and-one-half weeks for most patients. My doctors will have me speak with one of the Radiation Oncologists who will explain to me in greater detail the radiation treatments. However, these are generally well-tolerated and should not require hospitalization. Some patients may experience nausea and vomiting, a local skin reaction such as a sunburn, or mild shortness of breath during radiation treatment.

F. After the radiation treatments, my doctor will restart the anti-hormone medication, **Tamoxifen**. This will be continued indefinitely.

BENEFIT

The possible benefit of this treatment is that I would be able to receive a much higher dose of chemotherapy than I would with standard treatment. I understand that this may be a benefit because very high doses of chemotherapy may have a better chance of killing remaining breast cancer cells than normal doses. It is unknown how much better these high doses of chemotherapy will do against the breast cancer, and it is unknown

if this treatment will increase my chance of cure.

RISKS

I understand that the high dose chemotherapy which I will receive is dangerous. High dose chemotherapy can cause serious infections by weakening my immune defense system. These infections can usually, but not always, be successfully treated with antibiotics. There are also a number of other possible side effects, including nausea, vomiting, hair loss, bleeding, and damage to organs including the heart, the lungs, the kidneys, and the liver. My doctors will do everything possible to reduce these side effects. Nevertheless, I understand that I could have one or more serious side effects from this treatment, any of which could be permanent. Some, but not all, of the potential side-effects are listed below:

1. Peripheral Stem Cell Harvest

Peripheral stem cell harvest is a very safe procedure. I will need to be attached to a machine in the outpatient cancer clinic for approximately 4 hours per collection. During this time a nurse will be with me. I may notice a slight numbness in my hands or be weak during the collections. I may feel tired or weak following the collection. I will need to take care of the central line catheter that is used to attach me to the machine. Sometimes this catheter gets clogged or becomes infected. My doctors will treat this complication if it occurs, but I may need to be hospitalized briefly.

2. Stem Cell Infusion

Infusion of the stem cells may occasionally result in changes in heart rate or rhythm, blood pressure or respiratory rate. My doctor and nurse will be present in my room and my vital signs will be monitored continuously when I receive my stem cell infusion to prevent any serious complications.

3. Toxicity of Cyclophosphamide

High dose cyclophosphamide can cause nausea, vomiting, diarrhea, hair loss and skin rashes in most patients. These are all reversible and can be minimized with medications. Cyclophosphamide can cause or contribute to serious or potentially fatal damage to heart (1-3%), lungs (5%), liver (5-6%) and urinary bladder (1-3%). To prevent urinary bladder toxicity (cystitis), a catheter will be placed in my bladder during the infusion to drain the medication.

4. Toxicity of Thiotepa

High dose thiotepa may cause nausea, vomiting, headache, and mouth sores. Nerve problems are the most serious complications, but rarely occur.

5. Toxicity of Carboplatin

High dose carboplatin can cause nausea, vomiting, numbness in the hands and feet, and slight hearing difficulties (up to 30% of patients). The most common serious complication is kidney failure which is usually, but not always, reversible.

6. Toxicity of Consolidative Radiotherapy

Additional radiation will be given to the chest wall in an attempt to reduce the possibility of the breast cancer recurring in the local site. Previous experience with breast cancer treatment has shown that for patients with stage III disease, the chest wall is a likely site of relapse. Radiation may cause a variety of side-effects depending on the site of the body that is exposed to the radiation beam. Since part of the lung may be exposed to the radiation beam, shortness of breath may occur. Radiation following transplantation may also prolong the weakness experienced by most patients during the recovery phase or may lower the blood counts.

INFORMED CONSENT Page 8

7. Risk of Blood Transfusions

All patients undergoing bone marrow transplantation will require multiple transfusions of red blood cells and platelets until the new stem cells begin to grow and produce new blood cells. Patients may expect to receive over 5 units of red cells and 50 units of platelets (although some patients require less and some require more). All blood products used in the transplantation are obtained from the American Red Cross from volunteer donors. Blood products will be irradiated to prevent the development of transfusion-related-graft-versus-host disease. All blood products undergo a thorough screening by the American Red Cross for viruses such as hepatitis B and HIV (the virus that causes AIDS). Despite this screening (WHICH IS VERY GOOD) rare cases of transfusion related infection still occur. Unfortunately we can not predict which blood is contaminated, so every effort is made to reduce the number of transfusions during the transplant— but multiple transfusions will be required.

