CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010

DEPARTMENT OF MEDICAL ONCOLOGY AND THERAPEUTICS RESEARCH

TITLE: Dose-Intense Chemotherapy and Stem Cell Rescue in the Treatment of Inflammatory Breast Carcinoma

CITY OF HOPE PROTOCOL NUMBER/VERSION: **IRB # 96139** **VERSION: 18**

DATE(S)/ OF AMENDMENT(S)/REVISION(S):

		Version
C.O.H. Amendment #1	07/28/97	01
C.O.H. Amendment #2	02/06/98	02
C.O.H. Amendment #3	02/01/98	03
C.O.H. Amendment #4	03/20/98	04
C.O.H. Amendment #5	11/04/98	05
C.O.H. Amendment #6	07/28/99	06
C.O.H. Amendment #7	01/06/00	07
C.O.H. Amendment #8	04/27/00	08
C.O.H. Amendment #9	07/05/01	09
C.O.H. Amendment #10	09/13/01	10
C.O.H. Amendment #11	12/14/01	11
C.O.H. Amendment #12	04/29/02	12
C.O.H. Amendment #13	09/04/02	13
C.O.H. Amendment #14	12/10/02	14
C.O.H. Amendment #15	06/10/04	15
C.O.H. Amendment #16	05/25/05	16
C.O.H. Amendment #17	10/11/13	17
C.O.H. Amendment #18 (Change In PI)	12/21/17	18

SITE: Breast

HISTOLOGY: Inflammatory

STAGE (If applicable): Stage IIIB

MODALITY: Transplant

TYPE: Phase II

PRINCIPAL INVESTIGATOR: Joanne Mortimer, M.D.

PARTICIPATING CLINICIANS: Medical Oncology: Mihaela Cristea, M.D., Warren Chow, M.D., Marianna Koczywas, M.D., Stephen Koehler, M.D., Lucille Leong, M.D., Dean Lim, M.D., Robert Morgan, M.D., George Somlo, M.D., Nayana Vora, M.D., Jeffrey Weitzel, M.D., Yun Yen, M.D.

PARTICIPATING INSTITUTIONS: City of Hope National Medical Center

City of Hope Good Samaritan Hospital, Phoenix, AZ

TABLE OF CONTENTS

12/14/01

SCHEMA

Stratum 1

	Dox	Dox	Dox	Tax	Tax	Surgery	Tax	PBSCH	ACT	Mel/Cis
Week	1	3	5	7	9	11-12	14	16	18-22	24-27

Stratum 2

Week							14	16	18-22	24-27

Dox: Doxorubicin
Tax: Taxol
ACT: Doxorubicin/Cyclophosphamide/Taxol
Mel/Cis: Melphalan/Cisplatin
PBSCH: Peripheral Blood Stem Cell Harves

1.0 OBJECTIVES

1.1. Primary Objectives

1.1.1. To assess the feasibility of neoadjuvant dose-intense sequential chemotherapy followed by surgical resection, adjuvant therapy and tandem cycles of high-dose chemotherapy and stem cell rescue for patients with inflammatory stage IIIB breast carcinoma.

1.1.2. To assess the clinical and pathological remission rate (complete [CR], partial [PR], and overall [OR]) following neoadjuvant dose-intensive sequential chemotherapy in patients with inflammatory stage IIIB breast cancer.

1.1.3. To assess relapse-free and overall survival of patients with inflammatory stage IIIB breast carcinoma treated with neoadjuvant dose-intense sequential chemotherapy followed by surgical resection, adjuvant therapy and tandem cycles of high-dose chemotherapy and stem cell rescue.

1.2. Secondary Objectives

1.2.1. To perform biochemical, immunohistochemical and molecular analysis of primary breast tumors prior to neoadjuvant chemotherapy and at the time of definitive mastectomy.

1.2.2. To assess through a questionnaire and interview process the potential correlations between aggressive clinical presentation (i.e., inflammatory features) and hereditary background.

2.0 BACKGROUND

Approximately 180,000 new cases of breast cancer are diagnosed each year. One to three percent of newly detected cases will present with inflammatory disease. The diagnosis of inflammatory breast carcinoma is based on the presence of clinical features such as erythema and edema [peau d'orange], or thickening of the skin without a palpable mass developing over a relatively short time period. The pathological findings responsible for the presenting clinical characteristics include dermal lymphatic invasion. Prior to the introduction of systemic therapy, 5-year survival rates of patients with inflammatory carcinoma treated with surgical and/or radiotherapeutic means were under 5%. The inclusion of systemic chemotherapy first as adjuvant and more recently in the neoadjuvant setting seems to have changed the natural history of this disease. Doxorubicin-containing neoadjuvant regimens are capable of inducing clinical response in approximately 80% of patients, although complete remissions documented at the time of mastectomy occur in less than 15% of patients. While patients responding to neoadjuvant chemotherapy tend to have better local control rates than their non-responding counterparts, over 90% of patients can be rendered disease free with the combination of chemotherapy, surgery and radiation therapy. Despite an initial response to therapy, overall survival rates are 40-70% at 3 years and, at best 50%, at 5 years from the time of diagnosis.[1]

Preclinical data suggest that several drugs active against breast cancer such as cyclophosphamide, cisplatin, melphalan, thiotepa, BCNU, doxorubicin and possibly other classes of chemotherapeutic agents demonstrate steep dose-response curves *in vitro*. For standard dose chemotherapeutic regimens, a correlation has been established between the actual dose received and therapeutic outcome. Moderate decreases in doxorubicin dose and/or standard administration of cyclophosphamide, doxorubicin and 5-FU (CAF) result in decreased relapse-free and overall survival compared to standard treatment schedules.[3]

We performed a retrospective review of 58 patients treated at the COH since 1985 for inflammatory stage IIIB disease. We have observed three- and five-year relapse-free survival rates of 29% and 17% and overall survival rates of 43% and 31%, respectively (Curcio, et al., unpublished data). In contrast, we have recently reported our institutional experience in 22 patients with inflammatory breast cancer who underwent induction chemotherapy, surgical resection and high-dose chemotherapy consolidation with either doxorubicin or cisplatin together with VP-16 and cyclophosphamide (CAVP or CCVP). Patients received local-regional radiation therapy, and those with receptor positive tumors were treated with tamoxifen. The 3-year relapse-free and overall survivals, counting from initiation of high-dose chemotherapy, were 50% and 73%, respectively.[4] While these results compare favorably to historical controls, clearly there is a need for substantial improvement.

We have explored several combinations at the City of Hope consisting of 2 cycles of high-dose chemotherapy followed by stem cell support. Forty-five patients with high-risk primary or metastatic breast cancer have received 2 cycles of melphalan 20-150 mg/m²/cycle and cisplatin 200 mg/m²/cycle. Except for prolonged thrombocytopenia (at the highest dose level of 2 cycles of melphalan/cisplatin) no unusual toxicities, and more importantly, no grade 3 or 4 toxicities by the NCI autologous bone marrow transplant criteria were noted.[5] We have also tested, in an ongoing phase II study for patients with soft tissue and bone sarcomas, a tandem combination consisting of doxorubicin 150 mg/m² and ifosfamide 14 gm/m² as cycle 1 and melphalan 150 mg/m² and cisplatin 200 mg/m² as cycle 2. Both treatment cycles were supported with peripheral blood stem cells. To date, we have not encountered any significant toxicities other than mucositis similar in degree to that observed with our first generation high-dose combination of CAVP (Somlo, et. al, unpublished data).

In the design of effective chemotherapeutic combinations, sequence of drug delivery may also be important. Administration of doxorubicin, the agent with the highest reported response rate, followed by CMF for patients with stage II breast cancer, yielded a better outcome than the same 2 treatment regimens administered in an alternating fashion.[6] In our series of 114 patients treated with standard dose adjuvant chemotherapy followed by high-dose chemotherapy consolidation, we have also observed better overall outcome in patients who have received doxorubicin as part of their standard adjuvant chemotherapy.[4] Based on these findings, one can postulate that administration of the most effective agents early in the course of neoadjuvant/adjuvant chemotherapy is of therapeutic significance.

