CITY OF HOPE NATIONAL MEDICAL CENTER
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DEPARTMENT OF MEDICAL ONCOLOGY AND THERAPEUTICS RESEARCH

TITLE: Randomized Phase II Study Of Docetaxel, Adriamycin, And Cytoxan (TAC) Versus Adriamycin/Cytoxan, Followed By ABI/Carboplatin (ACAC) +/- Trastuzumab As Neoadjuvant Therapy For Patients With Stage I-III Breast Cancer.

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 05015 **PROTOCOL DATE: 03/17/09**

DATE(S)/ OF AMENDMENT(S)/REVISION(S):	Initial Review – Protocol dated 08/09/05 –	Packet: 00
COH Amendment 01	Dated 10/11/05 (Addition of Tower Group)	01
COH Amendment 02	Dated 03/22/06	02
COH Amendment 03	Dated 12/04/06	03
COH Amendment 04	Dated 04/26/07	04
COH Amendment 05	Dated 07/24/07 (Title page change only)	05
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SITE: Breast
HISTOLOGY: Adenocarcinoma
STAGE : II/III
MODALITY: Chemotherapy +/- immunotherapy
TYPE : Phase II

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IRB:	05015
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VERSION NUMBER:	14
VERSION DATE:	03/17/09
SITE:	Breast
HISTOLOGY:	Adenocarcinoma
STAGE:	I/II/III
MODALITY:	Chemotherapy +/- immunotherapy
TYPE:	Neoadjuvant therapy
ARMS:	TAC (Arm A) vs. ACAC (Arm B); ACAC + trastuzumab (Arm C)
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INTERNAL REVIEW BOARD NUMBERS:	
DATE OF AMENDMENTS/REVISIONS:	(1) 10/11/05 (2) 03/22/06 (3) 12/04/06 (4) 04/26/07 (5) 10/24/07 (6) 02/07/08 (7) 03/19/08 (8) 07/15/08 (9) 08/11/08 (10) 03/17/09

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a Cremophor EL-free of Paclitaxel for Injectable Suspension, is the first in its class of a biologically interactive compound combining a protein with a chemotherapeutic agent in the compound state. This composition provides a novel approach of increasing intra-tumoral concentration of the drug by a receptor-mediated transport process allowing transcytosis across the endothelial cell wall, thereby breaching the blood/tumor interface. This albumin-specific receptor mediated process involves the binding of a specific receptor (gp60) on the endothelial cell wall, resulting in activation of a protein caveolin-1, which initiates an opening in the endothelial wall with formation of a little caves or caveolae, with transport of the albumin-bound chemotherapeutic complex via these caveolae to the underlying tumor interstitium. A protein specifically secreted by the tumor (SPARC) binds and entraps the albumin, allowing release of the hydrophobic drug to the tumor cell membrane. Abraxane® is the first biologically interactive compound leveraging this gp-60/caveolin-1/caveolae/Sparc pathway to increase intra-tumoral concentration of the drug and reducing toxic drug in normal tissue. Abraxane® has undergone testing in patients with stage IV breast cancer, with encouraging preliminary results, leading to recent FDA approval of this agent in view of its ability to induce higher response rates in comparison to paclitaxel when given every 3 weeks at 260 mg/m^2 (versus paclitaxel 175 mg/m^2) (30). A Phase I study of ABI-007 (Abraxane®) administered weekly for 3 weeks followed by a 1 week rest in patients with advanced solid tumors has recently been completed (31). The MTDs for heavily and lightly pre-treated patients were 100 and 150 mg/m^2 respectively. Dose limiting toxicities included myelosuppression and peripheral neuropathy. Premedication was not required, and unexpected, non-taxane associated toxicities were not observed. In a Phase II trial in heavily pretreated patients with taxane-refractory metastatic breast cancer, objective antitumor responses occurred in 15% of women treated with Abraxane® 100mg/m^2 on this schedule (32). Toxicity was minimal even without premedications or growth factors (G-CSF) usually routinely required with paclitaxel or docetaxel in this group of patients. Patients were considered taxane-refractory if they had progressive cancer while receiving paclitaxel, docetaxel or both. Abraxane® given weekly was active in patients refractory to paclitaxel (16% overall response rate and 37% disease control) and patients refractory to docetaxel (24% overall response and 35% disease control). Grade 3/4 neutropenia without G-CSF in heavily pretreated patients was 15%. Other grade 3 toxicities, all below 4% were: anemia, neuropathy, nausea, vomiting and diarrhea. Results of this dose-finding study of weekly Abraxane® in heavily pretreated patients identified Abraxane® 100 mg/m2 as an appropriate dose for further study. It has proved activity in paclitaxel and docetaxel refractory patients with acceptable toxicity. Preliminary data (personal communication from Dr. Andrew Seidman, Memorial Sloan-Kettering Cancer Center) suggest that weekly administration of Abraxane® at 100 mg/m^2 and carboplatin at an AUC of 2 together with trastuzumab is feasible, and active, however, due to allergic reactions associated with carboplatin administration, steroid premedication may be required with such combination. 12

nausea, vomiting, pain, headache, dizziness, dyspnea, or rash. The symptoms occurred less frequently with subsequent infusions. Rarely, infusion reactions have results in death. 37

- Trastuzumab administration can result in the development of ventricular dysfunction and CHF. Left ventricular function should be evaluated in all subjects prior to and during treatment with trastuzumab. Discontinuation of trastuzumab treatment should be strongly considered in subjects who develop a clinically significant decrease in left ventricular function. The incidence and severity of cardiac dysfunction appears to increase in subjects who receive trastuzumab in combination with anthracycline and cyclophosphamide (28% versus 7% when used as a single agent). 37

- The incidence of generalized pain, abdominal pain, and back pain appears to be more frequent in subjects receiving trastuzumab in combination with chemotherapy. Occasionally pain at tumor sites has been reported. 38

- The following have also been reported in >5% of subjects: diarrhea (25% of these), myalgia, peripheral edema, and weakness. 38

- **Less Frequent Incidence (1 to 5%)** 38

- Allergic/hypersensitivity reactions that have been reported include rash, pruritus, urticaria, and anaphylaxis (rarely resulting in death) or anaphylactoid signs and symptoms (erythematous rash on the chest and beck, dyspnea, bronchospasm, angioedema, acute respiratory distress syndrome (ARDS), hypotension, wheezing, pleural effusions, pulmonary infiltrates, noncardiogenic pulmonary edema, and pulmonary insufficiency and hypoxia requiring supplemental oxygen or ventilatory support). 38

- **Rare Incidence (<1%)** 38

- Rare toxicities include abnormal liver function test, hepatitis, bone pain, tumor-site pain, pancytopenia, hypotension, anorexia, febrile neutropenia, worsening of pre-existing peripheral neuropathy, paresthesias, and thromboembolic disease. 38

- Pulmonary events that have been reported include pleural effusions, pulmonary infiltrates, and noncardiogenic pulmonary edema. Pulmonary insufficiency and hypoxia requiring supplemental oxygen or ventilatory support, including ARDS, and death have occurred. Most subjects with fatal events had significant, pre-existing pulmonary compromise secondary to intrinsic lung disease and/or malignant pulmonary involvement. Because it appears that subjects with significant pre-existing pulmonary compromise may be at greater risk, these subjects should be treated with extreme caution. Subjects experiencing any of the severe infusion-associated symptoms should have the trastuzumab infusion discontinued and appropriate medical therapy administered. Subjects should be closely monitored until complete resolution of their symptoms. In addition, subjects should be informed of the possibility of delayed severe reactions. 38

- 3.8.4 Nursing Guidelines 38

- The most common AEs related to trastuzumab are fever (> 38°C), chills, and occasional rigors, most often infusion-related after the initial dose. Treat with acetaminophen as necessary. Meperidine may be needed for rigors. Instruct the subject and family to report any fever >101°F. Subjects with underlying pulmonary pathology should be closely monitored. 38

- Transient, localized tumor-side pain may be experienced within 8 hours of infusion. Advise the subject that acetaminophen is helpful. 38

- Provide symptomatic management of the possible mild-to-moderate nausea/vomiting/diarrhea. Assess heart and lung sounds. Monitor vital signs (resting pulse, BP). Be alert to early signs of cardiotoxicity, i.e., dyspnea, steady weight gain, nonproductive cough, arrhythmias, tachycardia, and pulmonary rales. Trastuzumab may potentiate heart failure when administered in combination with *doxorubicin*. 39

- Advise subjects of possible fatigue, loss of strength, and weakness. Have subjects pace activities with frequent rest periods. 39

- 4.0 STAGING CRITERIA 39

DEFINITION OF TNM 39

- Q5. Does your pain feel like electric shocks? 103
- Q6. Does your pain feel like stabbing? 103
- Q8. Is your pain provoked or increased by brushing on the painful area? 103
- Q9. Is your pain provoked or increased by pressure on the painful area? 103

03/22/06

S C H E M A

Biopsy of primary, clip placement, core biopsies and dominant/sentinel node biopsy

HER-2 – patients will be randomized to
- **Arm A** (Docetaxel/Doxorubicin/Cytoxan; TAC)
- **Arm B** (Doxorubicin/Cytoxan → Carboplatin/Abraxane®;ACAC)

HER-2 + patients will receive **Arm C** (Doxorubicin/Cytoxan/Carboplatin/Abraxane® + Trastuzumab;ACAT)

Arm A

Docetaxel	75 mg/m^2 IV	*Q 21 days x 6 cycles*
Doxorubicin	50 mg/ m^2 IV	
Cytoxan	500 mg/ m^2 IV	

Arms B and C

Doxorubicin	60 mg/ m^2 IV	*Q 14 days x 4 cycles*
Cytoxan	600 mg/ m^2 IV	

Followed by

Carboplatin	AUC 2	*weekly x 3 and one week off*	*x 3cycles*
Abraxane®	100 mg/m^2	*weekly x 3 and one week off*	

*Trastuzumab (Arm C) 2 mg/kg Q *week x 12 weeks*

03/19/08

Neulasta™ or Neupogen® sc will be administered with all cycles of TAC and with all cycles of AC during ACAC; Neupogen will be administered during weeks 2 and 3 of each cycle of ACAC on Arms B & C.

*** Trastuzumab will be prescribed weekly together with carboplatin and Abraxane® to all patients with HER2 overexpressing breast cancer; the first dose of trastuzumab will be administered at a loading dose of 4 mg/kg .**

OBJECTIVES

03/19/08

1.1. To compare feasibility and efficacy - as defined by pathological complete response rate (pCR) - of docetaxel, doxorubicin, and cyclophosphamide (TAC) versus dose-dense doxorubicin and cyclophosphamide followed by carboplatin and Abraxane® (ACAC) in patients with HER-2 negative breast cancer.

03/19/08

1.2. To assess feasibility and efficacy as defined by Symmans Score response of dose-dense doxorubicin and cyclophosphamide followed by carboplatin, Abraxane®, and trastuzumab (ATACT), in patients with HER-2 positive breast cancer.

03/22/06

1.3. To determine the proportion of patients with a positive sentinel lymph node (SLN) before neoadjuvant (preoperative) chemotherapy will have metastases in nonSLNs (NSLNs) after neoadjuvant chemotherapy, and identify which clinicopathologic factors are associated with metastases in NSLNs after such chemotherapy in patients with a positive SLN before preoperative chemotherapy.

1.4. To assess quality of life in all participating patients across the treatment groups in order to define the less toxic regimen. To compare treatment-induced neuropathy/neuropathic pain in a subset of patients participating in this study.