8. Risk of Infection and Bleeding

During the critical period when the old bone marrow is dying from the chemotherapy and the new stem cells are just beginning to grow (usually the first 14-21 days) there is a significant risk of infection due to a low white blood cell count, and a risk of bleeding due to low platelet counts. The doctors will administer powerful antibiotics and transfuse platelets during this period. However, these complications comprise the most serious immediate risks of the transplantation and may be responsible for peri-transplant deaths.

9. Relapse of Breast Cancer

I understand that stage III breast cancer is difficult to cure with conventional treatments. Therefore, I am willing to accept the additional risks of this peripheral stem cell transplantation plan. I understand, however, that even if I go through this treatment I may not be cured of my cancer. Unfortunately, previous experience has shown that despite very aggressive treatment

over 50 percent of people with breast cancer will experience a relapse.

10. Long Term Effects High Dose Chemotherapy

The high dose chemotherapy treatment will probably result in an inability to have children and effect my hormones. Effects on the thyroid gland, menstrual cycle, breast milk production and growth rate may occur. Serious effects and death could result to any unborn child that has already been conceived. Therefore, my doctor cannot offer me the therapy if I become pregnant. There is less than a 1% chance that I could develop a second malignancy several years following high-dose chemotherapy. My doctor cannot predict, in any case, whether any of these long-term effects will happen to me, but my doctor will closely monitor my health following the transplant.

In summary, I understand that a small percentage of patients who decide to have a peripheral stem cell transplantation die from the side effects of the treatment. Approximately 10% of patients undergoing this particular type of treatment die from the side effects of the treatment. I must balance this very significant risk against the risk of dying from the breast cancer if I decide to have conventional treatment.

Even if I survive the treatment, I will probably not be able to have children in the future. There is also a risk of permanent damage to the heart, lungs, kidneys, skin, or any other organ system. I understand that my doctors will do everything possible to help me through the treatment without serious or long-lasting side effects, but that there is still a risk that these side effects could occur. I also understand that there is a risk that the cancer could come back even if I make it through the transplant.

If I am of childbearing age, I understand that I must not become pregnant at any time during this treatment because of the risk of damage to the unborn child. I understand that I will probably not be able to become pregnant ever again if I decide to have bone marrow transplantation. However, I also understand that, very occasionally, a woman will regain her fertility after stem cell transplantation. Since there is no way to predict which women will regain their fertility, or when this might happen, I understand that I should continue to use an accepted form of birth control. Attempting to have children after a stem cell transplantation is not recommended because my body may be too weak to withstand a pregnancy. In addition, there is the possibility of birth defects because of the very high doses of chemotherapy which I will receive.

ALTERNATE TREATMENTS

The treatment of stage III breast cancer is very complicated but usually involves the combination of chemotherapy, hormonal therapy, surgery, chemotherapy, radiation, and antihormone medications. The use of bone marrow transplants is also being explored in stage III breast cancer. The exact mixture and order of these various treatments is unknown. My doctors will discuss with me other chemotherapy drugs, as well as other sequences of these various treatments. The use of new experimental medications can also be considered in patients with stage III breast cancer. Other research protocols are also available.

CONFIDENTIALITY

I understand that the record of my progress while on the study will be kept in a confidential form at this hospital. The confidentiality of any central computer record will be carefully guarded and no information by which I can be identified will be released or published. Tissue samples and/or slides, may be sent to a central office for review. I understand that authorized representatives of The Food and Drug Administration (FDA), the National Cancer Institute (NCI) may inspect and copy the records.

CERTAIN COSTS

Procedures such as x-rays and laboratory tests purely related to the research will be explained. Some of the procedures or tests may result in added costs which may not be covered by insurance, and for which I may then be personally responsible. These possible additional costs will be discussed with me prior to the beginning of the study. In the event of physical injury resulting from this study, medical treatment to the extent that it is available can be provided. The financial burden for this treatment may be my personal responsibility. No monetary compensation will be provided for wages lost or for any other reason because of injury resulting from this study.