In recent years, paclitaxel has emerged as one of the most active single agents for the treatment of breast cancer with response rates in the range of 50% for patients with untreated metastatic disease.[7] This drug has been incorporated into ongoing prospective,

randomized trials as part of adjuvant therapy and as first-line treatment for metastatic disease. There is also recent information on the feasibility of employing G-CSF-supported sequential, dose-intense administration of doxorubicin, 80-90 mg/m², paclitaxel, 200-250 mg/m², and cyclophosphamide, 3.0 gm/m² q 14 days for 3 cycles.[8] An intergroup trial was recently initiated to test the feasibility and efficacy of 3 sequentially administered cycles each of doxorubicin 80 mg/m², paclitaxel, 200 mg/m², and cyclophosphamide 3.0 gm/m², as one arm of a randomized, prospective clinical trial for patients with stage II (4-9 axillary lymph nodes involved) breast cancer. Paclitaxel, when administered as 96-hour continuous infusion, demonstrated significant activity in patients who previously failed treatment with short taxane exposure.[9] The feasibility of paclitaxel dose escalation has been tested by several groups including one study in which paclitaxel was successfully added to high doses of cyclophosphamide and cisplatin.[10] In an ongoing trial, 3 cycles of stem cell supported high-dose chemotherapy consisting of paclitaxel (cycle 1), melphalan (cycle 2) and thiotepa, carboplatin and cyclophosphamide (STAMP V) have been administered to patients with metastatic breast cancer without significant toxicities with the Taxol dose still being escalated beyond 725 mg/m².[11] At our institution, doxorubicin 165 mg/m², cyclophosphamide 100 mg/kg, and paclitaxel up to 475 mg/m² has been tested in an ongoing phase I study, so far without any untoward toxicity.[12] Of note, we have found no clinically significant decrease in the left ventricular ejection fraction in over 120 patients with doxorubicin exposure of $\leq$ 180 mg/m² treated with high-dose (range, 150-165 mg/m² administered as continuous i.v. infusion over 96 hours) doxorubicin-containing regimens (CAVP, doxorubicin and ifosfamide, or doxorubicin, paclitaxel and cyclophosphamide) followed by local-regional radiation.

1/6/00

In order to safely undergo the second cycle of high-dose chemotherapy, patients undergo rigorous testing of their organ functions both before the first and second high-dose treatment cycle. To diminish the likelihood of any decrease of left ventricular ejection fraction following high-dose doxorubicin/paclitaxel/cyclophosphamide, patients will receive a 96 hour infusion of the cardioprotective agent dexrazoxane concomitant with doxorubicin. Dexrazoxane, when administered as short IV infusion together with doxorubicin or epirubicin protects against cardiotoxicity without any adverse effect on survival. [13,14] In our pilot data doxorubicin 165 mg/m^2 as 96-hour infusion and dexrazoxane 500 mg/m^2 give as 96-hour infusion without stem cell support resulted in adverse effects similar to our experience with a doxorubicin and cyclophosphamide combination. Of 10 patients treated with a variety of malignancies we observed only one case of asymptomatic, reversible, 10% decrease in left ventricular ejection fraction in a patient with thymoma with prior radiation to the mediastinum (unpublished data, Chow et al). We expect a 30-fold difference in drug concentrations (dexrazoxane versus doxorubicin) based on our pharmacokinetic measures which should result optimum cardioprotection.

Based on the above information, we plan to initiate a clinical study that will: administer neoadjuvant chemotherapy delivering the most active drug (doxorubicin) first, followed by paclitaxel; provide aggressive local control with modified radical mastectomy; administer paclitaxel and cyclophosphamide with G-CSF support and collect peripheral blood progenitor stem cells on recovery from the nadir; consolidate with tandem cycles of high-dose doxorubicin, cyclophosphamide and paclitaxel followed by melphalan and cisplatin with G-CSF primed stem cell rescue; complete local-regional control with

radiation therapy; and provide tamoxifen for 5 years for patients with receptor positive primary tumors.

We expect that a significant number of patients with inflammatory breast cancer will have received part or all of their neoadjuvant chemotherapy by their primary oncologist prior to evaluation at our institution. Hence, we plan to assess the efficacy of neoadjuvant chemotherapy as well as the total treatment strategy in patients receiving the entire treatment program (stratum 1) and will also evaluate the potential benefit of paclitaxel and cyclophosphamide followed by tandem cycles of high-dose chemotherapy in those patients who would be referred to the City of Hope National Medical Center following neoadjuvant chemotherapy and surgery (stratum 2).

We have reported our findings on the correlations between morphologic and more functional characteristics (grade, mitotic index, immunohistochemical analysis of p53 mutation, C-*erb*B-2 expression, receptor status, Ki-67 positivity) and relapse-free and overall survival following high-dose chemotherapy consolidation in 90 high-risk breast cancer patients treated with high-dose chemotherapy and stem cell support. We have proposed the presence of p53 mutation, estrogen and progesterone receptor negativity, and increased proliferation as adverse prognostic features.[15] Since the initial analysis, immunohistochemical staining for the presence of p-glycoprotein and MRP has also been performed on the same paraffin embedded tissue specimens. We plan to expand on our past work by examining the same biochemical markers in fresh tissue when available. In patients with inflammatory carcinoma, we have a unique opportunity to look for markers of both *de novo* and acquired drug resistance with the availability of biopsy tissue at the time of diagnosis and mastectomy.

Only a relatively small percentage (~5%) of breast cancer patients are members of families with BRCA1 or BRCA2 mutations, markers associated with hereditary breast cancer.[16] There are retrospective data suggesting that younger age at the time of diagnosis may be a manifestation of aggressive clinical disease.[17] Onset of disease at a relatively young age is also one of the potential hallmarks of hereditary clustering. We have treated over 300 patients with high-risk breast cancer (44 patients with stage IIIB inflammatory disease) on one of four high-dose chemotherapy protocols. The median age at the time of enrollment was 42 years (range, 25-63). Hence, as part of the initial screening for study eligibility we plan to ask patients to participate in an evaluation of family/hereditary background. For those patients fitting the profile of hereditary breast cancer, appropriate counseling, and if requested, family evaluation will be provided. In addition, both paraffin embedded and fresh tissue samples of the primary tumor and blood samples will be tested for surrogate signs of hereditary disease as they become available.

3.0 DRUG INFORMATION

3.1. Cyclophosphamide [NSC-26271]

3.1.1. Mode of Action: Cyclophosphamide is a weak alkylating agent. The drug undergoes enzymatic oxidation and additional multi-step processes resulting in end-products with increased alkylating properties.

3.1.2. Toxicities: Alopecia, nausea, vomiting, stomatitis, diarrhea, skin rash, pancytopenia, sterility, decreased gonadal function, hemorrhagic cystitis, syndrome of inappropriate antidiuretic hormone secretion, immune suppression, interstitial pulmonary fibrosis, leukemogenic potential, and at extremely high doses, myocardial necrosis.

3.1.3. Pharmaceutical data: Cyclophosphamide is available in 100 mg, 200 mg, and 500 mg ampules containing white powder. The drug can be reconstituted in normal saline or 5% dextrose and water. The drug should be dissolved and given in approximately 250 cc of diluent as rapid IV infusion over 1 hour.

3.1.4. Stability. Store at room temperature. Do not store at temperatures above 90 degrees F.

3.1.5. Cyclophosphamide is commercially available.

3.2. Doxorubicin [NSC 123127]

3.2.1. Mechanism of action: Doxorubicin, an anthracycline derivative, is known to engage in oxidation-reduction reactions, generating free radicals and to intercalate with DNA thus preventing DNA and RNA synthesis. In addition, the drug can cause alterations in membrane and topoisomerase II function.