03/22/06

1.5. To identify issues involved in the delays between awareness of symptoms of locally advanced breast cancer (Stage III A-C) and receiving medical treatment.

1.6. To identify specific mutations in tumor DNA in comparison to adjacent tissue and germ line DNA procured prior to, during, and subsequent to neoadjuvant chemotherapy, and to detect/measure the presence of such mutations in fragmented, circulating DNA from plasma, and correlate with the presence/characteristics of circulating tumor cells, in order to identify prognostic and predictive indicators of persisting/relapsed disease, and targets for therapy.

1.7. To assess protein profiles in tumor, adjacent tissue and plasma prior to, during, and at completion of neoadjuvant chemotherapy, in order to establish prognostic and predictive indicators of outcome, markers of persistent/relapsed disease, and targets for therapy.

03/19/08

1.8. To analyze tumor DNA to carry out microarray and RT-PCR analysis in addition to procuring genomic DNA from plasma to assess copy numbers/SNP/genomic polymorphism in genes for the purposes of establishing prognostic and predictive indicators of outcomes, markers of persistence/relapse disease, drug resistance, targets of therapy as well as drug metabolism.

1.9. To assess the prognostic and predictive value of conventional pathological features (stage, estrogen and progesterone receptor and HER-2 status, presence of lymphovascular invasion, high grade) in comparison to such values derived, based upon the molecular approaches.

12/4/06

1.11 To procure tumor from the definitive surgical specimen for the purpose of establishing breast cancer stem cell lines

2.0 BACKGROUND

In spite of the rising incidence of breast cancer disease, specific mortality has decreased over the past few years. There are two likely explanations for this phenomenon: increased awareness coupled with more effective screening allows for detection of early stage cancer with inherently better prognosis, and, modern day systemic therapy is better at preventing development, or, at the least allows for containment of systemic metastases (1,2).

2.1 Recent developments in systemic chemotherapy

The currently used neoadjuvant regimens have mostly evolved from experience in the adjuvant setting, or after these combinations have undergone testing in patients with stage IV advanced disease. More recently, there have been 2 adjuvant regimens/strategies that, when tested in prospective, randomized trials, resulted in improved relapse-free survival and trended toward better overall survival. Incorporation of docetaxel with doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan) (TAC) proved to be a superior regimen in comparison to 5-FU, Adriamycin, and Cytoxan (FAC, [relapse-free survival: 82% vs. 74%]) when both combinations were given every 21 days for 6 treatment cycles; there was also a suggestion of overall survival benefit in patients who presented with fewer, 1-3 axillary nodal metastases, in favor of TAC (3). The second approach, dose-dense (every 2 weeks) sequential, or combined administration of Adriamycin, Cytoxan, and subsequent biweekly paclitaxel yielded better 4-year relapse-free survival (82 % vs. 75%, RR: 0.74) and improved overall survival (RR:0.69) in comparison to administering the same agents every 3 weeks(4). Weekly administration of paclitaxel, in addition to every 3 week delivery of Adriamycin and Cytoxan -as part of another "dose-dense" strategy – may also be more effective in delaying relapse (5,6). Newer combinations consisting of a taxane and platinum are currently undergoing evaluation both in the neoadjuvant and adjuvant setting, based on confirmed activity of such combinations in the treatment of metastatic breast cancer. In such metastatic setting, combinations of carboplatin and a taxane have been found to be effective and safe when given on a variety of schedules, including weekly dose-dense administration (7-9).

03/19/08

2.2 Recent developments in systemic neoadjuvant chemotherapy

Recent data in the literature supports the notion that excellent pathological response to neoadjuvant chemotherapy may benefit patients in terms of relapse-free and overall survival (10, 11). Data from MD Anderson (12) suggests that complete pathological response as well a minimal residual disease labeled as scores 0 and 1 by the Symmans classification, carry the same prognosis. Administration of systemic chemotherapy before, rather then after surgical removal of a malignant tumor, does have theoretical advantages. Such neoadjuvant chemotherapy may allow breast preservation in patients presenting with large tumors who otherwise would have undergone mastectomy. The strategy may lead to better local control, prevent development of drug resistance, and, allows for better assessment of *in vivo* chemo-sensitivity. In general, overall survival has not been different among patients treated with identical duration/types of chemotherapy either prior to surgery, or in the adjuvant setting. However, based on results from NSABP B-18, a study comparing 4

cycles of adjuvant versus neoadjuvant Adriamycin and Cytoxan, accomplishment of complete pathological response (pCR) in patients treated on the neoadjuvant treatment arm was associated with improved relapse-free and overall survival (13,14). It has also become clear, that 4 cycles of an anthracycline containing regimen yield inferior complete pathological response rates (9.8%) in comparison to the same treatment, followed by 4 cycles of docetaxel (18.7%), as shown in NSABP B-27. More recently, 5 year follow-up data from this study revealed relapse free survival (Hazard Ratio [HR]: 0.33) and overall survival (HR: 0.45) advantage favoring patients who achieved pathological complete remissions in the breast as documented at the time of definitive surgery, suggesting that upfront aggressive and extended therapy will yield better outcome (15). Other studies comparing 8 cycles of an anthracycline-containing regimen to fewer cycles of the same regimen followed by either weekly paclitaxel, or docetaxel given every 3 weeks, concur with the findings of NSABP-27: there seems to be additional benefit derived from sequentially incorporating taxanes into a neoadjuvant regimen (16,17). While the above-described strategies do result in higher pCR rates, they remain relatively low at the 19-35% range. Intuitively, as well as based on the provocative data from the quoted NSABP studies, accomplishment of pCR could be the first step in a strategy leading to improved relapse-free and overall survival.

2.3 HER-2 overexpressing breast cancer

Approximately 25-30% of breast cancers can be characterized by overexpression of the HER-2 proto-oncogen, as documented either by immunohistochemical assessment of cell membrane or by FISH. This group of patients is faced with a worse prognosis, due to a greater likelihood of developing distant metastases. A humanized monoclonal antibody (trastuzumab, Herceptin®) has been developed and used successfully in the treatment of stage IV breast cancer (18-20). There is evidence of *in vitro* and *in vivo* synergism between trastuzumab, and some of the most effective chemotherapeutic agents, such as the taxanes, and platinum compounds (21). Accordingly, in the metastatic setting combinations of trastuzumab and taxanes with or without platinum compounds such as carboplatin vs. administration of the chemotherapeutic agents alone resulted in improved progression-free survival and a trend towards overall survival (19,22). Trastuzumab is currently undergoing evaluation in combination or in sequence to anthracycline, taxane, and platinum based therapies in the adjuvant and neoadjuvant setting in a variety of cooperative group trials. In one provocative, prospective, randomized study of paclitaxel followed by 5-fluorouracil, epirubicin, and Cytoxan (FEC) +/- trastuzumab in patients with HER2 overexpressing tumors, a surprisingly high pathological complete response rate of 65% had been described in the group treated with trastuzumab (23).

2.4 Recent developments in taxane delivery/formulation

Of the two taxanes in clinical use, paclitaxel has shown potentially improved activity when administered weekly, while the more traditional way of docetaxel administration is every 3 weeks (23-29). The toxicity profiles of the two drugs are somewhat different, and are also schedule dependent. Standard clinical practice calls for premedication with steroids, and, in the case of paclitaxel, (in order to alleviate cremophor vehicle-related problems) antihistamines are also part of the pre-treatment regimen. ABI-007 also known as Abraxane® (paclitaxel protein-bound particles for injectable suspension; (albumin-bound)) a Cremophor EL-free of Paclitaxel

for Injectable Suspension, is the first in its class of a biologically interactive compound combining a protein with a chemotherapeutic agent in the compound state. This composition provides a novel approach of increasing intra-tumoral concentration of the drug by a receptor-mediated transport process allowing transcytosis across the endothelial cell wall, thereby breaching the blood/tumor interface. This albumin-specific receptor mediated process involves the binding of a specific receptor (gp60) on the endothelial cell wall, resulting in activation of a protein caveolin-1, which initiates an opening in the endothelial wall with formation of a little caves or caveolae, with transport of the albumin-bound chemotherapeutic complex via these caveolae to the underlying tumor interstitium. A protein specifically secreted by the tumor (SPARC) binds and entraps the albumin, allowing release of the hydrophobic drug to the tumor cell membrane. Abraxane® is the first biologically interactive compound leveraging this gp-60/caveolin-1/caveolae/Sparc pathway to increase intra-tumoral concentration of the drug and reducing toxic drug in normal tissue. Abraxane® has undergone testing in patients with stage IV breast cancer, with encouraging preliminary results, leading to recent FDA approval of this agent in view of its ability to induce higher response rates in comparison to paclitaxel when given every 3 weeks at 260 mg/m^2 (versus paclitaxel 175 mg/m^2) (30). A Phase I study of ABI-007 (Abraxane®) administered weekly for 3 weeks followed by a 1 week rest in patients with advanced solid tumors has recently been completed (31). The MTDs for heavily and lightly pre-treated patients were 100 and 150 mg/m^2 respectively. Dose limiting toxicities included myelosuppression and peripheral neuropathy. Premedication was not required, and unexpected, non-taxane associated toxicities were not observed. In a Phase II trial in heavily pretreated patients with taxane-refractory metastatic breast cancer, objective antitumor responses occurred in 15% of women treated with Abraxane® 100mg/m^2 on this schedule (32). Toxicity was minimal even without premedications or growth factors (G-CSF) usually routinely required with paclitaxel or docetaxel in this group of patients. Patients were considered taxane-refractory if they had progressive cancer while receiving paclitaxel, docetaxel or both. Abraxane® given weekly was active in patients refractory to paclitaxel (16% overall response rate and 37% disease control) and patients refractory to docetaxel (24% overall response and 35% disease control). Grade 3/4 neutropenia without G-CSF in heavily pretreated patients was 15%. Other grade 3 toxicities, all below 4% were: anemia, neuropathy, nausea, vomiting and diarrhea. Results of this dose-finding study of weekly Abraxane® in heavily pretreated patients identified Abraxane® 100 mg/m2 as an appropriate dose for further study. It has proved activity in paclitaxel and docetaxel refractory patients with acceptable toxicity. Preliminary data (personal communication from Dr. Andrew Seidman, Memorial Sloan-Kettering Cancer Center) suggest that weekly administration of Abraxane® at 100 mg/m^2 and carboplatin at an AUC of 2 together with trastuzumab is feasible, and active, however, due to allergic reactions associated with carboplatin administration, steroid premedication may be required with such combination.

03/22/06

2.5 Rationale for the proposed study design

We hypothesize that accomplishment of pCR is a predictor of improved survival, as suggested by the results of NSABP -18 and 27. While the optimal neoadjuvant chemotherapy regimen has not been established, comparison of the "front-runner"

adjuvant strategies (6 cycles of docetaxel, doxorubicin, and cyclophosphamide [TAC]), versus a dose dense administration of Adriamycin and Cytoxan (AC), and a taxane may allow identification of the "better" regimen as defined by less toxicity using FACT-B and FACT-Taxane subscale -see Appendix- B, (33, 34), and by measurement of the pathological CR rate. We propose that, with the incorporation of a less toxic, and possibly more effective taxane (ABI-007 [Abraxane®]) and the platinum agent carboplatin, a higher pCR may be accomplished, without substantially increasing toxicity. To test this hypothesis, patients with HER-2 negative breast cancer will be randomized between TAC and dose-dense AC followed by weekly Abraxane® and carboplatin (ACAC). All patients with HER-2 overexpressing breast cancer will be treated with ACAC and, in addition, weekly trastuzumab (Herceptin®) will be co-administered with Abraxane® and carboplatin.