QUESTIONS

I am free to ask questions at any time about these procedures and to ask for additional information from the doctor identified on this consent sheet or his designated representative or other doctors involved in my care. If I have questions, I can reach my doctor, Dr. _____ at this telephone number (215) 728-_____

WITHDRAWAL

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

TERMINATION

My participation in the project may be terminated by my doctor without my consent if I am not

benefitting from the treatment or it develops that the treatment is not appropriate to my case or for other reasons at his discretion.

SIGNIFICANT FINDINGS

As the research progresses any significant new finding beneficial or otherwise, will be told to me and explained as related to my case.

If I have other questions about the research, or in the event of a research-related injury, I may contact the Institutional Review Board, which is concerned with protection of participants in research projects. I may reach the Board office by calling (215) 728-2931 9:00 am to 5:00 pm, Monday to Friday or by writing to the Institutional Review Board, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

By signing below, I indicate that I have read this form, received acceptable answers to any questions, and willingly consent to participate. I will receive a copy of this form:

SIGNATURE PARTICIPANT

DATE

SIGNATURE INFORMAT

DATE

SIGNATURE AUDITOR WITNESS

DATE

APPROVED BY THE
INSTITUTIONAL
REVIEW BOARD

JUN 28 1994

VOID ONE YEAR FROM ABOVE DATE
IRB No.

PATIENT CONSENT FORM

PHASE II TRIAL OF HIGH DOSE CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE, THIOTEPA, AND CARBOPLATIN AND PERIPHERAL BLOOD STEM CELL INFUSION IN WOMEN WITH LOCALLY ADVANCED INOPERABLE, AND INFLAMMATORY BREAST CANCER WHO ACHIEVE A PARTIAL RESPONSE TO INDUCTION CHEMOTHERAPY

(PHILADELPHIA BONE MARROW TRANSPLANT GROUP PROTOCOL PBT-3)

PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOLLOWED BY
MASTECTOMY FOR WOMEN ACHIEVING A CLINICAL PARTIAL RESPONSE
TO INDUCTION CHEMOTHERAPY (TREATMENT C)

My doctors have informed me that I have stage III breast cancer. I have previously been treated on the Philadelphia Bone Marrow Transplantation Protocol PBT-3. Thus far, I have received induction chemotherapy with adriamycin and/or taxol. Because my tumor did not completely shrink to this chemotherapy I did not undergo a mastectomy to remove my diseased breast.

My doctors are instead recommending that I receive very high doses of chemotherapy as part of a peripheral stem cell transplant. If my tumor shrinks to this very aggressive treatment my doctors will then recommend that I undergo a mastectomy to remove my diseased breast and receive radiation treatments to the chest wall.

PURPOSE

In hopes of shrinking the breast cancer to allow surgery (and to try to prevent the cancer from ever coming back) my doctor has recommended that I receive very high doses of chemotherapy as part of a peripheral blood stem cell transplant (PBSCT).

PROCEDURE

I have been told that there are several different parts to this treatment plan.

A. First, I would have to undergo a number of **special tests** to make sure that my heart, lungs, kidneys, liver, and other organs are strong enough to allow me to undergo PBSCT. These tests include breathing into a special machine, having approximately eight tablespoons of blood removed for blood tests, having a small sample of bone marrow removed from my hip (numbed up with local anesthetic similar to what my dentist uses), and having a number of special X-Rays.

B. If my doctors decide that these tests show that my organs are strong enough to

undergo bone marrow transplantation then the doctors will collect **stem cells** from me. Stem cells are the blood-making cells that will be needed to re-grow new bone marrow after I have received the high dose chemotherapy.