3.2.2. Toxicity: Alopecia, nausea, vomiting, mucositis, phlebitis at the site of injection, tissue necrosis at the site of extravasation are all frequently reported side effects. Severe cardiomyopathy occurs predominantly at higher cumulative doses, in excess of 550 mg/m^2. Acute cardiac toxicities, not predictable based on preceding medical evaluation, have also been described. Myelosuppression, predominantly leukopenia and granulocytopenia, occurs about 2 weeks post injection. Anemia and thrombocytopenia occur to a substantially lesser degree. Secondary hematological malignancy and dysplasia had been reported in patients following chemotherapy with doxorubicin-containing combination with an incidence of $< 0.25\%$.

3.2.3. Pharmaceutical data: Doxorubicin is supplied in 10 and 50 mg vials as a red-orange, freeze-dried powder with up to 2 years of storage ability. Reconstitution can be done in normal saline or sterile water. In this study, patients either will receive the daily dose over 24 hours, reconstituted in one liter of Dextrose containing fluid, or if portable pumps are feasible, the daily dose of doxorubicin will be reconstituted in 20 cc of 5% dextrose and the delivery system will be changed every 24 hours.

3.2.4. Stability: Doxorubicin is chemically stable for 24 hours at room temperature and for 48 hours under refrigeration. Reconstituted solution should be used within 8 hours. Preservatives should not be used with diluents due to the potential to worsen the effects of extravasation.

3.2.5. Doxorubicin is commercially available.

3.3. Paclitaxel [(Taxol®) NSC #125973]

3.3.1. Formulation: Sterile solution containing 6 mg/ml in a 5 ml (i.e. 30 mg) ampule. It is formulated in Cremophor EL® (polyoxyethylated castor oil and dehydrated alcohol U. S. P.). Intact paclitaxel vials should be stored at 2-8°C. After final dilution, paclitaxel solutions of 0.3-1.2 mg/ml are stable for 27 hours.

3.3.2. The dose-limiting toxicity of paclitaxel is neutropenia. Other known toxicities include myalgias, arthralgias, fatigue, nausea, vomiting, mucositis, stomatitis, diarrhea, pharyngitis, arrhythmia, liver function test abnormalities (elevated SGOT, SGPT, bilirubin, alkaline phosphatase), hepatic failure, hepatic necrosis, stomatitis, typhlitis, ischemic colitis, neutropenic enterocolitis, radiation recall reactions, ventricular tachycardia, atrial arrhythmia, heart block, myocardial infarction, hypotension, hypertension (possibly related to concomitant Dexamethasone), mood changes, hepatic encephalopathy, encephalopathy, sensory changes (taste), peripheral neuropathy, myelosuppression, seizures, anaphylactoid reactions, urticaria, flushing, pruritus, light-headedness, myopathy, alopecia, skin rash, and vision changes (sensation of flashing lights, blurred vision). Local infiltration with Taxol will cause mild local symptoms (erythema, induration, tenderness, and rarely, ulceration) that usually resolve within a week. When Taxol has been administered with G-CSF, there have been reports of ischemic or infarcted colon, sometimes with other GI tract involvement.

3.3.3. Paclitaxel must be administered in glass or polyolefin containers. Nitroglycerin administration sets (polyethylene lining) are also necessary for Taxol administration. Do not use ordinary PVC tubing. Taxol solutions should be filtered during administration with a 0.22 micron in-line filter (e.g. Millex-GV, Millipore; IVEX-2, Abbott).

3.3.4. Taxol is commercially available.

3.4. G-CSF [(Filgrastim, Neupogen®) NSC #61429]

G-CSF or Filgrastim is a 175 amino acid protein manufactured by recombinant DNA technology. It is produced by E. Coli bacteria containing the human granulocyte colony stimulating factor gene.

3.4.1. Intact vials must be stored under refrigeration at 2-8°C. Prolonged exposure of G-CSF to temperatures outside this range will inactivate the drug. Freezing G-CSF may compromise the biological activity of the molecule.

3.4.2. G-CSF vials contain no preservatives. Discard vials left at room temperature for greater than 24 hours. Store opened vials under the refrigeration at 2-8°C.

3.4.3. Mild to severe bone pain, hypotension, anemia, taste perversion and pain at the subcutaneous injection site are the most notable side effects.

3.4.4. Administration is by subcutaneous or IV bolus injection.

3.4.5. Special Handling: G-CSF injection should not be shaken; transportation in institutional pneumatic tube systems or other high speed mechanical devices is also not advised.

3.4.6. G-CSF is commercially available.

3.5. CDDP, Cisplatin [Cisplatinum II Diamminedichloride, NSC 119875]

3.5.1. Mode of Action: The proposed main mechanism of action is interference with DNA function by cross-linking complementary strands of DNA.

3.5.2. Toxicity: The major acute morbidity is nausea and emesis. Myelosuppression is described and seems to be dose dependent. Renal toxicity is manifested mostly as reversible rise of serum BUN and creatinine and electrolyte-losing nephropathy due to failure of urinary concentration. Toxicities associated with large cumulative doses of CDDP are peripheral and occasional autonomic neuropathy, high frequency hearing loss, ataxia, blurry vision and defect in color vision.

3.5.3. Pharmaceutical Data: Each 20 ml vial (Bristol) contains 10 mg CDDP(white lyophilized cake); 90 mg NaCL; 100 mg mannitol (USP); and HCL for pH adjustment. Upon reconstitution with 10 ml of sterile water (USP) each ml of the resulting solution will contain 1 mg of CDDP, 10 mg mannitol, 9 mg NaCl at a pH of 3.5 - 4.5. At 22-C the reconstituted solution is stable for at least 8 hours. Administration is by intravenous infusion in 3% saline at 25 mg/hour.

3.5.4. Supplier: This drug is commercially available.

12/10/02 3.6. Alkeran®, Melphalan [L-Phenylalanine Mustard, L-Pam]

3.6.1. Mode of Action: In common with other nitrogen mustards, melphalan reacts with DNA to produce either DNA-DNA or DNA-proteins cross-linked products probably by binding at the N-7 position of guanine.

3.6.2. Supply, Reconstitution and Administration: Melphalan is commercially available by Burroughs Wellcome Company in sterile vials containing 50 mg lyophilized drug as the hydrochloride salt. It is formulated with 20 mg povidone per 50 mg vial. Sterile diluent is supplied which contains per 10 ml: sodium citrate 0.20 g, propylene glycol 6 ml, ethanol (95%) 0.526 ml, sterile water q.s. 10 ml. Reconstituted vials (undiluted solutions) are stable for 90 minutes. Melphalan diluted in NS to 0.1-0.45mg/ml is stable for only 60 minutes. Melphalan is unstable when diluted with NS to 2mg/ml. The rate of infusion should be 30 minutes or less.

3.6.3. Pharmaceutical Data: The drug is supplied in sterile vials containing 50 mg lyophilized melphalan as the hydrochloride salt. Alkeran® for injection is formulated with 20 mg povidone per 50 mg vial. Sterile diluent is supplied which contains per 10 ml: sodium citrate 0.20 g, propylene glycol 6.00 ml, ethanol (95%) 0.526 ml, sterile water q.s. 10 ml.

Alkeran® should be reconstituted with the diluent to a final concentration of 5 mg/ml. Reconstituted Alkeran® should be diluted only with 0.9% saline, to a concentration no greater than 2 mg/ml. Administration of reconstituted Alkeran® should be completed within one hour of reconstitution. The rate of infusion should be 10 mg/min or less in order to avoid hypotension from the propylene glycol in the diluent.

3.7. Mesna (NSC-113891)

3.7.1. Mode of Action

Sodium-2-mercapto-ethane sulfonate (Mesna) is a urothelial protectant. It binds to acrolein, the urotoxic metabolite of cyclophosphamide and it also inhibits the breakdown of its 4-hydroxy metabolites. It is excreted exclusively in the urine.

3.7.2. Supply, reconstitution and administration

Mesna is available from Bristol-Myer Squibb as a 100 mg/ml solution (400 mg/ml ampule). It may be administered intravenously by bolus and /or continuous infusion.

3.7.3. Toxicity

Nausea and vomiting are the only observed side effects.