03/22/06

2.6 Scientific questions/ hypotheses

There are many important research questions that can be addressed best in the context of a neoadjuvant treatment trial, since serial assessment of malignant tissue first for diagnosis, and then for removal of the previously involved region of the breast is already part of standard clinical approach. Capitalizing on this opportunity, procurement of cancerous and adjacent "host" environmental tissue, blood, and possibly other sources such as urine, both prior to and subsequent to therapy will allow for better definition of the tumor biology, predictors of treatment response, markers of relapse, target selection, and effective treatment strategies. Here are just some of the scientific questions one can attempt to answer in the setting of the proposed study:

2.6.1 Do the presence of specific DNA mutations in the tumor –in comparison to the non-malignant host environment - allow for predicting outcome, provide a method for early detection of relapse, and identify targets/tools of therapy?

We will look for specific mutations in genes known to be relevant in the development/progression of breast tumors; potential targets include genes for nuclear transcription factors (p53), apoptosis (PTEN, Phosphatidylinositol 3-kinase subunit alfa (PI3-KCA), intracellular transducers (Ras), receptors (HER-2, epidermal growth factor receptor (EGFR) and the insulin –like growth factor I) (35-54). We will apply bi-directional pyrophosphorolysis-activated allelic specific amplification (Bi-PAP-A) measurement in comparison to adjacent tissue and germ line DNA (55). Once identified, we will examine the frequency of such mutations in plasma and the cellular components of blood, and assess their significance. Listed are some of the possible findings:

1. Larger tumors tend to have higher levels of the tumor-associated mutation in their blood.
2. The frequency of the tumor-associated mutation will be reduced most dramatically in those patients that had the best response to chemotherapy.
3. The mutation frequency in blood may increase during chemotherapy in some patients.
4. Those patients with increased mutation frequency as a result of the chemotherapy have a poor prognosis because the increased frequency

reflects an increase frequency of cells with metastatic potential that are shed into the blood.

5. Those patients with increased mutation frequency in plasma will have a better prognosis since this component reflects fragmented DNA from dead cells.
6. Those patients whose tumor progresses on chemotherapy will show an increased tumor mutation frequency in blood.
7. In an individual patient, the frequency of specific mutations may increase during chemotherapy as a result of the general mutagenic affects of these agents.
8. A subset of patients will be highly susceptible to the mutagenic effects of chemotherapy and these patients will have a poor prognosis since the residual cancer is mutagenized, providing increased genetic diversity for selection of metastatic clones.
9. An increase or decrease in circulating tumor DNA copy numbers will also correlate with the presence/caracteristics of circulating tumor cells.

2.6.2 Will identification of protein expression patterns allow for identification of predictors of outcome, markers of relapse, and targets/tools of therapy?

Analysis of laser capture microdissected (LCM) tumor and host tissue will allow for extraction of proteins from frozen, or ethanol-preserved tissue, procured before, during, and after completion of neoadjuvant chemotherapy in order to subject the specimens to protein array analysis for the purpose of delineating and assessing the activity level of cellular pathways participating in cell cycle, apoptosis, or drug metabolism (56-58). Proteomic analysis of the invasive tumor and host environment will allow generation of molecular profile and assessment of signal transduction activity based on the detected pattern of proteins. Low molecular weight protein profiling by surface enhanced laser desorption ionization (SELDI), electron spray mass spectrometry (ES-MS), and matrix-assisted laser desorption mass spectrometry (MALDI-MS) may provide diagnostic, early and late warning signals, and targets of therapy tissue, serum, or body fluid. Two-dimensional gel electrophoresis and immunohistochemical means as well and protein microarray may lead to specific validation of the proteomic profile of the tumor, surrounding tissue, and body fluids (59-66). Further understanding of the relationship of the relevant protein and genetic markers could lead to more accurate estimation of risk of recurrence, resistance to therapy, and guide effective intervention.

Studying of protein profile in serum may help to answer important biological and clinical questions. Preliminary work suggest that such “signals” may be helpful in differentiating non-malignant and malignant processes in patients with ovarian and breast pathology (67-69). We will search for signals of such protein profiles in plasma prior to, during, at completion of neoadjuvant chemotherapy, and during follow-up.

Prior experience by our group has revealed predictors of adverse outcome by assessing tumor protein profile applying immunohistochemical methods. For example, we have shown that overexpression of HER-2 protein, presence of

mutant p53, and low level expression of p21 and p27 have been associated with worse outcome in patients with high-risk primary breast cancer (70). Applying surface enhanced laser desorption ionization (SELDI), electron spray mass spectrometry (ES-MS), or matrix-assisted laser desorption mass spectrometry (MALDI-MS) will allow establishment of patterns of expression, and identification of specific proteins using immunohistochemistry, 2D gel electrophoresis and protein microarray. One can then proceed to assess functional status as defined for example by degree of phosphorylation, and evaluate any correlation with adverse outcome (i.e. incomplete pathological response) (71-74). Our findings may lead to a change in therapeutic strategy and identification of new treatment targets as well as development of novel diagnostic panels.

2.6.3 Will identification of patterns of gene expression and microarray analysis allow prediction of outcome and identification of targets of therapy?

The anthracycline doxorubicin is an essential component of most current adjuvant and neoadjuvant treatment regimens. It works, in part, through inhibition of topoisomerase 2 (TOP2) function and stabilization of the DNA-TOP2 complex, eventually leading to DNA Double-strand break (DSB) upon colliding with the replication fork or the transcription apparatus (75).

Genomic DNA is continuously exposed to endogenous and exogenous agents that elicit DNA damage. Cellular response to genotoxic stress ranges from cell cycle arrest allowing for DNA repair, to apoptosis, if the damage is irreparable (76,77). Replication of damaged DNA is prevented by cell cycle checkpoint controls, which are essential parts of the DNA-damage signaling network. Repair of DNA DSB, as seen upon doxorubicin treatment, is carried out mainly through the actions of members of the phosphatidylinositol kinase-related kinase (PIKK) family: ATM, ATR and DNA-PK. These kinases share sequence homology and many of the same substrate, but they are activated differently based on the type of genotoxic stress. ATM is an integral part of the cell cycle checkpoint control, resulting in the activation of either apoptotic or anti-apoptotic signaling pathway(s) (76,77). ATM primarily responds to agents that cause DNA double-strand breaks (DSBs), whereas ATR plays a role in response to drug-induced changes manifesting in the formation of bulky adducts to DNA, stalling of replication forks and generation of single-stranded DNA breaks. DNA-PK holoenzyme binds to DNA strand breaks, recruits and activates DNA-PKcs, and plays an essential role in non-homologous end-joining (NHEJ) processes.

The initial response to DNA damage is recruitment of several proteins to the damage site, with members of the MRN complex: Mre11, Rad50, and Nbs1 (77) playing prominent roles. For example, ATM undergoes autophosphorylation at S1981 and subsequently phosphorylates other downstream substrates, such as Chk2, which in turns phosphorylates several cyclin-dependent kinase (CDK) compounds, c-Abl, p53, and BRCA1. This series of events will eventually result in cell accumulation in a specific cell cycle phase or leads to apoptosis (78-81).

High-mobility group A2 (HGMA2), also known as HMGI-C, is a non-histone chromosomal architectural transcription factor belonging to the HMGA family, which consists of 3 additional members: HMGA1a, HMGA1b, and HMGA1c (83). HMGA2 level is elevated during embryogenesis, but is usually undetectable in normal adult tissues.

HMGA2 expression is reported to be an indicator for poor prognosis and metastasis of breast cancer. Our preliminary results indicate that the differential responsiveness of breast cancer cells to doxorubicin correlates well with the steady-state level of HMGA2. The degree of expression of H2AX S139 phosphorylation, a TOP2 binder may also reflect on the status of damage signaling network. HMGA2 confers selective sensitivity for doxorubicin in HS578T breast cancer cells, but not in breast cancer KCC1419 cells, possibly via modulation of the cellular response to doxorubicin-elicited genotoxicity. HMGA2 expression in breast cancer cells may provide a useful therapeutic marker for predicting chemosensitivity to doxorubicin treatment (82-86).

2.6.4 Are DNA or RNA expression panels by microarray analysis or by RT-PCR profile helpful in providing prognostic models and predicting therapeutic response?

Gene expression profiles generated based on cDNA cloning from RNA extracted from frozen tissue can be performed regardless of whether the source of RNA is the entire tumor, fine needle/core biopsy, or microdissected specimens. The majority of information currently in the literature has been derived from tumor tissue without separating cancer cells from the "host" environment. Microarray analysis results in expression profiles that allow for separation of breast tumors into categories both for the purpose of predicting outcome and potentially guide therapy. Indeed, attempts currently are underway to validate the prognostic and predictive value of limited sets/clusters of genes procured from primary breast tumors (87-95). We have identified overexpression of the epidermal growth factor receptor (EGFR) by RT-PCR analysis performed of a 9-gene panel, as a potential adverse indicator of outcome in patients with high-risk breast cancer (70). Others have successfully used RT-PCR technology for generating a validated prognostic scoring system in patients with early stage receptor positive breast cancer using a panel of 21 primers for genes including the estrogen and progesterone receptors, and HER-2 (96). Such profiling may identify predictors of response to individual therapeutic agents and help in choosing the optimal therapy (97-102). We will procure RNA from fresh and preservative-embedded cancerous and "host" tissue. Decreasing the number of "targeted" genes down to a panel 6-10 genes, coupled with expression analysis by proteomics and immunohistochemical assessment may identify predictors and prognosticators of outcome as well as targets of therapy.

2.6.5 Will identification of loss of heterozygosity (LOH) in genes coding for specific enzymes associated with drug metabolism allow better drug and patient selection?

Genomic variations of key enzymes involved in drug metabolism may explain some of the variability in treatment outcome. We, and others, have been exploring methods to identify predictors of drug resistance (103,104). In our prior pilot experience a specific CYP polymorphism was seen in high-dose paclitaxel-treated patients (105). We will analyze genomic DNA for the presence polymorphism in genes associated with drug metabolism prior to, during, and following completion of neoadjuvant chemotherapy, in order to identify predictors of complete response to a taxane-containing neoadjuvant regimen.

2.6.6 **Will conventional prognostic and predictive markers become obsolete?** Conventional pathological, immunohistochemical, and clinical features (stage, estrogen and progesterone receptor, presence of lymphovascular invasion, high grade) and information from the above described molecular approaches will be analyzed and compared for the purpose of generating prognostic and predictive models of outcome. As part of this assessment, we would like to know what proportion of patients with a positive SLN before preoperative chemotherapy will have metastases in their NSLN after preoperative chemotherapy? What are the clinicopathologic factors associated with metastases in NSLNs after preoperative chemotherapy in patients with a positive SLN before preoperative chemotherapy?

Sentinel lymph node (SLN) biopsy has largely replaced routine ALND for women with clinically negative axilla. Several single- and multi-institutional reports have documented the accuracy of SLN biopsy by performing ALND after SLN biopsy (106-112). These studies have demonstrated a low false negative rate. Thus, if the SLN does not contain metastases, then the risk of non-sentinel lymph node (NSLN) metastases is very low. ALND and its associated morbidities may be avoided in patients with a negative SLN biopsy.