I would first have a **Central Line catheter** placed. This is a minor surgical procedure performed by a surgeon during which a special intravenous tube is placed under my skin, usually in the upper chest area near the shoulder. The Central Line is needed for all of the special procedures described below. The Central Line can be taken out after the transplantation is finished. The catheter can be placed by a surgeon as an outpatient (without hospitalization) under local anesthesia. Once the central line is working I can undergo the **Peripheral Blood Stem Cell Collections**. For these stem cell collections, I will have to come to the clinic approximately three to ten times. During these visits, my central line would be connected to a special machine called an apheresis machine. This is somewhat similar to a kidney dialysis machine. It takes special types of blood cells, called "stem cells," out of my blood. Each of the collection procedures takes 4-6 hours. The stem cell harvest is inconvenient because of the time involved. There is usually no discomfort from the stem cell harvest. Serious side effects from the stem cell harvest itself are very rare but I may notice a slight numbness of my lips or hands during the collections or I may notice weakness following the collection. A nurse will be with me the entire time I am attached to the collection machine. After each collection the doctors will count the number of stem cells obtained and decide if there are enough to re-grow my marrow or whether I need to have additional collections. The doctors will also give me medicines, called growth factors, in an attempt to increase the number of stem cells in my blood. This is called "mobilization" and is often helpful in making the collections better.

(If not enough healthy stem cells are obtained by this collection method, my transplant doctors may wish to obtain stem cells directly from the bone marrow in my hips. If this treatment is needed, I would come into the hospital for three days for a **Central Line Placement** and the **Bone Marrow Harvest**. Both of these procedures would be done after I am put to sleep in the operating room. The Central Line Placement is a minor surgical procedure performed by a surgeon during which a special intravenous tube is placed under my skin, usually in the upper chest area near the shoulder. The Central Line is needed for all of the special procedures described below. The Central Line can be taken out after the bone marrow transplantation is finished. During the Bone Marrow Harvest bone marrow will be taken from both hips, under general anesthesia, for about 1-3 hours. Bone marrow is required to re-grow my marrow which will be eliminated by high doses of chemotherapy. About 1 to 3 quarts of bone marrow mixed with blood, representing less than 3% of my total marrow, will be collected. The hips are usually somewhat tender for a few days after the procedure, but most patients

are able to go home the next day.)

C. After the stem cell collections are completed, I would then have to come into the hospital for approximately four to six weeks for **Peripheral Blood Stem Cell Transplant** itself. During the entire hospital stay it is necessary to draw approximately three tablespoons of blood per day to check the function of my bone marrow, kidneys, liver, and other organs. The blood can be drawn through the central line, which does not hurt. The first thing which will happen to me in the hospital is that I will receive my **high-dose chemotherapy**. The actual names of the high dose chemotherapy drugs are "cyclophosphamide", "thiotepa" and "carboplatin." Each of the medications are given intravenously through the central line by continuous infusion for four days.

Then, there will be two days to recover from the effects of the chemotherapy and to allow the medications to get out of my body. After two days, I will receive the actual **stem cell transplantation**. This is a simple procedure performed right in my hospital room in which my doctors give me my stem cells through the central line. These were the cells that were collected from me prior to the transplant from the catheter (during the multiple collections). These healthy cells, which were not exposed to the effects of the high dose chemotherapy, are the cells that will rescue my bone marrow.

The next step is **waiting for my bone marrow to grow back**. This usually takes 2-3 weeks. During this period of time I will have to stay in the hospital in a special isolation room to prevent any germs from getting in my body. The special isolation room is a little bit smaller than a regular hospital room. Everyone who comes to see me including nurses, doctors, and family must wear a mask and gown and sterile gloves. I will probably have a fever because my immune system will be weak due to the high dose chemotherapy. My doctors will treat the fever with special, powerful antibiotics. While I am waiting for my bone marrow to grow back i will probably feel weak and tired. I may have a poor appetite, a sore mouth, or diarrhea. My doctors have many special medicines and liquid nutrition which can often help patients feel better during this waiting period. While I am waiting for my new bone marrow to grow back my blood counts will fall dramatically and I will require many blood and platelet transfusions. I understand that occasionally, patients become so sick that they need to go to the intensive care unit during this time.

Another important aspect of my treatment is that I will be offered the opportunity to receive special bone marrow hormones called "growth factors" during my stem cell transplantation. These growth factors are virtually identical to growth factors which my own body makes. They are used to make the stem cells grow faster after they are replaced into my body.