1/6/00 3.8. Dexrazoxane (ICRF 187)

3.8.1. Chemistry: 2,6-Piperazinedione, 4,4'-(1 methyl-1,2-ethanediyl) bis, (+)-, (S)- or Dexrazoxane (also know as ADR-529), is a white to off-white powder with a molecularweight of 286.3 and a melting point of 193˚C to 194˚C. The molecular formula of this compound is $C_{11}H_{16}N_4O_4$.

3.8.2. Mechanism of Action: Dexrazoxane acts in part by chelation of metals and divalent cations. This agent also inhibits DNA synthesis, possibly by acting as a bifunctional alkylator. Inhibition of topoisomerase II has also been demonstrated as an action of Dexrazoxane.

3.8.3. Toxicology: Phase I studies performed to date have revealed myelosuppression to be the primary toxicity, with leukopenia more prominent than thrombocytopenia. Reversible liver function abnormalities, mold nausea and vomiting, alopecia, low grade fever, malaise, and pain at the injection site have also been noted. Increased urinary clearances of metals have been found.

3.8.4. Pharmaceutical Data: Dexrazoxane is supplied in vials as a sterile lyophilized powder for injection. Each vial contains 500 mg Dexrazoxane and upon reconstitution with 50 mL M/6 sodium lactate for injection USP, yields 50 mL withdrawable solution containing 10 mg Dexrazoxane per mL. The dosage form is packaged in 50 cc amber vials that contain no preservatives. The shelf-life of the unreconstituted vial at ambient temperature is one year. Based on a stability study performed at the City of Hope, the diluted Dexrazoxane at a concentration of 0.1 mg/ml to 1 mg/ml is stable for 24 hours. The product is to be administered via the intravenous route, with no special tubing required. The reconstituted solution is physically compatible with M/6 sodium lactate, 5% dextrose injection, D.5 normal saline, 0.45% saline, and D.5-0.45% saline. The pH of the reconstituted solution is 4.5 ± 0.3. Extravasation injury has not been a problem with the administration of this drug.

3.8.5. Supplier: The formulation is commercially available.

4.0 PATIENT ELIGIBILITY

4.1. PATIENTS MUST MEET THE FOLLOWING ELIGIBILITY CRITERIA:

4.1.1. Patients must have histologically proven breast cancer with clinical features of inflammation, erythema, pain or hypersensitivity, edema (peau d'orange) or thickening of the skin, or

4.1.2. Dermal/epidermal invasion should be present in the biopsy specimen.

4.1.3. Patients must have stage IIIB disease.

4.1.4. Patients must have a performance status of 80% or greater (see attached Performance Status Scale).

4.1.5. Patients with a physiological age $\leq$ 60 years are eligible.

07/01 4.1.6. Bilirubin $\leq$ 1.5 mg/dl and SGOT or SGPT $\leq$ 2 x the upper limit of normal.

11/98 4.1.7. Creatinine $\leq$ 1.2 mg/dl and creatinine clearance $\geq$ 70 cc/mL.

4.1.8. Neutrophil count $\geq$ 1.500/µl, platelet count $\geq$ 100,000/˜l.

4.1.9. Patients must have a left ventricular ejection fraction $\geq$ 55% on MUGA scan.

4.1.10. Patients must have a FEV 1 of $\geq$ 60% predicted, a room air $pO_2 > 85$ mmHg, a room air $pCO_2 \leq 43$ mmHg, and a DLCO $\geq$ 60% of the lower limit of predicted value.

4.1.11. Pretreatment laboratory and x-ray parameters must have been performed within 4 weeks prior to the initiation of therapy.

4.1.12. All patients must have signed the informed consent in accordance with institutional and federal guidelines.

09/01

4.1.13. Patients must be within 18 months from the time of diagnosis and within 12 months of their last chemotherapy.

4.2. PATIENTS ARE INELIGIBLE IF THEY FULFILL ANY THE FOLLOWING CRITERIA:

4.2.1. History of prior malignant disease in the past 5 years, except for squamous or basal cell skin carcinoma, and stage I or *in situ* cervical carcinoma.

4.2.2. Organic CNS dysfunction.

4.2.3. History of any previous valvular heart disease or arrhythmia.

4.2.4. Previous history of hemorrhagic cystitis.

4.2.5. Current pregnancy.

4.2.6. Patients with a known and potentially disabling psychosocial history are ineligible.

4.2.7. Patients with prior radiation to the left chest wall are ineligible.

4.2.8. Patients with hepatitis B Ag positivity or a positive HIV antibody are ineligible.

4.2.9. Patients who are candidates for treatment on stratum 1 should have received $\leq$ 1 cycle of chemotherapy.

7/28/99

4.2.10. Patients who are candidates for treatment on stratum 2 must have received $\leq$240 mg/m^2 doxorubicin and $\leq$750 mg/m^2 paclitaxel given as a short infusion or $\leq$420 mg/m^2 given as continuous i.v. infusion ($\geq$96 hours).

4/29/02

4.2.11. Patients with a history of inflammatory bowel disease are excluded.

5.0 PRETREATMENT EVALUATION

5.1. Physical evaluation and both laboratory and radiographic evaluation will be performed according to the study calendar listed under Section 9. Patients will be evaluated prior to neoadjuvant therapy and prior to peripheral blood stem cell collection (stratum 1) or if they already underwent modified radical mastectomy, prior to the first cycle of paclitaxel on this protocol (stratum 2).

5.2. Pretreatment testing/requirements.

5.2.1. History and physical examination.

5.2.2. Radiographic evaluation as indicated (CT of chest, abdomen or pelvis, MRI of the head and bone scan).

5.2.3. Pulmonary function test, ABG's

5.2.4. MUGA scan, EKG

5.2.5. Hepatitis and HIV panel, SMA 18, PT, PTT, CBC, differential and platelet count.

5.2.6. Twenty-four hour creatinine clearance.

5.2.7. Bilateral bone marrow biopsies, unilateral aspirate, cytogenetics.

5.2.8. ABO, Rh and HLA typing.

5.2.9. Urine pregnancy test for premenopausal female patients

5.2.10. Double lumen central venous catheter placement, size $\geq$ 12.0 French.

6.0 DESCRIPTIVE FACTORS

6.1. Stratification

6.1.1. Patients who have received all phases of treatment including neoadjuvant therapy on this protocol (stratum 1) versus those patients enrolled on study after completion of surgery (stratum 2).

6.2. Descriptive Factors

6.2.1. Pathological CR versus PR at the time of modified radical mastectomy.

6.2.2. Estrogen and/or Progesterone receptor positive versus receptor negative primary tumors.

6.2.3. Pathological and clinical versus clinical only signs of inflammatory disease.

6.2.4. Postmenopausal (>12 months since last period [without hysterectomy] or prior bilateral oophorectomy]) versus pre- and perimenopausal patients

7.0 TREATMENT PLAN

Ideal body weight will be used when calculating dose for all chemotherapeutic agents.

7.1. Patient Registration

After all pretreatment evaluations have been performed and within four weeks of anticipated neoadjuvant chemotherapy (stratum 1) or paclitaxel therapy (stratum 2) patients can be entered on study. Eligibility requirements must be reviewed by a member of the Department of Biostatistics.

7.2. Stratification of Treatment Schema

There will be two treatment strata. All patients enrolling after $\leq$ 1 cycle of prior neoadjuvant chemotherapy will undergo all treatment phases (patients already

treated with 1 cycle of doxorubicin-containing chemotherapy will receive only 2 cycles of neoadjuvant doxorubicin); patients who have received more than 1 cycle of neoadjuvant chemotherapy will enroll following modified radical mastectomy.

02/98

Patients who have received more than 1 cycle of neoadjuvant or adjuvant chemotherapy and have undergone modified radical mastectomy will be enrolled to start with Paclitaxel 140 mg/m² IV over 96 hours as priming, and will continue on protocol with stem cell harvest and then tandem cycles of high-dose chemotherapy.