Approximately, 40% of patients with SLN metastases will also have metastases in NSLNs confirmed by ALND (113-121). Table 1 lists several factors associated with increased risk of NSLN metastases. Of these, tumor size (> 2 cm) and SLN metastasis size (> 2 mm) are the strongest prognostic factors.

Table 1. Factors associated with increased risk of NSLN metastases

Factor	Increase risk
Primary tumor size	> 2 cm
Size of largest metastasis in SLN	> 2 mm
Lymphovascular invasion	present
Ratio of number of positive SLNs: total number of SLNs identified	increased
Number of SLNs with metastases	> 1
Extracapsular nodal extension	present
Multicentric breast cancer	present

Estrogen receptor status	negative
Method of detection of SLN metastases	frozen section > H&E > IHC

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 was initiated to determine whether preoperative chemotherapy results in improved survival (13,14). Women (n =1,523) were randomized to receive either postoperative doxorubicin and cyclophosphamide (AC) or preoperative AC. While the investigators were not able to demonstrate a statistically significant improvement in overall survival, they did find an increased rate of lumpectomies (68% vs. 60%, $p = 0.002$) in the group receiving preoperative AC. In addition, there was a 37% increase in the incidence of pathologically negative nodes after preoperative AC (57% vs. 41%, $p < 0.001$) (122).

Another randomized trial, NSABP B-27, determined the effect of adding doxetaxel (T) to preoperative AC. In this study, the addition of preoperative T after preoperative AC increased the proportion of patients with negative axillary lymph nodes (58.2% vs. 50.8%, $p < 0.001$). Of patients with a complete pathologic response, only 15.5% had positive nodes by pathology. Downstaging had been observed by other investigators as well (15).

The results of SLN biopsy after preoperative chemotherapy have been variable. While some studies (123-127) have demonstrated low false negative rates after preoperative (neoadjuvant) chemotherapy, others have reported false negative rates greater than 10% (128-131). Several explanations have been proposed. Preoperative chemotherapy may selectively eradicate metastases in the SLN, but not in other axillary nodes. As a result, a negative SLN biopsy may not accurately reflect the status of the axilla; so metastases in NSLNs may go untreated. Another explanation is that preoperative chemotherapy causes scarring and fibrosis in the lymphatic vessels and lymph nodes, thus diverting tracer agents to NSLNs and leading to false-negative results (132).

To overcome the limitations of SLN biopsy after preoperative chemotherapy, many surgeons are now performing SLN *before* preoperative chemotherapy. This strategy eliminates the potential for selective eradication of metastases in the SLN or for scarring and fibrosis to lead to inaccurate lymphatic mapping. Sabel et al. demonstrated the feasibility of this approach in 25 patients who underwent 26 SLN biopsy procedures before preoperative chemotherapy (133). The SLN identification rate was 100%. SLN metastases were identified in 13 patients; of those, 8 had NSLN metastases confirmed by ALND after preoperative chemotherapy. Schenk et al. preformed SLN biopsy on 21 patients before preoperative chemotherapy (134). SLN metastases were identified in 9 patients; of those, 6 had positive NSLNs confirmed by ALND after preoperative chemotherapy.

Currently, the standard of care in patients with a positive SLN before preoperative chemotherapy is a complete axillary node dissection after chemotherapy. Since many patients with a positive SLN biopsy do not have NSLN metastases, and preoperative chemotherapy can eradicate lymph node metastases in many patients, some patients may have a very low risk (<10%) of NSLN metastases, and may be able to avoid mandatory axillary node dissection. Presently, no study has evaluated clinical or pathologic factors that might predict the presence or absence of metastases in NSLNs, when the SLN is performed before preoperative chemotherapy. We aim to determine the proportion of patients with a positive SLN before preoperative chemotherapy who have NSLN metastases after preoperative chemotherapy and to determine clinicopathologic factors which may be associated with these NSLN metastases.

2.6.7 Neurotoxicity as part of Quality of Life Assessment

Clinical experience indicates that there is a wide spectrum of severity in cancer chemotherapy-associated peripheral neuropathies. Patients with pre-existing neuropathies are particularly at risk (135); and clearly the drug administered, dose-intensity, and total cumulative dose are all important determinations of severity. Nerve length, as given by subject height, also has a positive correlation to the severity of nerve damage (136). Peripheral neuropathy from paclitaxel procedures primarily sensory symptoms and signs in the distal distribution of the longest nerves of the body, a postulated "dying back" axonopathy resulting from disruption of axoplasmic transport from the drug's effect on microtubule assembly (137). A number of methods have been used to assess the incidence and severity of paclitaxel neuropathy. Most investigational studies have used practical measures such as neurological examination, or standard toxicity scale measurements. Significant interobserver variability occurs in grading peripheral neurological examination, or standard toxicity scale measurements. Significant interobserver variability occurs in grading peripheral neuropathy by health care professionals (138); and as in other aspects of quality of life (139), the patients' own assessment of neuropathy symptoms and functional limitations may be more meaningful. Nerve conduction and quantitative sensory tests have been carried out in patients receiving high dose paclitaxel (140); but these objective measures have not been extensively used or validated in clinical studies of chemotherapy associated neuropathies. In the present protocol, these objective measures will be used to assess docetaxel/paclitaxel neuropathy, compare the degree of neuropathy in the 2 regimens (TAC versus ACAC), and correlate changes with quality of life measures.

2.6.8 Barriers Study: Identification of delays of care between awareness of symptoms and receiving medical treatment by an oncologist**.**

Despite decreased mortality of breast cancer partially attributed to increased awareness and more effective screening, many patients still present for treatment after extended delays. Understanding that these delays may lead to

a possible higher stage and possible worse prognosis, we would like to interview a subgroup of patients to identify some issues involved in the barriers leading to treatment delay. The central issues we would like to evaluate are issues involved in the delay of treatment once symptoms appear, and the outcomes of having a delayed diagnosis. The patient population we will evaluate are those with stage III (A-C) breast cancer who were aware of symptoms or of the possibility of breast cancer and still had a delay of seeking or receiving treatment for 3 months or more. The framework with which we would like to examine the barriers to treatment include:

A) Patient barriers - fears of diagnosis, belief that treatment will be painful, fear of bodily mutilation or harm, other cultural beliefs about cancer and revealing the diagnosis, and sense of fatalism.
B) Professional barriers – delays by primary care physicians in getting appropriate diagnosis and treatment.
C) System Barriers – issues such as managed care, access to care, financial issues and women's understanding of the healthcare system.

The standard of care we will use for appropriate initial evaluation and diagnosis is the National Comprehensive Cancer Network guidelines. We understand the sensitive nature of these questions and special care will be taken to understand patient feelings and to not evoke a sense of guilt or shame.

2.6.9 Breast Cancer Stem Cells: Originators of Metastasis and Resistance?

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While a substantial portion of patients will demonstrate complete disappearance of pathologically detectable tumors (CR) after completion of neoadjuvant chemotherapy, relapses even in this patient population, and especially in those with persistent disease at the time of completion of chemotherapy, are frequent. One can postulate many reasons for such lack of efficacy with our current best chemotherapy, including pharmacodynamic, pharmacokinetic, and pharmacogenomic differences in tumors and in patients. It is also possible, that the most resistant tumor cells are actually clonogenic and pluripotent tumor stem cells, with inherent and/or acquired treatment resistance and ability to self-renew and invade. Delineation of mechanisms of such resistance and ability to multiply and metastasize is of crucial importance, in order to target this tumor population. Tumor conglomerates still persisting after aggressive neoadjuvant therapy are most likely to be enriched for such cancer stem cells, hence procurement of such tumors together with the establishment of cell lines for the purpose of characterization of specific signal pathways as targets of effective therapeutic intervention is crucial. (141-145) Implantation of the tumor cells will be carried out in the Clarke Laboratory after procurement of tumor tissue according to the described methodology below and will be transported to the Clarke laboratory in Stanford.

The City of Hope will have access to all xenograft tumors established from tumors sent to Stanford. Cells harvested in the xenograph will be processed, stored, used to establish tumors as secondary recipients and once that secondary recipient is made available to City of Hope we will establish a xenographt line here. Frozen cells will be available as well. Collaborative data will be accessible to selected members of City of Hope and appropriate of City of Hope will be included as authors in all manuscripts that arise from this materials, such as Stanford.

3.0 DRUG INFORMATION

3.1. Doxorubicin [NSC 123127]

3.1.1. Mechanism of Action: Doxorubicin, an anthracycline 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.

3.1.2. 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 450 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%.

3.1.3. 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.

3.1.4. 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.

3.1.5. Doxorubicin is commercially available.

3.2 Docetaxel (Taxotere®) (RP56976) (NSC-628503)

DESCRIPTION

3.2.1 Origin: In the late 1960's, the National Cancer Institute large-scale plant screening program found that a crude extract of the bark from the Pacific yew, *Taxus brevifolia*, had activity against the P388 mouse leukemia. In 1971, Wani, Taylor et al. isolated and characterized paclitaxel (Taxol ®), the active principle of the extract. It has become evident that paclitaxel (Taxol®) has activity against several human malignancies including refractory ovarian caner and breast cancer.

Several years ago, researchers at Rhone-Poulenc Rorer with the cooperation of the French "Centre National de Recherche Scientifique (CNRS)" were able to prepare doxetaxel (Taxotere ®), a semisynthetic analog of paclitaxel, using a precursor extracted from the needles of the European yew, *Taxus baccata*, a renewable source.

3.2.1 Name and Chemical Information:

Chemical Name: 4-acetoxy-2α-benzoyloxy-5β, 20-epoxy-1, 7β, 10β-trihydroxy-9-oxotax-11-ene-13α-yl-(2R,3S)-3-tert-butoxycarbonylamino-2-hydroxy-3 phenylpropionate

-Empirical formula: $C_{43}H_{53}O_{14}N$
-Molecular weight: 807.9
-Appearance: White powder

3.2.2 Mechanism of action: In vitro, docetaxel promotes tubulin assembly in microtubules and inhibits depolymerization thus stabilizing microtubules, which is different from the action of other spindle poisons in clinical use. This can lead to bundles of microtubules in the cell, which by blocking cells in the M phase of the cell cycle, results in the inability of the cells to divide.

Comparing docetaxel and paclitaxel using the "tubulin in vitro assay," the concentration required to provide 50% inhibition of microtubule disassembly (or IC50) is 0.2μM for docetaxel and 0.4 μM for paclitaxel.

3.2.3 TOXICOLOGY

The major toxic effect of docetaxel, which limits dose, is neutropenia. Other toxic effects, which may be seen, include leukopenia, thrombocytopenia, anemia, asthenia, dysgeusia, myalgia, arthralgia, nail changes and conjunctivitis. Severe anaphylactoid reactions, characterized by a flush associated with hypo-or hypertension, with out without dyspnea, may occur. Other toxicities include cutaneous reactions (e.g., skin rash, desquamation following localized prurignious maculopapular eruption, skin erythema with edema), hypersensitivity reactions (flushing, pruritus, fever, chills, rigors, lower back pain), dyspnea with restrictive pulmonary syndrome, pleural effusions, arrhythmias, pericardial effusions, fluid retention syndrome, ascites, myopathy, digestive tract toxicities (nausea, vomiting, oral mucositis, diarrhea, anorexia), alopecia, extravasation reaction (erythema, swelling, tenderness, pustules), reversible peripheral phlebitis, peripheral edema, reversible increase in liver function tests, hepatic failure and neurotoxicity (reversible dysesthesias or paresthesias, peripheral neuropathy, seizure, headache, lethargy or somnolence). Patients with SGOT > 1.5 times normal and alkaline phosphatase > 2.5 times normal appear to have decreased docetaxel clearance and appear to be more likely to suffer sever toxicity, including drug-related death.