Children under the age of 16 are not allowed to visit the bone marrow transplantation ward because of the risk of the chicken pox virus and other childhood viruses for patients with very weak immune systems.

D. After the bone marrow grows back and I am feeling better I will be discharged from the hospital. However I will need to stay in close contact with the transplant doctors. The doctors will need to monitor my blood. The recovery period usually lasts 3 to 6 months, but many patients are able to go back to work during the recovery period. During the recovery period, I will probably still feel somewhat weak and tired. I will need to come to the clinic for frequent checkups. I may need blood transfusions from time to time. My doctor may recommend special antibiotics or other treatments to help my immune system recover. My immune system will probably be back to normal after twelve months. During the recovery period my doctor will ask me to avoid crowds, public swimming areas, public restrooms, exposure to farm animals, gardening, and excessive exposure to dirt and dust. My skin may remain sensitive for a short period, and my doctors may advise me to use special sunscreens or avoid excessive sun exposure. During the recovery period my care will be gradually transferred to the physician who originally referred me to Temple.

E. After I have recovered from the transplant, if my tumor has shrunk, my doctors will recommend that I have surgery to remove the breast. This surgery is called a **mastectomy**. The surgery will be performed in the hospital using standard surgical techniques.

My doctors will have one of their surgical colleagues talk to me about the mastectomy and its risks at the appropriate time. However, generally this surgery is safe and carries only a small risk of potential complications. The most frequent complications include swelling of the arm on the side of the surgery, local skin infection at the area of the surgical cutting, and the risks associated with anesthesia. If my tumor does not shrink during the transplant, my doctors will discontinue this study and will discuss with me other treatment options. They may or may not recommend a mastectomy depending on other factors. The doctors will discuss with me these options.

F. After I have recovered from the transplant and the mastectomy I will be offered **additional radiation treatments** to my chest wall (the site of my prior breast) in hopes of preventing the disease from coming back in this area. Radiation is a very effective anti-breast cancer treatment. This radiotherapy will be performed as an outpatient over 25-28 sessions. This will take approximately five to five-and-one-half weeks for most patients. My doctors will have me speak with one of the Radiation Oncologists who will explain to me in greater detail the radiation treatments. However, these are generally well-tolerated and should not require hospitalization. Some patients may experience nausea and vomiting, a local skin reaction such as a sunburn, or mild shortness of breath during radiation treatment.

G. After the radiation treatments, my doctor will restart the anti-hormone medication, **Tamoxifen**.

As with every cancer treatment, peripheral stem cell transplantation has risks as well as benefits. The possible benefit of this treatment is that I would be able to receive a much higher dose of chemotherapy than I would with standard treatment. I understand

that this may be a benefit because very high doses of chemotherapy may have a better chance of killing any remaining breast cancer cells than normal doses. It is unknown how much better these high doses of chemotherapy will do against the breast cancer, and it is unknown if this treatment will increase my chance of cure.

RISKS

I understand that the high dose chemotherapy which I will receive is dangerous. High dose chemotherapy can cause serious infections by weakening my immune defense system. These infections can usually, but not always, be successfully treated with antibiotics. There are also a number of other possible side effects, including nausea, vomiting, hair loss, bleeding, and damage to organs including the heart, the lungs, the kidneys, and the liver. My doctors will do everything possible to reduce these side effects. Nevertheless, I understand that I could have one or more serious side effects from this treatment, any of which could be permanent. Some, but not all, of the potential side-effects are listed below:

1. Peripheral Stem Cell Harvest

Peripheral stem cell harvest is a very safe procedure. I will need to be attached to a machine in the outpatient cancer clinic for approximately 4 hours per collection. During this time a nurse will be with me. I may notice a slight numbness in my hands or be weak during the collections. I may feel tired or weak following the collection. I will need to take care of the central line catheter that is used to attach me to the machine. Sometimes this catheter gets clogged or becomes infected. My doctors will treat this complication if it occurs, but I may need to be hospitalized briefly.

2. Stem Cell Infusion

Infusion of the stem cells may occasionally result in changes in heart rate or rhythm, blood pressure or respiratory rate. My doctor and nurse will be present in my room and my vital signs will be monitored continuously when I receive my stem cell infusion to prevent any serious complications.