Neoadjuvant Chemotherapy

Doxorubicin	75 mg/m² IV over 96 hours ; Days 1-4, 15-19, 29-32
Paclitaxel	140 mg/m² IV over 96 hours; Days 43-47, 57-60
G-CSF	5 µg/kg sc, days 5-10, 20-25, 33-38, 48-55 and 61-68 administration may continue beyond the days listed if the absolute granulocyte count is $\leq$ 1000/µl
Ofloxacin	400 mg po, BID on days 48-55 and 61-68

Modified Radical Mastectomy — Between Days 70 and 80 after initiation of chemotherapy

Paclitaxel — 140 mg/m^2, IV over 96 hours; Days 100-104

G-CSF	5 µ/kg, sc, Days 105-110
07/01 G-CSF	10 µ/kg, sc, Days 111-116

Harvesting and Cryopreservation of Stem Cells

PBSC collection — Days 113-116

A total of $\geq$ 14 x 10^8 mononuclear cells/kg with a $CD34^+$ cell content of $\geq$ 6 x 10^6/kg will be procured.

In case of technical problems, or time, or geographical constraints, autologous bone marrow can be collected as an adjunct, instead of peripheral stem cells following the third cycle of paclitaxel. The peripheral stem cells and autologous bone marrow will be processed and cryopreserved according to standard procedures at the COH stem cell apheresis and cyropreservation facilities.

High-Dose Chemotherapy

Cycle 1 – Doxorubicin, Cyclophosphamide and Taxol (ACT)

	Day –7	Admission, check laboratory and x-ray data, history and physical.
		Start oral ofloxacin 400 mg bid.
		Prophylactic antibiotics will be continued daily, until patient is removed from isolation, or systemic antibiotics need to be started.
		Oral amphotericin 10 mg/5 cc 6 x per day and Nystatin vaginal suppositories daily will be given.
1/6/00		Doxorubicin 165 mg/m^2 continuous IV infusions will be started at **noon**, concomitant with 500 mg/m^2 of dexrazoxane as continuous infusion.
1/6/00	**Days – 6 through – 3**	Continuous infusional doxorubicin and dexrazoxane for a total of 96 hours.
	Day – 3	**At 15:00 pm:** Mesna (40% of cyclophosphamide dose) IV will be started and will be given over 15 minutes. The same dose will be repeated at 3 hours. Then Mesna (240% of cyclophosphamide dose) will be infused over 21 hours.
		Following the administration of the first dose of Mesna, cyclophosphamide 100 mg/kg will be given IV over 2 hours in 500 cc of D5/NS.
	Day – 2	Following premedication with 20 mg po decadron 12 and 6 hours prior to administration, and 1 hour after 25 mg of Benadryl IV and 300 mg of po Tagamet, **at 15:00** a 24-hour continuous infusion of Taxol, 725 mg/m^2, will be started.
02/98		
	Day – 1	start G-CSF 5 µg/kg, daily, IV.
	Day – 0	reinfusion of ~50% of peripheral stem cells.
	G-CSF	5 µg/kg IV, daily, until AGC > 1000/µl x 3 days
		Starting as early as possible at 4 to 6 weeks from the first day of cycle.
	Cycle 2 – Melphalan and Cisplatin	
	Day – 11	Admission, history and clinical evaluation, SMA-18, Mg^{++}, CBC, differential and PLT count, reticulocyte count, urinalysis, EKG, MUGA scan, and additional laboratory tests as needed. Chest x-ray and additional radiographic tests as necessary. Start prophylactic antibiotic: Ofloxacin

400 mg bid; administration of the antibiotic will continue daily until patient removed from isolation, or systemic antibiotics are started for neutropenic fevers. Oral Amphotericin solution, 10 mg in 5 cc, will be given six times daily. Patients will be given Nystatin vaginal suppositories daily, and menstruating women will receive norethindrone acetate 15 mg orally once a day.

12/10/02

At 11 AM intravenous hydration with normal saline at 200 cc/hr will be started, and end after 6 hours, IV melphalan 75 mg/m^2 will be infused in $\leq$30 minutes. Immediately following the infusion of melphalan, CDDP 100 mg/m^2 at a rate of 25 mg/hr will be infused in 3% saline. Prior to delivery of CDDP, mannitol 25 gm, and if necessary, IV furosemide will be given. IV hydration will continue with normal saline and standard electrolytes at a rate of 200 cc/hour for an additional 8 hours. Mannitol and furosemide will be used as necessary to maintain diuresis. Appropriate antinausea medications will be given.

Day – 10 to – 5

Continue with IV hydration to maintain euvolemia, and antinausea medications as needed. G-CSF 5 micrograms/kg/day will be given intravenously and discontinued on day -6.

Day – 4

Repeat sequence of hydration, melphalan and CDDP as specified on day -11.

Day – 3 to 0

Continue with IV hydration and antinausea medications as needed.

02/98

Day – 3

Approximately 12.5% of the total amount of stem cells collected will be reinfused. G-CSF 5 µg/kg daily will be given intravenously following reinfusion of stem cells. If the number of frozen stem cells available does not allow partition into this range, then a minimum of one bag will be given. The frozen peripherally harvested cells will be removed from liquid nitrogen and thawed in a 37°C water bath. Each bag will be rapidly infused, undiluted, through standard IV tubing without a filter. Hydrocortisone 50 mg and Diphenhydramine 25 mg will be administered prior to reinfusion.

Day – 0

The remaining 37.5% of the total amount of stem cells collected) will be reinfused as above. If there are an odd number of total frozen stem cell bags, the larger number of bags will be given with this cycle. Administration of G-

02/98

CSF will be discontinued following granulocyte recovery to ≥ 1000/microliter for 3 consecutive days.

Radiation Therapy

To start at 4 to 7 weeks from the beginning of cycle 2.

Tamoxifen, 20 mg/day, within 2 weeks of being discharged from hospital following cycle 2 (patients with ER or PR + tumors).

8.0 TOXICITIES AND DOSE MODIFICATIONS

The NCI Common Toxicity Grading will be used to assess toxicities except for the high-dose chemotherapy / stem cell support-phase when toxicities will be graded by the NCI/autoBMT Toxicity Grading.

8.1. Doxorubicin

8.1.1. If on the scheduled day of administration the granulocyte count is less than 1,000/µl or the platelet count is less than 50,000/µl, doxorubicin will be delayed. The granulocyte and platelet counts will be checked at least biw and doxorubicin will be administered when these minimal levels are achieved. If adequate blood counts are not achieved by three weeks from the prior doxorubicin dose, contact the Principal Investigator.

8.1.2. The dosage of doxorubicin will not be reduced unless the patient develops neutropenic fever (absolute granulocyte count below 1,000/µl at the time of a documented temperature of 38.5°C or greater). The subsequent doses of doxorubicin will be reduced by 25% from the previously administered dose. Doses may not be re-escalated.

7/28/99

8.1.3. The maximum cumulative dose of doxorubicin is 240 mg/m², prior to high-dose chemotherapy. Doxorubicin will be discontinued and a patient will be taken off study if: congestive heart failure appears, persistent arrhythmia (including sinus tachycardia with no demonstrable cause) appears or heart size increases by 50% on PA chest x-ray.

8.1.4. In case of Grade 2 or 3 mucositis, dysphagia, and diarrhea doxorubicin will be held until toxicity resolves to Grade 1 or less at which point chemotherapy will be administered at a full dose. In case of grade 4 toxicity subsequent doses of doxorubicin will be reduced by 25% from the previously administered dose. Doses may not be re-escalated.

8.1.5. For bilirubin > 1.5 times the institutional upper limit of normal hold therapy; if bilirubin remains > 1.5 times the institutional upper limit of normal beyond one week, call the principal investigator to discuss the need for additional workup and further treatment.

8.2. Paclitaxel

8.2.1. If on the schedule day of administration the granulocyte count is less than 1,000/µl or the platelet count is less than 50,000/µl, Taxol will be delayed. The granulocyte and platelet counts will be checked at least every other day, and Taxol will be administered when these minimal levels are achieved. If adequate blood counts are not achieved by three weeks from the prior doxorubicin dose, contact the Principal Investigator.