3.2.4 PHARMACOLOGY

Docetaxel is a cytotoxic anticancer drug and, as with other potentially toxic compounds, caution should be exercised when handling and preparing docetaxel solutions. The use of gloves is recommended. Please refer to **Handling and Disposal** section.

If docetaxel concentrate, initial diluted solution, or final dilution for infusion should come into contact with the skin, immediately and thoroughly wash with soap and water. If docetaxel concentrate, initial diluted solution, or final dilution for infusion should come into contact with mucosa, immediate and thoroughly wash with water.

Docetaxel for Injection Concentrate requires two dilutions prior to administration. Please follow the preparation instruction provided below. **Note:** Both the docetaxel for Injection Concentrate and the diluent vials contain an overfill.

How Supplied: Docetaxel for Injection Concentrate is supplied in a single-dose vial as a sterile, pyrogen-free, non-aqueous, viscous solution with an accompanying sterile, non-pyrogenic, diluent (13% enthanol in Water for Injection) vial. The following strengths are available: 80 mg and 20 mg strengths are available.

Storage: Store between 2 and 25°C (36 and 77°F). Retain in the original package to protect from bright light. Freezing does not adversely affect the product.

Preparation of the Initial Diluted Solution: Gather the appropriate number of vials of docetaxel for Injection Concentrate and diluent (13% Ethanol in Water for Injection). If the vials were refrigerated, allow them to stand at room temperature for approximately 5 minutes. Aseptically withdrawn the contents of the appropriate diluent vial into a syringe and transfer it to the appropriate vial of docetaxel for Injection Concentrate. **If the procedure is followed as described, an Initial diluted solution of 10 mg docetaxel/ will result.** Gently rotate the initial diluted solution for approximately 15 seconds to assure full mixture of the concentrate and diluent. The initial diluted docetaxel solution (10 mg doxetaxel/ml) should be clear; however, there may be some foam on top of the solution due to the polysorbate 80. Allow the solution to stand for a few minutes to allow any foam to dissipate. It is not required to all foam dissipate prior to continuing the preparation process. The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.

Preparation of the Final Dilution for Infusion: Aseptically withdraw the required amount of initial diluted docetaxel solution (10 mg docetaxel/ml) with a calibrated syringe and inject into a 250 ml infusion bag or bottle of other 0.9% Sodium Chloride solution or 5% Dextrose solution to produce a final concentration of 0.3 to 0.74 mg/ml. If a dose greater than 200 mg of docetaxel is required, use a large volume of the infusion vehicle so that a concentration of 0.74 mg/ml doxetaxel is not exceeded. Thoroughly mix the infusion by manual rotation. As with all parenteral products, docetaxel should be inspected visually for particulate matter or discoloration prior to

administration whenever the solution and container permit. If the docetaxel for Injection initial diluted solution or final dilution for infusion is not clear or appears to have precipitation, these should be discarded.

Contact of the docetaxel concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP (di-2-ethylhexyl phthalate), which may be leached from PVC infusion bags or sets, the final docetaxel dilution for infusion should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

Administration: The drug will be administered to the patients as a 1-hour IV infusion under ambient room temperature and lighting conditions. Please refer to the approved package labeling for complete prescribing and toxicity information.

Stability: The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.

Docetaxel infusion solution, if stored between 2 and 25°C (36 and 77°F) is stable for 4 hours. Fully prepared docetaxel infusion solution (in either 0.9% Sodium Chloride solution or 5% Dextrose solution) should be used within 4 hours (including the 1 hour IV administration).

Handling and Disposal: Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

Supplier: This drug is commercially available for purchase by a third party.

3.3 ABI-007; Abraxane® (paclitaxel protein-bound particles for injectable suspension; (albumin-bound)).

3.3.1 General: Abraxane® is a unique protein formulation of a non-crystalline, amorphous form of paclitaxel in an insoluble compound state. Abraxane® has been developed to reduce the toxicities associated with Taxol® (paclitaxel) Injection (in which paclitaxel-from the native crystalline form-is in solution with CremophorEL/ethanol as the solvent) while maintaining or improving its chemotherapeutic effect.

3.3.2 The Product: Abraxane® is a Cremophor-free formulation of paclitaxel albumin for injectable suspension. Each 50 mL vial contains 100 mg of paclitaxel, and human albumin, as a white to off-white sterile lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection USP.

3.3.3 Preclinical and Clinical Experience:Preclinical Studies with Abraxane®

Preclinical studies comparing Abraxane® to Taxol demonstrated dramatically lower acute toxicities, with an LD_{10} approximately 3-fold higher for Abraxane® compared to Taxol. At equal doses there was less myelosuppression and improved efficacy in a xenograft tumor model of human mammary adenocarcinoma. At equitoxic doses of paclitaxel, Abraxane® was found to be markedly more efficacious than Taxol.

Clinical Studies with Abraxane®
Every 3 Weeks Schedule

In a phase I study, the maximum tolerated dose (MTD) of Abraxane® was determined to be 300 mg/m^2 by 30 minute infusion every 3 weeks, without premedication or G-CSF support. Two multicenter phase II studies have evaluated 2 dose levels of Abraxane® (300 mg/m^2, n=63, and 175 mg/m^2, n=43) in patients with metastatic breast cancer. The overall response rates in these 2 phase II trials were 40% (95% CI 25-54%) for the 175 mg/m^2 dose, and 48% (95% CI 35-60%) for the 300 mg/m^2 dose. Of 39 patients receiving 300 mg/m^2 as first-line therapy for metastatic breast cancer, 64% (95% CI 49-79%) responded. This was contrasted with a 45% response rate in similar patients at the lower dose level. Grade 4 neutropenia was noted in 24% of patients at the higher dose level, occurred primarily during the first cycle and resolved rapidly (146).

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A Phase III trial in patients with metastatic breast cancer compared Abraxane® 260 mg/m^2 to Taxol 175 mg/m^2 given every 3 weeks (30). Significantly higher efficacy for Abraxane® vs Taxol was demonstrated as measured by overall response rates (33% vs 19%, $p<0.001$) and time to tumor progression (5.2 vs 3.7 months, $p=0.029$). The overall median survival was 65.0 weeks for the Abraxane® group and 55.3 weeks for the Taxol group ($p=0.329$). In 2nd-or greater-line patients, median survival in the Abraxane® arm was significantly improved (56.4 vs 46.7 weeks, $p=0.016$). Despite a 49% higher dose of paclitaxel administered to patients in the Abraxane® group than in the Taxol group, the incidence of treatment-related Grade 3/4 neutropenia was significantly less (25% vs. 31% and 9% vs. 22%, respectively). The Abraxane® group also had a higher mean neutrophil nadir (1.67 vs 1.31 x 10^9/L; $p = 0.046$) suggesting that Cremophor® may have contributed to this toxicity. Grade 3 sensory neuropathy occurred in 10% of Abraxane® treated patients and 2% of those treated with Taxol. No grade 4 sensory neuropathy occured in either study group. Of the patients who developed grade 3 sensory neuropathy while on Abraxane®, 58% had documented rapid improvement in symptoms after a median of 22 days and 42% of patients were able to resume treatment at a reduced dose. Only 3% of patients that received Abraxane® discontinued treatment due to peripheral neuropathy.

Weekly for 3 Weeks, Every 4 Weeks Schedule

A Phase I study of Abraxane® administered weekly for 3 weeks followed by 1 week rest in patients with advanced solid tumors has been completed (31). The MTDs for heavily and lightly pre-treated patients were 100 and 150mg/m^2 respectively. These doses are significantly higher than paclitaxel.

Dose limiting toxicities included myelosuppression and peripheral neuropathy. Pre-medication was not required, and unexpected, non-taxane associated toxicities were not observed.

In a Phase II trial in heavily pretreated patients with taxane-refractory metastatic breast cancer, objective antitumor responses occurred in 15% of women treated with Abraxane® 100mg/m^2 on this schedule (32). Toxicity was minimal even without premedications or growth factors (G-CSF) usually routinely required with paclitaxel or docetaxel in this group of patients. Patients were considered taxane-refractory if they had progressive cancer while receiving paclitaxel, docetaxel or both. Abraxane® given weekly was active in patients refractory to paclitaxel (16% overall response rate and 37% disease control) and patients refractory to docetaxel (24% overall response and 35% disease control). Grade 3/4 neutropenia without G-CSF in heavily pretreated patients was 15%. Other grade 3 toxicities, all below 4% were: anemia, neuropathy, nausea, vomiting and diarrhea. Results of this dose-finding study of weekly Abraxane® in heavily pretreated patients identified Abraxane® 100 mg/m^2 as an appropriate dose for further study. It has proven activity in paclitaxel and docetaxel refractory patients with acceptable toxicity (32).

3.3.4 Human Toxicity: Please refer to the Clinical Investigator's Brochure for details of the Adverse Reactions in the overall Safety Database for Abraxane® (147).

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Table 1. Frequency of Adverse Events in a Randomized Phase III Study on an Every-3-Weeks Schedule

	Percent of Patients	
	ABRAXANE® 260/30min[b] (n=229)	Paclitaxel Injection 175/3h[c,d] (n=225)
Bone Marrow		
Neutropenia		
< 2.0 x 10^9/L	**80**	**82**
< 0.5 x 10^9/L	**9**	**22**
Thrombocytopenia		
< 100 x 10^9/L	**2**	**3**
< 50 x 10^9/L	**<1**	**1**
Anemia		
< 11 g/L	**33**	**25**
< 8 g/L	**1**	**<1**
Infections	**24**	**20**
Febrile Neutropenia	**2**	**1**
Bleeding	**2**	**2**
Hypersensitivity Reaction[e]		
All	**4**	**12**
Severe[f]	**0**	**2**
Cardiovascular		
Vital Sign Changes[g]		

	Percent of Patients	
	ABRAXANE® 260/30min[b] (n=229)	Paclitaxel Injection 175/3h[c,d] (n=225)
Bradycardia	**<1**	**<1**
Hypotension	**5**	**5**
Severe Cardiovascular Events[f]	**3**	**4**
Abnormal ECG		
All patients	**60**	**52**
Patients with Normal Baseline	**35**	**30**
Respiratory		
Cough	**6**	**6**
Dyspnea	**12**	**9**
Sensory Neuropathy		
Any Symptoms	**71**	**56**
Severe Symptoms[f]	**10**	**2**
Myalgia / Arthralgia		
Any Symptoms	**44**	**49**
Severe Symptoms[f]	**8**	**4**

	ABRAXANE® 260/30min[b] (n=229)	Paclitaxel Injection 175/3h[c,d] (n=225)
Asthenia		
Any Symptoms	**47**	**38**
Severe Symptoms[f]	**8**	**3**
Fluid Retention/Edema		
Any Symptoms	**10**	**8**
Severe Symptoms[f]	**0**	**1**
Gastrointestinal		
Nausea		
Any symptoms	**30**	**21**
Severe symptoms[f]	**3**	**<1**
Vomiting		
Any symptoms	**18**	**9**
Severe Symptoms[f]	**4**	**1**
Diarrhea		
Any Symptoms	**26**	**15**
Severe Symptoms[f]	**<1**	**1**
Mucositis		
Any Symptoms	**7**	**7**
Severe Symptoms[f]	**<1**	**0**
Alopecia	**90**	**94**
Hepatic **(Patients with Normal Baseline)**		
Bilirubin Elevations	**7**	**7**
Alkaline Phosphatase Elevations	**36**	**31**
AST (SGOT) Elevations	**39**	**32**
Injection Site Reaction	**1**	**1**

[a] Based on worst grade

[b] Abraxane® dose in mg/m^2/duration in minutes

[c] paclitaxel injection dose in mg/m^2/duration in hours
[d] paclitaxel injection pts received premedication
[e] Includes treatment-related events related to hypersensitivity (e.g., flushing, dyspnea, chest pain, hypotension) that began on a day of dosing.
[f] Severe events are defined as at least grade 3 toxicity
[g] During study drug dosing.