3. Toxicity of Cyclophosphamide

High dose cyclophosphamide can cause nausea, vomiting, diarrhea, hair loss and skin rashes in most patients. These are all reversible and can be minimized with medications. Cyclophosphamide can cause or contribute to serious or potentially fatal damage to heart (1-3%), lungs (5%), liver (5-6%) and urinary bladder (1-3%). To prevent urinary bladder toxicity (cystitis), a catheter will be placed in my bladder during the infusion to drain the medication.

4. Toxicity of Thiotepa

High dose thiotepa may cause nausea, vomiting, headache, and mouth sores. Nerve

problems are the most serious complications, but rarely occur.

5. Toxicity of Carboplatin

High dose carboplatin can cause nausea, vomiting, numbness in the hands and feet, and slight hearing difficulties (up to 30% of patients). The most common serious complication is kidney failure which is usually, but not always, reversible.

6. Toxicity of Consolidative Radiotherapy

Additional radiation will be given to the chest wall in an attempt to reduce the possibility of the breast cancer recurring in the local site. Previous experience with breast cancer treatment has shown that for patients with stage III disease, the chest wall is a likely site of relapse. Radiation may cause a variety of side-effects depending on the site of the body that is exposed to the radiation beam. Since part of the lung may be exposed to the radiation beam, shortness of breath may occur. Radiation following transplantation may also prolong the weakness experienced by most patients during the recovery phase or may lower the blood counts.

7. Risk of Blood Transfusions

All patients undergoing bone marrow transplantation will require multiple transfusions of red blood cells and platelets until the new stem cells begin to grow and produce new blood cells. Patients may

expect to receive over 5 units of red cells and 50 units of platelets (although some patients require less and some require more). All blood products used in the transplantation are obtained from the American Red

Cross from volunteer donors. Blood products will be irradiated to prevent the development of transfusion-related-graft-versus-host disease. All blood products undergo a thorough screening by the American Red Cross for viruses such as hepatitis B and HIV (the virus that causes AIDS). Despite this screening (WHICH IS VERY GOOD) rare cases of transfusion related infection still occur. Unfortunately we can not predict which blood is contaminated, so every effort is made to reduce the number of transfusions during the transplant--- but multiple transfusions will be required.

10. Risk of Infection and Bleeding

During the critical period when the old bone marrow is dying from the chemotherapy and the new stem cells are just beginning to grow (usually the first 14-21 days) there is a significant risk of infection due to a low white blood cell count, and a risk of bleeding due to low platelet counts. The doctors will administer powerful antibiotics and transfuse platelets during this period. However, these complication comprise the most serious immediate risks of the transplantation and may be responsible for peri-transplant deaths.

11. Relapse of Breast Cancer

I understand that stage III breast cancer is difficult to cure with conventional treatments. Therefore, I am willing to accept the additional risks of this peripheral stem cell transplantation

man. I understand, however, that even if I go through this treatment I may not be cured of my cancer. Unfortunately, previous experience has shown that despite very aggressive treatment over 50 percent of people with breast cancer will experience a relapse.

12. Long Term Effects High Dose Chemotherapy

The high dose chemotherapy treatment will probably result in an inability to have children and affect my hormones. Effects on the thyroid gland, menstrual cycle, breast milk production and growth rate

may occur. Serious effects and death could result to any unborn child that has already been conceived. Therefore, my doctor cannot offer me the therapy if I become pregnant. There is less than a 1% chance that I could develop a second malignancy several years following high-dose chemotherapy. My doctor cannot predict, in any case, whether any of these long-term effects will happen to me, but my doctor will closely monitor my health following the transplant.

In summary, I understand that a small percentage of patients who decide to have a peripheral stem cell transplantation die from the side effects of the treatment. Approximately 10% of patients undergoing this particular type of treatment die from the side effects of the treatment. I must balance this very significant risk against the risk of dying from the breast cancer if I decide to have conventional treatment.