8.2.2. With the development of a peripheral neuropathy doses will be reduced by 20% (from previously administered dose) for Grade 3 or greater neurotoxicity.

Doses will similarly be reduced for neutropenic fever, or any other Grade 3 toxicity attributable to a prior dose of paclitaxel.

All Grade 3 or higher toxicity must have resolved to Grade 1 or less before paclitaxel may be restarted.

8.3. High-dose Chemotherapy Cycles

8.3.1. No dose modifications will be made during the first cycle of high-dose chemotherapy with doxorubicin, cyclophosphamide and paclitaxel.

8.3.2. Patients should meet all staging and functional screening requirements following completion of the first high-dose chemotherapy cycle prior to the beginning of the second cycle of high-dose chemotherapy.

8.3.3. Melphalan and Cisplatin (Mel/Cis)

Chemotherapy Dosage Adjustments

8.3.3.1 Renal criteria:

Serum Creatinine	Creatinine Clearance (measured or calculated)	Dose (melphalan)	Dose (Cisplatin)
< 1.4 mg/dl	> 75 cc/min	100%	100%
≥ 1.4 ≤ 1.6 mg/dl	≤ 75-50 cc/min	75%	75%
≥ 1.6 mg/dl	≤ 50 cc/min	0%	0%

8.3.3.2 Neurologic criteria:

grade 1 toxicity	100%	75 mg/m^2
grade 2 toxicity	100%	0

Mel/Cis will be delivered at earliest 4 weeks from day 1 of ACT. Treatment can be given once the neutrophil count is > 1500/ µl, plt > 100,000/µl. A maximum of 7 weeks delay will be allowed for recovery from treatment-related toxicities. Any patient requiring > 7 week delay (beyond 7 weeks from day 1 ACT) will go off study.

11/4/98

8.4. G-CSF

G-CSF will be held if WBC counts go beyond 20,000 between chemotherapy cycles and following high-dosechemotherapy cycles. G-CSF will also be held in the setting of stem cell mobilization if the total white count reaches 50,000 and the dose will be reduced to 50% if the total white cell count in the setting of stem cell priming is between 25,000 to 50,000/µl.

8.5. Supportive care

8.5.1. All patients will have central intravenous catheters placed.

8.5.2. Low bacteria diet and prophylactic ofloxacin 400 mg bid orally and amphotericin 5 mg i.v. and acyclovir 250 mg/m^2 will be prescribed.

8.5.3. In the event of neutropenic fever or documented infection, appropriate i.v. antibiotic treatment will be initiated.

8.5.4. All blood products will be irradiated. Blood product support will be provided in an attempt to maintain the Hematocrit above 24% and the platelet count above 20,000 unless otherwise indicated.

8.5.5. Parenteral nutrition will be initiated depending on the patient's needs. The patient's caloric requirements will be assessed with the assistance of the dietary service.

8.5.6. Physical examination and laboratory as well as radiographic evaluation will be performed as outlined in the Study Parameters section.

In evaluating toxicity, the NCI Common Toxicity Scale will be used, with modifications appropriate to the setting of high dose chemotherapy with peripheral blood stem cell / autologous bone marrow support (see attached Toxicity Grading Scale). Since stem cell / bone marrow reinfusion is being used to alleviate dose limiting hematologic toxicity, hematopoietic toxicity and similarly, evaluation of gastrointestinal toxicity will be based on guidelines for studies involving stem cell, or autologous transplant criteria adopted from the NCI autologous bone marrow toxicity scale (Appendix B).

12/14/01

8.5.7. Adverse Drug Reaction (ADRs) will be reported as outlined in Appendix C (Reporting Guidelines for Adverse Drug Reactions).

9.0 STUDY PARAMETERS

9.1. Neoadjuvant Chemotherapy and Surgery

		Day 1	Day 8	Day ** 15	Day 22	Day 29	Day 36	Day 43	Day 50	Day 57	Day 64	Day 70-80
REQUIRED STUDIES	Pre Study**	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11-12
History and Physical	X			X		X		X		X		X
Pelvic Examination/Pap Smear	X*											
Weight and Performance Status	X			X		X		X		X		X
Toxicity Notation				X		X		X		X		X
LABORATORY												
CBC/Differential/Platelets+	X		X	X	X	X	X	X	X	X	X	X
Bilateral bone marrow biopsies												X
Measured creatinine clearance	X											
Hepatitis/HIV	X											
SMA 18	X			X		X		X		X		X
Urinalysis and pregnancy test	X											
PFTs/DLCO/ABG	X											
ER/PgR assay	X											X
Pathology submission	X											X
Molecular Pharmacology Studies: Biopsy for ≥ 10 mg tumor tissue obtained prior to chemotherapy for PCR studies at surgery	X											X
X-RAYS AND SCANS												
Mammogram	X											
Chest x-ray	X											
Bone scan	X											
CT chest/abd/pelvis#	X											
MUGA	X											
ECG	X											
TREATMENT												
Surgery												X
Doxorubicin		X		X		X						
Paclitaxel								X		X		

09/01

* A pelvic examination within six months prior to registration is required prestudy. Women who have had their uterus completely removed are exempt from the requirement for prestudy pelvic examinations.

\+ CBC, differential and platelets must be performed weekly starting Day 8 during therapy.

\# Repeat pelvic CT only if indicated.

** Patients with only one prior cycle of neoadjuvant chemotherapy will have prestudy testing performed and begin treatment on Day 15.

09/01

9.2. Paclitaxel and PBSC collection

REQUIRED STUDIES		Day 100	Day 107	Day 114

	Pre Study	Wk 14	Wk 15	Wk 16
History and Physical		X		
Weight and Performance Status		X		
Toxicity Notation	X			
LABORATORY				
CBC/Differential/Platelets	X		X	
Measured creatinine clearance	X			
SMA 18	X			
09/01 PFTs/DLCO/ABG	X			
X-RAYS AND SCANS				
Chest X-ray	X			
Bone Scan	X			
09/01 Bone marrow biopsies	X			
CT chest/abd/pelvis#	X			
09/01 Brain MRI	X			
MUGA				
ECG	X			
TREATMENT				
Paclitaxel		X		
Cyclophosphamide				
PBSC Collection				X
G-CSF		X	X	X
09/01 Mammogram*				

\# Pelvic CT only if indicated.

09/01 * If the prior mammogram had been performed more than 6 months ago."

9.3. High-dose chemotherapy with ACT and PBSC

REQUIRED STUDIES	Pre HDCT	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 7	Day 0	Day 7
History and Physical	X											
Weight and Performance Status	X											
Toxicity Notation		X	X	X	X	X	X	X	X	X	X	X
LABORATORY												
CBC/Differential/Platelets	X		X	X	X	X	X	X	X	X	X	X++
Measured creatinine clearance												
SMA 18	X											
PFTs/DLCO												
X-RAYS AND SCANS												
Chest x-ray	X											
ECG	X											
[1]Nerve Conduction Test Audiogram	X											
TREATMENT												
PBSC									X			
G-CSF								X	X	X	X	X+
CHEMOTHERAPY												
Cyclophosphamide						X						
Doxorubicin		X	X	X	X							
Taxol							X					

09/01

\+ Through AGC > 1000/µl x 3 days
++ Daily till completion of therapy and recovery
[1] Amended 3/98

9.4. High-dose chemotherapy with Mel/Cis and PBSC

REQUIRED STUDIES	Pre HDCT	Day -11	Day -10	Day -9	Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 7
History and Physical	X													
Weight and Performance Status	X													
Toxicity Notation		X	X	X	X	X	X	X		X	X	X	X	X
LABORATORY														
CBC/Differential/Platelets	X		X	X	X	X	X	X	X	X	X	X	X	X++
Measured creatinine clearance	X													
SMA 18	X													
X-RAYS AND SCANS														
Chest x-ray	X													
Bone Scan#														
CT chest/abd/pelvis#														
MUGA	X													
ECG	X													
[2]Nerve Conduction Test	X													
TREATMENT														
Melphalan		X							X					
Cisplatin		X							X					
PBSC										X			X	
G-CSF			X	X	X	X								

6/10/04

\# If indicated.
++ Daily till completion of therapy and recovery
[2] Amended 3/98

9.5. Follow-up

	Day 30 From Start of RX	Every 3 Months	Every 6 Months
H & P	X	X	
CBC, diff, plat	X	X	
SMA-7	X	X	
SMA-12, Mg	X	X	
CXR	X		X
Muga Scan + EKG	X		X[a]
Nerve Conduction Studies	X		X[a]
Mammogram			

a Repeat thereafter at discretion of treating MD.