Pregnancy
Abraxane® may cause fetal harm when administered to a pregnant woman. A developmental toxicity study in rats showed that no gross external, soft tissue or skeletal fetal alterations were caused by Abraxane® at doses of 0.5 mg/kg/day. Higher doses of Abraxane® resulted in significant maternal toxicity. This was evidenced by increased mortality, reduction in body weight gain, reduced terminal body weight, reduced food intake, and embryo-fetal lethality. Dose-related increases in malformations, variations, fetal deaths and/or resorptions occurred in pregnant rats that were administered intravenous doses of 1 or 2 mg/kg/day of Abraxane®.
There are no adequate and well-controlled studies of Abraxane® in pregnant women. If Abraxane® is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

Nursing Mothers
It is not known whether paclitaxel (and therefore Abraxane®) is excreted in human milk. It has been reported that following IV administration of 14C-paclitaxel to rats on days 9 to 10 postpartum, concentrations of radioactivity in milk were higher than in plasma and declined in parallel with the plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, it is recommended that nursing be discontinued when receiving Abraxane® therapy.

3.3.5 Pharmaceutical Information: **Abraxane® Administration**
NOTE: It is not a requirement to use filter needles in the preparation of, or in-line filters during the administration of Abraxane®. In any event, filters of pore-size less than 15 micrometers must not be used.

Abraxane® will be reconstituted by appropriate study personnel and administered to the patient in the study site setting at 1-week intervals. The investigator will calculate the body surface area (BSA) of the patient in order to determine the total amount of paclitaxel to be administered.

Reconstitution and use of Abraxane®:
1. Calculate the patient's body surface area at the beginning of the study and if the weight changes by >10%.
2. Calculate the total dose (in mg) to be administered by:
   Total Dose (mg) = BSA x (study dose mg/m^2)
3. Calculate the total number of vials required by:
   Total Number of Vials = $\frac{\text{Total Dose (mg)}}{\text{100 (mg/vial)}}$

Round up the number of vials to be reconstituted to the next higher whole number when a fractional number of vials is obtained by the above formula (eg, if the total number of vials = 4.05 or 4.5, then 5 vials would be reconstituted).

4. Using sterile technique, prepare the vials for reconstitution.

5. Swab the rubber stoppers with alcohol.

6. Reconstitute each Abraxane® vial by using a sterile syringe to inject 20 mL of 0.9% Sodium Chloride Injection, USP or equivalent into each vial over a period of not less than 1 minute (Note: Change the syringes after reconstituting every 3 vials).
   - **Slowly** inject the 20mL of 0.9% Sodium Chloride Injection, USP,, using the sterile syringe directing the solution flow onto the **inside wall** of the vial.
   - **DO NOT INJECT** the 0.9% Sodium Chloride Injection, USP solution directly onto the lyophilized cake as this will result in foaming.
   - Once the injection is complete, allow the vial to sit for a **minimum of 5 minutes** to ensure proper wetting of the lyophilized cake/power.
   - **Gently** swirl and/or invert the vial **slowly** for at east **2 minutes** until complete dissolution of any cake;/powder occurs. **Avoid** generation of foam.
   - If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.
   - Each mL of reconstituted product will contain 5 mg of paclitaxel.

7. Calculate the exact total dosing volume (to the nearest mL) of 5 mg/mL suspension required for the patient:
   Dosing volume (mL) = Total dose (mg)/5 (mg/mL)

8. The reconstituted sample should be milky and homogeneous without visible particulates. If unsuspended powder is visible, the vial should be **gently** inverted again to ensure complete resuspension, prior to use.

9. Once the exact volume of reconstituted Abraxane® has been withdrawn from the vials, discard any excess solution left over in accordance with standard operating procedures.

10. Inject the calculated dosing volume of reconstituted Abraxane® suspension into an empty sterile, standard PVC IV bag using an injection port. Inject perpendicularly into the center of the injection port to avoid dislodging plastic material into the IV bag. Repeat steps 10 and 11 until the patient's entire required dose is injected into the IV bag.

11. Administer the calculated dosing volume of reconstituted Abraxane® suspension by IV infusion over 30 minutes. The use of in-line filters is

not necessary. If used, in-line filters with pore sizes of <15µ should not be used.

12. Unopened vials of Abraxane are stable until the date indicated on the package when stored between 20°C to 25°C (68°F to 77°F), in the original package. Reconstituted Abraxane should be used immediately, but may be refrigerated at 2 °C to 8 °C (36°F to 46°F) for a maximum of 8 hours if necessary. If not used immediately, each vial of reconstituted suspension should be bright light. Discard any unused portion. Neither freezing nor refrigeration adversely affects the reconstituted the stability of the product. Some settling of the reconstituted suspension may occur. Ensure complete resuspension by mild agitation before use. Discard the reconstituted suspension if precipitates are observed. The suspension for infusion prepared as recommended in an infusion bag is stable at ambient temperature (approximately 25°C) and lighting conditions for up to 8 hours.

13. Store the vials in original cartons at 20°C to 25°C (68°F to 77°F). Retain in the original package too protect from bright light.

3.4 Cyclophosphamide (Cytoxan®) [NSC-26271]

3.4.1 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.

3.4.2 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.

3.4.3 Pharmaceutical Data: Cyclophosphamide is available in 1 and 2 gram vials containing white powder. The drug can be reconstituted in normal saline or 5% dextrose and water. The drug should be diluted in approximately 250ml of diluent and infused over 1 hour.

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

3.4.5 Cyclophosphamide is commercially available.

3.5 Carboplatin (Paraplatin®) (NSC-241240) (CBDCA)

3.5.1 Description

Carboplatin (CBDCA) is a hydrophilic platinum coordination compound and is an analog of cisplatin, producing intrastrand DNA cross-links.

3.5.2 Toxicology

Human Toxicology: Side effects of carboplatin (CBDCA) include myelosuppression, nausea, vomiting, abdominal pain, diarrhea and constipation. Other toxicities include allergic reaction (including hypersensitivity, i.e., rash, urticaria, erythema, pruritus, bronchospasm and hypotension), peripheral neuropathy, paresthesia, loss of hair, hearing loss, visual disturbances and change in taste. Serum creatinine elevations and blood urea elevations have occurred, as well as abnormal liver function tests and decreased serum electrolyte values. Although rare, pain, asthenia, cardiovascular, respiratory, genitourinary and mucosal side effects have occurred in some patients. Cancer-associated hemolytic uremic syndrome has been reported rarely. Carboplatin may cause fetal harm; therefore women of childbearing potential should be advised to avoid becoming pregnant. The renal effects of nephrotoxic compounds may be potentiated by carboplatin. Carboplatin is contraindicated in patients with a history of severe allergic reactions to cisplatin or other platinum-containing compounds or mannitol. This drug should not be used in patients with severe bone marrow depression or significant bleeding. The occurrence of acute leukemia has been reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.

3.5.3 Pharmacology

Pharmacokinetics: The difference in potencies of carboplatin and cisplatin are due to differences in equation rates. The initial half-life is 1.1–2.0 hours and the post-distributional half-life is 2.6–5.9 hours. Sixty-five percent of the dose is excreted in the urine within twelve hours. Carboplatin is not bound to plasma proteins.

Formulation: Carboplatin is supplied as a sterile lyophilized powder available in single-dose vials containing 50 mg, 150 mg and 450 mg of carboplatin for administration by intravenous injection. Each vial contains equal parts by weight of carboplatin and mannitol. Immediately before use, the content of each vial must be reconstituted with either Sterile Water for Injection, USP, 5% Dextrose in Water, or 0.9% Sodium Chloride Injection, USP, according to the following schedule:

Vial Strength	Diluent Volume
50 mg	5 mL
150 mg	15 mL
450 mg	45 mL

These dilutions all produce a carboplatin concentration of 10 mg/ml. Carboplatin can be further diluted to concentrations as low as 0.5 mg/ml with 5% Dextrose in Water or 0.9% Sodium Chloride Injection, USP (NS).

Storage and Stability: Unopened vials of carboplatin for injection are stable for the life indicated on the package when stored at controlled room temperature 15–30 ˚C, and protected from light. When reconstituted as directed, the solution of carboplatin exhibits no decomposition for 8 hours at room temperature (25 ˚C). Like cisplatin, this drug should not be given

through aluminum needles. **Caution:** The single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded eight hours after dilution.

Administration: Intravenous.

Supplier: Carboplatin is commercially available.

3.6 Filgrastim (Neupogen®) (r-metHuG-CSF) (NSC - 614629)

3.6.1 Description: Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. NEUPOGEN® is the Amgen Inc. trademark for filgrastim, recombinant methionyl human granulocyte colony stimulating factor (r-metHuG-CSF).

3.6.2 Contraindications: NEUPOGEN® is contraindicated in patients with known hypersensitivity to E. coli-derived proteins, filgrastim, or any component of the product.

3.6.3 Adverse Reactions: The only consistently observed clinical toxicity described with Neupogen® is medullary bone pain. Other clinical toxicities that have been described include skin rash, and cutaneous vasculitis. Since commercial introduction of Neupogen®, there have been rare reports of allergic-type reactions. Biochemical abnormalities that may occur include increases in alkaline phosphatase, uric acid, and lactate deydrogenase.

3.6.4 Dilution/Storage: If required, NEUPOGEN® may be diluted in 5% dextrose. NEUPOGEN® diluted to concentrations between 5 and 15 mcg/mL should be protected from adsorption to plastic materials by addition of Albumin (Human) to a final concentration of 2 mg/mL. **Do not dilute with saline at any time; product may precipitate.**

NEUPOGEN® should be stored in the refrigerator at 2-8 degrees Centigrade (36-46 degrees Fahrenheit). Do not freeze. Avoid shaking. Prior to injection, NEUPOGEN® may be allowed to reach room temperature for a maximum of 24 hours. Any vial left at room temperature for greater than 24 hours should be discarded.

3.6.5 How Supplied: Commercial NEUPOGEN® is available in 1 mL and 1.6 mL vials at a concentration of 300 mcg/mL. Discard unused portions. Use only one dose per vial; do not reenter the vial. Do not save unused drug for later administration.

3.7 Pegfilgrastim (Neulasta™)

3.7.1 DESCRIPTION

Pegfilgrastim (Neulasta™) is a covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol. Studies on cellular proliferation, receptor binding, and neutrophil function

demonstrate that filgrastim and pegfilgrastim have the same mechanism of action. Pegfilgrastim has reduced renal clearance and prolonged persistence in vivo compared with filgrastim.

Pegfilgrastim is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia.

Pegfilgrastim was evaluated in 2 randomized, double-blind, active-control studies, using doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2 administered every 21 days for up to 4 cycles in the treatment of patients with high-risk stage II or stage III/IV breast cancer. Study 1 investigated the utility of a fixed dose of pegfilgrastim. Study 2 used a weight-adjusted dose. In the absence of growth-factor support, similar chemotherapy regimens have been reported to result in a 100% incidence of severe neutropenia [absolute neutrophil count (ANC) <0.5 x 10^9/L] with a mean duration of 5 to 7 days, and a 30 to 40% incidence of febrile neutropenia.