Even if I survive the treatment, I will probably not be able to have children in the future. There is also a risk of permanent damage to the heart, lungs, kidneys, skin, or any other organ system. I understand that my doctors will do everything possible to help me through the treatment without serious or long-lasting side effects, but that there is still a risk that these side effects could occur. I also understand that there is a risk that the cancer could come back even if I make it through the transplant.

If I am of childbearing age, I understand that I must not become pregnant at any time during this treatment because of the risk of damage to the unborn child. I understand that I will probably not be able to become pregnant ever again if I decide to have bone marrow transplantation. However, I also understand that, very occasionally, a woman will regain her fertility after stem cell transplantation. Since there is no way to predict which women will regain their fertility, or when this might happen, I understand that I should continue to use an accepted form of birth control. Attempting to have children after a stem cell transplantation is not recommended because my body may be too weak to withstand a pregnancy. In addition, there is the possibility of birth defects because of the very high doses of chemotherapy which I will receive.

Tamoxifen has been used to treat women with breast cancer for many years, and most side effects are well known. Side effects include hot flashes, vaginal discharge or bleeding, nausea and vomiting, dizziness, tremor, rashes, decrease in blood counts, bone pain and a tendency to develop blood clots. Rarely, Tamoxifen can cause an increase in the calcium in the body causing thirst, nausea, vomiting, abdominal pain and

confusion. Other rare side effects include mild reversible changes in vision, distaste for food, depression, light headedness, blood clots, abnormal PAP smears, ovarian cysts, increased lipids in the bloodstream, and minimally increased chance of developing liver & endometrial cancer (cancer of the uterus).

ALTERNATE TREATMENTS

The treatment of stage III breast cancer is very complicated but usually involves the combination of chemotherapy, hormonal therapy, surgery, chemotherapy, radiation, and antihormone medications. The use of bone marrow transplants is also being explored in stage III breast cancer. The exact mixture and order of these various treatments is unknown. My doctors will discuss with me other chemotherapy drugs, as well as other sequences of these various treatments. The use of new experimental medications can also be considered in patients with stage III breast cancer. Other research protocols are also available.

CONFIDENTIALITY

I understand that the record of my progress while on the study will be kept in a confidential form at this hospital. The confidentiality of any central computer record will be carefully guarded and no information by which I can be identified will be released or published. Tissue samples and/or slides, may be sent to a central office for review. I understand that authorized representatives of The Food and Drug Administration (FDA), the National Cancer Institute (NCI) may inspect and copy the records.

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Procedures such as x-rays and laboratory tests purely related to the research will be explained. Some of the procedures or tests may result in added costs which may not be covered by insurance, and for which I may then be personally responsible. These possible additional costs will be discussed with me prior to the beginning of the study. In the event of physical injury resulting from this study, medical treatment to the extent that it is available can be provided. The financial burden for this treatment may be my personal responsibility. No monetary compensation will be provided for wages lost or for any other reason because of injury resulting from this study.

QUESTIONS

I am free to ask questions at any time about these procedures and to ask for additional information from the doctor identified on this consent sheet or his designated representative or other doctors involved in my care. If I have questions, I can reach my doctor, Dr. _____ at this telephone number (215) 728-_____

WITHDRAWAL

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

TERMINATION

My participation in the project may be terminated by my doctor without my consent if I am not benefitting from the treatment or it develops that the treatment is not appropriate to my case or for other reasons at his discretion.

SIGNIFICANT FINDINGS

As the research progresses any significant new finding beneficial or otherwise will be told to me and explained as related to my case.

If you have other questions about the research, or in the event of a research-related injury. I may contact the Institutional Review Board, which is concerned with protection of participants in research projects. I may reach the Board office by calling (215) 728-2931 9:00 am to 5:00 pm. Monday to Friday or by writing to the Institutional Review Board, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111. By signing below, I indicate that I have read this form, received acceptable answers to any questions, and willingly consent to participate. I will receive a copy of this form.

SIGNATURE PARTICIPANT

DATE

SIGNATURE INFORMANT

DATE

SIGNATURE AUDITOR WITNESS

DATE

APPROVED BY THE
INSTITUTIONAL
REVIEW BOARD

JUN 28 1994

WED ONE YEAR FROM ABOVE DATE
IRB No.