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

10.1. Complete Clinical Response following neoadjuvant chemotherapy: Clinically undetectable tumor. Partial Clinical Response following neoadjuvant chemotherapy: Palpable tumor or signs of persistent inflammation.

10.2. Complete Pathological Response: Lack of tumor in the modified radical mastectomy specimen. Partial Pathological Response: Any evidence of residual tumor in the modified radical mastectomy specimen.

10.3. Disease-free Survival: From date of mastectomy to date of relapse or death.

10.4. Overall Survival: Survival is measured from the date mastectomy to the date of death.

10.5. Relapse: Appearance of any new lesions during or after protocol treatment. Whenever possible, relapses should be documented histologically.

11.0 STATISTICAL CONSIDERATION

This is a pilot study with the goal of assessing the feasibility and efficacy of administering a complete treatment plan consisting of neoadjuvant sequential chemotherapy, modified radical mastectomy, paclitaxel, cyclophosphamide adjuvant therapy (followed by G-CSF primed peripheral stem cell collection) and 2 cycles of high-dose chemotherapy and peripheral blood stem cell rescue with subsequent local-regional radiation treatment and tamoxifen for patients with receptor positive neoplasms. Based on current referral patterns to the Departments of General and Oncologic Surgery and Medical Oncology and Therapeutics Research, it is expected that approximately 15 patients per year will be accrued to this study. During the first year of this study we estimate the ratio of eligible patients between stratum 1 versus 2 at 5:10. Once the referring oncologists in the greater Los Angeles / Orange County area become familiar

with the design we estimate at least an equalization in the number of patients between the 2 strata. As the majority of relapses are expected to occur within 3 years, a goal of 60 patients accrued in 8 years will be set. Patients will be analyzed for toxicity, disease-free and overall survival and the ability to deliver the treatment phases according to schedule.

12/10/02

As reported in Section 2.0, 3-year relapse-free survival improved from 29% to 50% in the current cohort of COH patients treated with single cycle high-dose chemotherapy compared to an historical group of COH patients treated with standard therapy. Patient accrual will be evenly split between the two strata over a period of 8 years, hence, a comparison of strata within the study could detect an improvement from 50% to 80% in 3-year relapse-free survival (at p=.05 and 80% power), while a comparison of all 60 patients to historical data could detect an improvement from 50% to 72% in 3-year relapse-free survival.

If complete response rates are near 30% among the expected 30 subjects undergoing the entire treatment at COH (stratum 1), then the estimated rate would be accurate to within 17%, sufficient to distinguish the complete response rate from a historical rate of about 10%. The sample size is thus sufficient to establish feasibility at practical referral rates, and to detect a marked improvement in efficacy.

We will be utilizing the Division of Cancer Treatment's (DCT)/National Cancer Institute's (NCI) Common Toxicity Criteria for evaluating toxicity of all organ systems with the exception of the hematologic system and gastrointestinal system, where we will follow the guidelines for studies involving autologous bone marrow transplantation as recommended by the Cancer Therapy Evaluation Program (CTEP).

Criteria for early termination of this feasibility study include > than 2 patients with regimen-related toxicity ≥ grade 4 arrhythmia, CHF, myocardial infarction, tamponade or > 2 patients with ≥ grade 3 hematologic or gastrointestinal toxicities.

12.0 PATHOLOGY REVIEW

All available pathology specimens will be reviewed by the City of Hope Department of Pathology to confirm the primary diagnosis. In addition, morphological and immunohistochemical analysis of biopsy and mastectomy specimens will be carried out assessing grade, receptor status, p53, Ki-67, C-*erb*B-2, MDR1, MRP. In addition, both paraffin embedded and fresh tissue samples will be tested for surrogate signs of hereditary disease i.e. BRCA1 and 2 as these tests become available.

13.0 REGISTRATION GUIDELINES

Once all pretreatment evaluations have been performed, patients will be entered on study after review of patient eligibility by a member of the Department of Biostatistics. Patients may be screened for registration by calling the City of Hope Department of Biostatistics, ext. 2468.

14.0 DATA SUBMISSION SCHEDULE

All primary data will be maintained by the Department of Biostatistics, City of Hope Cancer Research Center. These will include eligibility checklist, prestudy and initial

flow sheet, pathology report, study specific flow sheets as well as off-study information.

15.0 ETHICAL AND REGULATORY CONSIDERATIONS

This study is to be approved by the Institutional Review Board according to City of Hope ethical and regulatory guidelines. All patients will have signed an informed consent for participation in research activities, and they will have been given a copy of the Experimental Subject's Bill of Rights.

When results of a study such as this are reported in medical journals or at meetings, identification of those taking part will be withheld. Medical records of patients will be maintained in strictest confidence according to current legal requirements. However, they may be made available for review, as required by the Food and Drug Administration (FDA) or other authorized users only under the guidelines established by the Federal Privacy Act. The National Cancer Institute or the FDA may review these records.

16.0 MOLECULAR PHARMACOLOGY

In a recent evaluation of the expression of several genes in primary breast cancer specimens from patients with high-risk primary disease treated with both standard chemotherapy and high-dose chemotherapy with stem cell support (Somlo et al ASCO 1996), expression of p53, estrogen, and progesterone receptor protein were found to have prognostic significance. Furthermore, in a recent unpublished evaluation of mRNAs from breast and gastric cancer specimens by Danenberg and colleagues at the USC/Norris Comprehensive Cancer Center, expression of the DNA repair gene *ERCC*1 was found to correlate closely with relapse-free survival in patients treated with platinum-containing chemotherapy regimens. For this reason, patients will be requested to allow a biopsy of their primary tumor specimen to be performed for research purposes in addition to that required for definitive diagnosis of inflammatory breast cancer. A specimen of > 10 mg of tissue will be obtained either by multiple punch biopsies of the skin or by incisional biopsy under local anesthesia after appropriate consent has been obtained. At least 24 hours prior to consenting patients for this procedure, Mary Carroll, R.N. must be contacted at extension 2960 in the Department of Medical Oncology and Therapeutics Research so that the standard procedure for specimen handling developed with the COH Division of Pathology can be implemented. All specimens will be divided at the time of biopsy for standard light microscopy to assess the extent of tumor in the biopsy specimen and to prepare slides for subsequent immunohistochemistry. The remaining material will be quick frozen and the mRNA processed using the standardized procedures in the Analytical Pharmacology Core Facility of the COH Cancer Research Center. Relative quantitation RT-PCR will then be performed in Dr. Doroshow's laboratory to assess the expression of several genes including *MDR*1, *ERCC*1, and ribonucleotide reductase prior to the initiation of chemotherapy in these specimens. Every attempt will be made to perform assessments of gene expression in patients who do not respond to chemotherapy and from the surgical specimens obtained at the end of the induction period of treatment.

17.0 FAMILY HISTORY / HEREDITARY BREAST CANCER EVALUATION

At the COH the median age of high-risk breast cancer patients -including those with inflammatory carcinoma- is 42 years. A clinical feature of hereditary breast cancer is

earlier age at the time of diagnosis. A unique opportunity is presented at our institution to study a significant number of young breast cancer patients presenting with high-risk breast cancer. We plan to include this group of patients with inflammatory breast cancer in our ongoing projects on cancer genetics.. Patients and family members will be asked to signed consent form 2 in order to construct an extended pedigree including family history of cancer. Risk Factor and Family History Questionnaires will be presented to the participants (These questionnaires are included in a separate protocol and authored by Dr. Weitzel, Director of Clinical Cancer Genetics). In addition, tumor samples both prior to and after neoadjuvant chemotherapy and blood samples of patients and appropriate family members will be obtained to search for germ line and somatic mutations of already known genetic markers, tumor suppressor genes and hitherto unidentified genes/markers.