In study 1,157 subjects were randomized to receive a single subcutaneous (SC) dose of 6 mg of pegfilgrastim on Day 2 of each chemotherapy cycle or filgrastim 5 mg/kg/day SC beginning on Day 2 of each cycle. In study 2,310 subjects were randomized to receive a single SC injection of pegfilgrastim at 100 mg/kg on Day 2 or filgrastim 5 mg/kg/day SC beginning on Day 2 of each cycle of chemotherapy.

Both studies met the primary objective of demonstrating that the mean days of severe neutropenia (ANC <0.5 x 10^9/L) of pegfilgrastim-treated patients did not exceed that of filgrastim -treated patients by more than 1 day in Cycle 1 of chemotherapy. The rates of febrile neutropenia were 13% and 9% for pegfilgrastim vs 20% and 18% for filgrastim in studies 1 and 2, respectively.

Other secondary endpoints included days of severe neutropenia in Cycles 2 to 4, the depth of ANC nadir in Cycles 1 to 4, and the time to ANC recovery after nadir. In both studies, the results for the secondary endpoints were similar between the 2 treatment groups.

The safety and efficacy of once-per-cycle pegfilgrastim was also found to be comparable to daily filgrastim in phase 2 studies in patients with non-small cell lung cancer being treated with carboplatin and paclitaxel and patients with non Hodgkin's lymphoma (NHL) or Hodgkin's lymphoma being treated with ESHAP (etoposide, methylprednisolone, high-dose cytarabine, cisplatin) or CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy.

3.7.2 TOXICOLOGY

The most common adverse event attributed to pegfilgrastim in clinical trials was medullary bone pain, reported in 26% of subjects, which was comparable to the incidence in filgrastim -treated patients. This bone pain was generally reported to be of mild-to-moderate severity. Approximately 12% of all

subjects utilized non-narcotic analgesics and less than 6% utilized narcotic analgesics in association with bone pain. No patients withdrew from the study due to bone pain. Reversible elevations in LDH, alkaline phosphatase and uric acid have been observed in clinical trials. Pegfilgrastim has been associated with leukocytosis (defined as WBC >100. x 10^9/L) in <1% of 465 subjects with nonmyeloid malignancies, when observed is was not associated with any adverse event. Transient thrombocytopenia has also been noted in patients receiving filgrastim.

Pegfilgrastim is contraindicated inpatients with known hypersensitivity to E. coli-derived proteins, pegfilgrastim, filgrastim, or any other component of the product.

Rare cases of splenic rupture have been reported following the administration of the parent compound of pegfilgrastim, filgrastim, for PBPC mobilization in both healthy donors and patients with cancer. Some of these cases were fatal. Pegfilgrastim has not been evaluated in this setting. Patients receiving pegfilgrastim who report left upper abdominal or shoulder tip pain should be evaluated for an enlarged spleen or splenic rupture.

Adult respiratory distress syndrome (ARDS) has been reported in neutropenic patients with sepsis receiving filgrastim, the parent compound of pegfilgrastim, and is postulated to be secondary to an influx of neutrophils to sites of inflammation in the lungs. Neutropenic patients receiving pegfilgrastim who develop fever, lung infiltrates, or respiratory distress should be evaluated for the possibility of ARDS. In the event that ARDS occurs, pegfilgrastim should be discontinued and/or withheld until resolution of ARDS and patients should receive appropriate medical management for this condition.

Allergic-type reactions, including analyphylaxis, skin rash, and urticaria, occurring on initial or subsequent treatment have been reported with the parent compound of pegfilgrastim, filgrastim. In some cases, symptoms have recurred with rechallenge, suggesting a causal relationship. Allergic-type reactions to pegfilgrastim have not been observed in clinical trials. If a serious allergic reaction or anaphylactic reaction occurs, appropriate therapy should be administered and further use of pegfilgrastim should be discontinued.

Severe sickle cell crisis have been reported in patients with sickle cell disease (specifically homozygous sickle cell anemia, sickle/hemoglobin C disease, and sickle b+ thalassemia) who received filgrastim, the parent compound of pegfilgrastim, for PBPC mobilization or following chemotherapy. One of these cases was fatal.

There are no adequate and well-controlled studies in pregnant women. The risks of the study drug to an unborn or newborn child, are not known. In addition, it is not known whether pegfilgrastim is secreted in human milk. Therefore, pregnant or nursing mothers may not take part in this study.

No formal drug interaction studies between pegfilgrastim and other drugs have been performed. Drugs such as lithium may potentiate the release of neutrophils; patients receiving lithium and pegfilgrastim should have more frequent monitoring of neutrophil counts.

The maximum amount of pegfilgrastim that can be safely administered in single or multiple doses has not been determined. Single doses of 300 mcg/kg have been administered SC to 8 normal volunteers and 3 patients with non-small cell lung cancer without serious adverse effects. These subjects experienced a mean maximum ANC of 55 x 10^9/L, with a corresponding mean maximum WBC of 67 x 10^9/L. The absolute maximum ANC observed was 96 x 10^9/L with a corresponding absolute maximum WBC observed of 120 x 10^9/L. The duration of leukocytosis ranged from 6 to 13 days. Leukapheresis should be considered in the management of symptomatic individuals.

For prescribing information and a comprehensive list of adverse events associated with pegfilgrastim, refer to the drug package insert.

3.7.3 PHARMACOLOGY

Pegfilgrastim (Neulasta™) is a clear, colorless, sterile liquid. It is supplied as a preservative-free solution containing 6 mg (0.6 mL) of pegfilgrastim (10 mg/mL) in a single-dose syringe with a 27 gauge, ½ inch needle with an UltraSafe® Needle Guard. The formulation is 10 mg pegfilgrastim (PEG-metHuG-CSF) per mL of solution containing acetate (0.35 mg), sorbitol (30 mg), polysorbate 20 (0.02 mg), and sodium (0.02 mg) in water for injection, USP, pH 4.0. Each dispensing pack contains 1 pre-filled syringe.

Storage and Stability: Pegfilgrastim should be stored refrigerated at 2 to 8ºC (36 to 46ºF); syringes should be kept in their carton to protect from light until time of use. Shaking should be avoided. Before injection, pegfilgrastim may be allowed to reach room temperature for a maximum of 48 hours but should be protected from light. Pegfilgrastim left at room temperature for more than 48 hours should be discarded. Freezing should be avoided; however, if accidentally frozen, pegfilgrastim should be allowed to thaw in the refrigerator before administration. If frozen a second time, pegfligrastim should be discarded.

Administration: No preparation is required for administration of pegfilgrastim. Each subject will receive a fixed dose of 6 mg of pegfilgrastim. The entire contents of the 0.6 mL pre-filled syringe should be administered subcutaneously irrespective of the subject's actual weight.

Supplier: Pegfilgrastim is commercially available and should be purchased by a third party.

3.8 Trastuzumab (Herceptin®); NSC #688907

Trastuzumab will be given at 4 mg/kg as a loading dose, and then a dose of 2 mg/kg will be repeated every week. The drug will be administered over 90 minutes; subsequent doses will be given over 30 minutes, if the loading dose was well tolerated.

3.8.1 Description

Herceptin®, manufactured by Genentech Inc., is commercially available and is supplied as a freeze-dried preparation at a nominal content of 440 mg per vial for parenteral administration. The drug is formulated in histidine, trehalose, and polysorbate 20. Each 440-mg vial of trastuzumab is supplied with 20ml bacteriostatic water for injection, USP, 1.1% benzyl alcohol. Herceptin® vials must be stored in the refrigerator (2° C to 8°C). Do not freeze.

The reconstituted formulation (440-mg vial) is designed for multiple use. Unused drug may be stored for 28 days at 2°C to 8°C (36°F to 46°F). Reconstituted trastuzumab should be clear to slightly opalescent and colorless to pale yellow.

3.8.2. Preparation for Use

Trastuzumab may be sensitive to shear-induced stress (e.g., agitation or rapid expulsion from a syringe). DO NOT SHAKE. Vigorous handling of solutions of trastuzumab results in aggregation of the protein and may create cloudy solutions. A 19-guage or larger needle should be used during drug admixture to avoid sheer-induced stress. Reconstitute each vial as follows, using aseptic technique:

1. Using a sterile syringe, slowly inject 20 mL of BWFI into the vial directing the stream into the lyophilized cake. The vacuum in the vial will automatically draw the water from the syringe into the vial;

2. Swirl the vial gently to aid reconstitution. DO NOT SHAKE. The reconstituted solution contains 21 mg/mL trastuzumab and will be added to 250 mL of 0.9% sodium chloride injection, USP.

3.8.3 Known Potential Toxicities

More Frequent Incidence (> 5%)

Infusion-associated symptoms: Chills and/or fever, occasionally accompanied by rigors, occurred in approximately 40% of subjects during the first infusion of trastuzumab. The symptoms were usually mild to moderate in severity and were treated with acetaminophen and diphenhydramine (with our without reduction in the rate of trastuzumb infusion). Meperidine for rigors is effective. Other signs and/or symptoms may include nausea, vomiting, pain, headache, dizziness, dyspnea, or rash. The symptoms occurred less frequently with subsequent infusions. Rarely, infusion reactions have results in death.

Trastuzumab administration can result in the development of ventricular dysfunction and CHF. Left ventricular function should be evaluated in all subjects prior to and during treatment with trastuzumab. Discontinuation of trastuzumab treatment should

be strongly considered in subjects who develop a clinically significant decrease in left ventricular function. The incidence and severity of cardiac dysfunction appears to increase in subjects who receive trastuzumab in combination with anthracycline and cyclophosphamide (28% versus 7% when used as a single agent).

The incidence of generalized pain, abdominal pain, and back pain appears to be more frequent in subjects receiving trastuzumab in combination with chemotherapy. Occasionally pain at tumor sites has been reported.

The following have also been reported in >5% of subjects: diarrhea (25% of these), myalgia, peripheral edema, and weakness.

Less Frequent Incidence (1 to 5%)

Allergic/hypersensitivity reactions that have been reported include rash, pruritus, urticaria, and anaphylaxis (rarely resulting in death) or anaphylactoid signs and symptoms (erythematous rash on the chest and beck, dyspnea, bronchospasm, angioedema, acute respiratory distress syndrome (ARDS), hypotension, wheezing, pleural effusions, pulmonary infiltrates, noncardiogenic pulmonary edema, and pulmonary insufficiency and hypoxia requiring supplemental oxygen or ventilatory support).

Rare Incidence (<1%)

Rare toxicities include abnormal liver function test, hepatitis, bone pain, tumor-site pain, pancytopenia, hypotension, anorexia, febrile neutropenia, worsening of pre-existing peripheral neuropathy, paresthesias, and thromboembolic disease.

Pulmonary events that have been reported include pleural effusions, pulmonary infiltrates, and noncardiogenic pulmonary edema. Pulmonary insufficiency and hypoxia requiring supplemental oxygen or ventilatory support, including ARDS, and death have occurred. Most subjects with fatal events had significant, pre-existing pulmonary compromise secondary to intrinsic lung disease and/or malignant pulmonary involvement. Because it appears that subjects with significant pre-existing pulmonary compromise may be at greater risk, these subjects should be treated with extreme caution. Subjects experiencing any of the severe infusion-associated symptoms should have the trastuzumab infusion discontinued and appropriate medical therapy administered. Subjects should be closely monitored until complete resolution of their symptoms. In addition, subjects should be informed of the possibility of delayed severe reactions.