DRAFT (7/1/94)

EASTERN COOPERATIVE ONCOLOGY GROUP

Induction with Adriamycin or Paclitaxel in
Inoperable Locally Advanced and Inflammatory Breast Cancer to
Evaluate for Multidrug Resistance
A Registration Study

STUDY CHAIR: Lori J. Goldstein, M.D.

STUDY CO-CHAIR:

MODALITY CO-CHAIR(S):

STATISTICIAN:

(MODALITY ORIENTED)
COMMITTEE CHAIR:

(DISEASE ORIENTED)
COMMITTEE CHAIR: William Wood, M.D.

STUDY PARTICIPANTS
ECOG GROUP
Intergroup

ACTIVATION DATE
(DATE)

SCHEMA

ECOG 7194

Induction with Adriamycin(Taxol or Adriamycin/Taxol) in
 Inoperable Locally Advanced and Inflammatory Breast Cancer to
 Evaluate for Multidrug Resistance
 A Registration Study

Pre-Study Requirements

Histologically or Cytologically documented adenoca
 of the female breast
 Measurable or evaluable disease
 Stage III breast cancer
 Any T with N₂ or N₃, M₀
 T₃ N₁, M₀
 T₄, any N, M₀
 No clinical or imaging evidence of distant
 metastasis
 No previous or concomitant malignancy except
 inactive non-melanoma skin cancer, in situ
 carcinoma of the cervix, or other cancer if
 disease free for ≥ 10 years
 No previous breast cancer
 Age ≥ 18 years old
 Informed consent/IRB Approval
 Performance Status ECOG 0 -1
 Expected survival > 6 months

Required initial parameters

Granulocytes ≥ 1500/ui
 Platelet count ≥ 100,000/ui
 Hemoglobin ≥ 10 gm/dl
 BUN < 1.5 X nl
 Creatinine < 1.5 X nl
 Bilirubin normal
 Normal left ventricular ejection fraction
 ER and Pr known or pending
 Pre-chemotherapy CT scan of chest
 No previous chemotherapy, hormonal
 therapy or radiotherapy to the
 proposed treatment area
 No other serious medical or psychiatric
 disease
 No history of congestive heart failure,
 recent myocardial infarction, or
 symptomatic cardiac arrhythmias
 requiring medication
 No history of chronic hepatic disease
 Non-pregnant, non-lactating

Stratification
 Disease:
 Locally Adv.
 Inflammatory

Menopausal
 Status:Pre- or
 Post

ER Status:
 positive or
 negative

R
E
G
I
S
T
E
R

Diagnostic Biopsy for
 Molecular analysis of Drug
 Resistance¹

THEN

Adriamycin(doxorubicin)
 30 mg/m² IV Day1,2,3
 Repeat every 3 weeks X 4

OR

Taxol(paclitaxel)
 175 mg/m² by continuous
 infusion over 24 hours^{2,3}
 Repeat every 3 weeks X 4

E
V
A
L
U
A
T
E

Surgery

Mastectomy
 or
 Incisional
 or
 Core Biopsy

Specimen analyzed
 for molecular analysis
 of Drug Resistance¹

Further
 treatment with
 Radiation and/or
 Chemotherapy
 to be
 determined by
 individual
 investigator

- 1 Biopsies(Core or Incisional) and representative portions of the mastectomy specimens(optimally 500-1000 mg or 1 cm³; will be frozen in liquid nitrogen or dry ice and stored at -70⁰ C and shipped on dry ice to:

Lori J. Goldstein, M. D.
Department of Medical Oncology
Fox Chase Cancer Center
7701 Burholme Avenue
Philadelphia, PA 19107

- 2 Use the patient's actual weight when calculating surface area.
- 3 Taxol must be filtered. In-line filtration with a 0.2 micron filter is required. The entire dose will be administered (diluted in 0.9% sodium chloride injection, USP or 5% dextrose injection, USP) in 1000 cc solution over 24 hours. Taxol must be prepared in glass bottles and administered with nitroglycerin administration sets (polyethylene lined PVC tubing). See Section 8.14.