Patients will be enrolled on the treatment protocol regardless of their agreement to participate in the family survey / molecular genetics evaluation.

18.0 MINORITIES AND WOMEN

The City of Hope has a plan in place to increase minority recruitment to our studies in compliance with the National Institute of Health policy for recruitment of women and minorities.

The table below shows the distribution by sex and race of the patients accrued to therapeutic clinical studies at City of Hope for the past five years with the same primary site of disease targeted for this protocol (breast). Our goal is to maintain our high accrual of women while continuing to increase the accrual of minority subjects.

Accrual by Site, 1989 – 1994								
		By Sex		By Ethnicity				
Site	Total	Female	Male	White	Hispanic	Black	Asian	Other
Breast	1060	1059 (100%)	1 (0%)	801 (76%)	110 (10%)	36 (3%)	70 (7%)	43 (4%)

Based on our typical accrual, the table below reflects our goal for the accrual of women and minorities on this research protocol.

Accrual Goal for Women and Minorities								
		By Sex		By Ethnicity				
Site	Accrual Goal	Female	Male	White	Hispanic	Black	Asian	Other
Breast	42	42 (100%)	0 (0%)	25 (60%)	7 (17%)	3 (6.5%)	4 (10%)	3 (6.5%)

19.0 BIBLIOGRAPHY

1. Hortobagyi GN, Singletary SE, McNeese NM. Treatment of locally advanced and inflammatory breast cancer. In Diseases of the Breast, (Jay Harris, Marc Lippman, Monica Morrow, and Samuel Hellman, eds), Lippincott-Raven Publishers, Philadelphia, PA, pp 585-599, 1996.

2. Peters WP. Autologous bone marrow transplantation for breast cancer. In Bone Marrow Transplantation, (Stephen Forman, Karl Blume E. Donnall Thomas, eds), Blackwell Scientific Publications, Boston, Massachusetts, pp 789-802, 1994.

3. Wood WC, Budman DR, Horzun AH, Cooper MR, Younger J, Hart RD, Moore A, Ellerton JA, Norton L, Ferree CR, Ballow AC, Frei III E, Henderson IC. Dose and dose intensity of adjuvant chemotherapy for stage II, node-positive breast carcinoma. N Engl J Med 330:1253-1259, 1994.

4. Somlo G, Doroshow JH, Forman SJ, Maryon T, Lee J, Chow W, Hamasaki V, Leong L, Morgan Jr. R, Margolin K, Raschko J, Shibata S, Tetef M, Yen Y, Simpson J, Molina A. High-dose chemotherapy and stem cell rescue in the treatment of high-risk breast cancer: prognostic indicators and progression-free and overall survival. Manuscript submitted.

5. Somlo G, Doroshow JH, Ahn C, Chow W, Hamasaki V, Leong L, Margolin K, Morgan R, Raschko J, Shibata S, O'Donnell M, Smith E, Tetef M, Forman S, Yen Y, Molina A, Sniecinski, 1. Double cycle high-dose melphalan (MEL) and cisplatin (CDDP) with peripheral stem cell rescue (PSCR) in patients with breast cancer (BC) and other malignancies. Proc Am Soc Clin Oncol 15:352, 1996.

6. Bonadonna G, Zambetti M, Valagussa P. Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes. Ten-year results. JAMA 273:542-547, 1995.

7. Reichman BS, Seidman AD, Crown JPA, et al. Paclitaxel and recombinant human granulocyte colony-stimulating factor as initial chemotherapy for metastatic breast cancer. J Clin Oncol 11:943-1951, 1993.

8. Hudis C, Seidman A, Raptis G, Baselga J, Fennelly D, Theodoulou M, Gilewski T, Currie V, Moynahan M, Surbone A, Sklarin N, Robles M, Riccio L, Yao T-J, Uhlenhopp M, Norton L. Sequential (seq) dose-dense (DD) doxorubicin (A) - > paclitaxel (T) - > cyclophosphamide 0 is less toxic than A - > concurrent (Con) T + C as adjuvant therapy in resected node positive (+) breast cancer (NPBC). Proc Am Soc Clin Oncol 15:119, 1996.

9. Seidman AD, Hachhauser D, Gollub M, Edelman B, Yao T-J, Hudis CA, Francis P, Fennelly D, Gilewski TA, Maynahan ME, Currie V, Baselga J, Tong W, O'Donaghue M, Salvaggio R, Auguste L, Spriggs D, Norton L. Ninety-six-hour paclitaxel infusion after progression during short taxane exposure: A phase II pharmacokinetic and pharmacodynamic study in metastatic breast cancer. J Clin Oncol 14:1877-1884, 1996.

10. Stemmer SM, Cagnoni PJ, Shpall EJ, Bearman SI, Matthes S, Dufton C, Day T, Taffs S, Hami L, Martinez C, Purdy MH, Arron J, Jones RB. High-dose paclitaxel,

cyclophosphamide, and cisplatin with autologous hematopoietic progenitor-cell support. A phase I trial. J Clin Oncol 14:1463-1472, 1996.

11. Vahdat LT, Balmeceda C, Papadopoulos KP, Garrett TJ, McGovern T, Antman KH, Hesdorffer CS. Tandem high dose chemotherapy (HDC) with escalating paclitaxel (P), melphalan (M) and cyclophosphamide (C), thiotepa, and carboplatin (CTCB) with peripheral blood progenitor (PBP) support in responding metastatic breast cancer. ASBMT, p 37, 1996, 2nd Annua Meeting, San Diego, CA.

12. Somlo G, Doroshow JH, Synold T, Chow W, Hamasaki V, Leong L, Margolin K, Morgan R, Raschko J, Forman SJ, Shibata S, TetefM, Yen Y. High-dose Adriamycin (A), cyclophosphamide (C) and paclitaxel (T) for patients (PTS) with high-risk and responsive stage IV breast cancer (BC). ASBMT, p 90, 1996, 2nd Annual Meeting, San Diego, CA.

13. Swain S, Whale F, Gerber M, Wiesberg S, York M, Spicer D, Jones S, Wadler S, Desai A, Vogel C, Speyer J, Mittelman A, Reddy S, Pendergradd K, Velz-Garcia E, Ewer M, Bianchine J, Gams. Cardioprotection with dexrazoxane for doxorubicin-containing chemotherapy in advanced breast cancer. J Clin Oncol 15: 1318-1340,1997

14. Lopez M, Vici P, Di Lauro L, Conti F, Paoletti G, Ferraironi A, Sciuto R, Gianarelli D, Maini C. Randomized prospective clinical trial of high-dose epirubicin and dexrazoxane in patients with advanced breast cancer and soft tissue carcinomas. J Clin Oncol 16: 86-92, 1998

15. Somlo G, Simpson J, Doroshow J, Forman S, Niland J, Leong L, Morgan R, Raschko J, Lee J, Margolin K, Hamasaki V, Shibata S, Chow W, Tetef M, Molina A, Battifora H. Immunohistochemical (IH) expression of P53, estrogen (ER) and progesterone (PR) receptors and MIB1 as well as increased mitotic index (MI) are predictors of outcome after high-dose chemotherapy (HDCT) and stem cell rescue (SCR) for high-risk breast cancer (HRBC) patients (PTS). Proc Am Soc Clin Oncol 15:126, 1996.

16. Weber BL, Garber JE. Familial breast cancer. In Diseases of the Breast, (Jay Harris, Marc Lippman, Monica Morrow, and Samuel Hellman, eds), Lippincott-Raven Publishers, Philadelphia, PA, pp 168-185, 1996.

17. Albain KS, Allred DC, Clark GM. Interactions between very young age and prognostic factors for disease-free survival (DFS) in the presence or absence of adjuvant breast cancer therapy. Proc Am Soc Clin Oncol 15:107, 1996.