3.8.4 Nursing Guidelines

The most common AEs related to trastuzumab are fever (> 38°C), chills, and occasional rigors, most often infusion-related after the initial dose. Treat with acetaminophen as necessary. Meperidine may be needed for rigors. Instruct the subject and family to report any fever >101°F. Subjects with underlying pulmonary pathology should be closely monitored.

Transient, localized tumor-side pain may be experienced within 8 hours of infusion. Advise the subject that acetaminophen is helpful.

Provide symptomatic management of the possible mild-to-moderate nausea/vomiting/diarrhea. Assess heart and lung sounds. Monitor vital signs (resting pulse, BP). Be alert to early signs of cardiotoxicity, i.e., dyspnea, steady weight gain, nonproductive cough, arrhythmias, tachycardia, and pulmonary rales. Trastuzumab may potentiate heart failure when administered in combination with *doxorubicin*.

Advise subjects of possible fatigue, loss of strength, and weakness. Have subjects pace activities with frequent rest periods.

4.0 STAGING CRITERIA

DEFINITION OF TNM

Primary Tumor (T)

Definitions for classifying the primary tumor (T) are the same for clinical and for pathologic classification. If the measurement is made by the physical examination, the examiner will use the major headings (T1, T2, or T3). If other measurements, such as mammographic or pathologic measurements, are used, the subsets of T1 can be used. Tumors should be measured to the nearest 0.1-cm increment.

Table 1. TNM Staging System for Breast Cancer

Changes in Breast Cancer staging, Sixth Edition of the American Joint Committee on Cancer Staging Manual

Primary Tumor (T)	
• TX	• Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	Ductal carcinoma in situ
Tis (LCIS)	Lobular carcinoma in situ
Tis (Paget)	Paget's disease of the nipple with no tumor
Note: Paget's disease associated with a tumor is classified according to the size of the tumor	
T1	Tumor ≤ 2 cm in greatest dimension
T1mic	Microinvasion 0.1 cm or less in greatest dimension
T1a	Tumor more than 0.1 cm but not more than 0.5 cm in greatest dimension
T1b	Tumor more than 0.5 cm but not more than 1 cm in greatest dimension
T1c	Tumor more than 1 cm but not more than 2 cm in greatest dimension
T2	Tumor >2 cm but not >5 cm in greatest dimension
T3	Tumor >5 cm in greatest dimension
T4	Tumor of any size with direct extension to (a) chest wall or (b) skin, only as described below
T4a	Extension to chest wall not including pectoralis muscle
T4b	Edema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same level
• T4c	Both T4a and T4b

• T4d	Inflammatory carcinoma
Regional Lymph Nodes Clinical (N)	
• NX	Regional lymph nodes cannot be assessed (e.g., previously removed)
N0	No regional lymph node metastasis
N1	• Metastasis to movable ipsilateral axillary lymph node (s)
N2	Metastases in ipsilateral axillary lymph nodes fixed or matted, or in *clinically apparent*[a] ipsilateral internal mammary nodes in the *absence* of clinically evident axillary lymph node metastasis
N2a	Metastases in ipsilateral axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastasis only in *clinically apparent*[a] ipsilateral internal mammary nodes and in the *absence* of clinically evident axillary lymph node metastasis.
• N3	Metastasis in ipsilateral infraclavicular lymph node (s) with or without axillary lymph node involvement, or in *clinically apparent*[a] ipsilateral internal mammary lymph node (s) and in the *presence* of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node (s) with or without axillary or internal mammary lymph node involvement.
N3a	Metastasis in ipsilateral infraclavicular lymph node (s)
N3b	Metastasis in ipsilateral internal mammary lymph node (s) and axillary lymph node (s)
N3c	Metastasis in ipsilateral supraclavicular lymph node (s)
Note: Isolated tumor cells are defined as single tumor cells or small cell clusters not greater than 0.2 mm, usually detected only by immunohistochemical (IHC) or molecular methods but which may be verified on hematoxylin and cosin stains. Isolated tumor cells do not usually show evidence of malignant activity, e.g., proliferation or stromal reaction. [a]*Clinically apparent* is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination or grossly visible pathologically.	
Pathologic (PN)[b]	
• PNX	Regional lymph nodes cannot be assessed (eg, previously removed or not removed for pathologic study)
PN0	No regional lymph node metastasis histologically, no additional examination for isolated tumor cells.
PN0(i-)	No regional lymph node metastasis histologically, negative IHC
PN0(i+)	No regional lymph node metastasis histologically, positive IHC, no IHC cluster >0.2 mm
PN0(mol-)	No regional lymph node metastasis histologically, negative molecular findings (RT-PCR)[c]
• PN0(mol+)	No regional lymph node metastasis histologically, positive molecular findings (RT-PCR)[c]
PN1	Metastasis in one to three axillary lymph nodes and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
PN1mi	Micrometastasis (greater than 0.2 mm, none greater than 2.0 mm)
PN1a	Metastasis in one to three axillary lymph nodes
PN1b	Metastasis in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
PN1c	Metastasis in one to three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent

PN2	Metastasis in four to nine axillary lymph nodes, or in clinically apparent* internal mammary lymph nodes in the absence of axillary lymph node metastasis
PN2a	Metastasis in four to nine axillary lymph nodes (at least one tumor deposit >2.0 mm)
PN2b	Metastasis in clinically apparent* internal mammary lymph nodes in the absence of axillary lymph node metastasis
PN3	• Metastasis in 10 or more axillary lymph nodes, or in infraclavicular lymph nodes, or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axilliary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes; or in ipsitlateral supraclavicular lymph nodes.
PN3a	Metastasis in 10 or more axillary lymph nodes (at least one tumor deposit ≥2.0 mm), or metastasis to the infraclvicular lymph nodes
PN3b	Metastasis in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three auxiliary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
PN3c	Metastasis in ipsilateral supraclavicular lymph nodes
[b] Classification is based on axillary lymph node dissection with or without sentinel lymph node dissection. Classification or grossly visible pathologically lymph node dissection without subsequent axillary node dissection is designated "(sn)" for "sentinel node" [e.g., pN0 (I+)(sn)]. [c] RT-PCR, reverse transcriptase-polymerase chain reaction.	

Stage Grouping:

Stage Grouping	Tumor (T)	Node (N)	Metastasis (M)
0	Tis	N0	M0
I	T1[a]	N0	M0
IIA	T0	N1	M0
	T1[a]	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1[a]	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

Note: Stage designation may be changed if postsurgical imaging studies reveal the presence of distant metastases, provided that the studies are carried out within 4 mo of diagnosis in the absence of disease progression and provided that the patient has not received neoadjuvant therapy. [a]T1 includes T1mic

5.0 PATIENT ELIGIBILITY

Patients are eligible if they fulfill all of the listed criteria:

04/26/07

5.1. Patients must have histologically proven stage I (limited to T1C N0 M0), II or III infiltrating ductal and lobular carcinoma; inflammatory breast cancer is allowed.

5.2. Patients must not have received any anti-estrogen treatment for any indication other than breast cancer prevention (with tamoxifen, raloxifen, or an aromatase inhibitor), and/or could not have received such therapy for at least five years prior to enrollment into this study.

12/4/06

5.3. Patients must have an ECOG performance status of < 2 or KPS of ≥ 70.

5.4. Patients must be ≥ 18 years old.

5.5. Bilirubin $\leq$ the upper limit of normal (except for patients with Gilbert's disease), instead of the current bilirubin ≤ 1.5 mg/dl.

5.6. Creatinine ≤ 1.2 mg/dl and calculated or measured creatinine clearance ≥ 70 cc/min.

5.7. Neutrophil count ≥ 1500/ul, platelet count $\geq 100{,}000$/ul.

5.8. Patients must have an ejection fraction $\geq 50\%$ on MUGA scan.

12/4/06

5.9. Pretreatment laboratory parameters must have been performed within 1 week and x-ray parameters must have been performed within 4 weeks prior to the initiation of therapy

5.10. All patients must have signed an informed consent document in accordance with institutional and federal guidelines.

03/22/06

5.11. In regards to the exploratory barriers study, patients must have histologically proven stage III breast cancer who have experienced a 3 month or greater delay from initial symptoms/suspicion of possible breast disease as identified the principal investigator.

Patients are ineligible if they fit any of the listed criteria:

5.12. History of prior malignant disease, (except for squamous or basal cell skin carcinoma, and stage I or *in situ* cervical carcinoma) within the previous 5 years. History of non-invasive (*in situ)* breast carcinoma within the previous 5 years.

5.13. History of any significant (and active) cardiovascular disease

5.14. > Grade 1 neuropathy, pre-existing from any medical condition

5.15. Any intercurrent/co-morbid medical or psychological condition, which, in the opinion of the principal investigator would interfere with safety and timely delivery of the assigned, planned treatment.

5.16. Current pregnancy. Patients are to utilize effective contraceptive methods (other then hormonal methods) while undergoing treatment on this study.

5.17. Patients with prior radiation to the chest wall or local regional areas (i.e., prior radiation for lymphoma, etc) are ineligible.

5.18. Patients with a documented sensitivity to E. coli-derived products.

6.0 DESCRIPTIVE FACTORS/STRATIFICATION/RANDOMIZATION SCHEME

6.1 Patients will be stratified by stage and receptor status

6.2 Descriptive factors include HER-2 and ER/PR receptor status, grade, age, the number of axillary nodes involved with metastasis, and presence of vascular invasion.

6.3 Patients with HER-2 negative tumors will be randomized to TAC (Arm A) or ACAC arm (Arm B) by phone call to the Department of Biostatistics at the time of accession to the protocol. Patients with HER-2 overexpressing tumors will be receiving ACAC and trastuzumab (Arm C). Randomization for those with HER-2 negative disease will be by permuted blocks within strata. Patients who withdraw prior to treatment will be replaced at a randomly selected subsequent registration occasion. The analysis will thus be on an as-treated basis.

7.0 TREATMENT PLAN

04/26/07

7.1 Patients with biopsy-proven, clinically/radiographically stage I (limited to T1C N0 M0), II (including T2N0) and stage III breast carcinoma (as agreed upon by the primary breast surgeon, medical oncologist, and diagnostic radiologist) will be offered participation and asked to sign the IRB-approved, informed consent form.

12/4/06

7.2 All patients will undergo clip placement and core-biopsies of the primary site/s. If a patient already underwent core biopsy and clip placement, and is otherwise eligible for study participation, repeat core biopsy and/or clip placement could be eliminated as per PI judgment. If there is evidence of lymph node involvement by imaging, ultrasound-guided biopsy of such lymph node will be performed. ER/PR receptor HER-2 status will be evaluated in addition to standard pathological assessment. An attempt to procure 8-12 cores (as per COH practice) through a single biopsy entry site will be made in order to procure sufficient fresh frozen tissue (and if abundant tissue is procured embedded tissue) specimens. HER-2 + is defined as 3+ by IHC, or amplified by FISH.

7.3 Once the primary diagnosis is established, and provided that ultrasound-guided lymph node biopsy is negative or could not be performed, sentinel node mapping/biopsy will be carried out to establish the presence or absence of lymph node metastasis.

7.4 Placement of a central venous device is recommended prior to initiating chemotherapy.

7.5 Patients with HER-2 negative disease will be randomized to undergo treatment with either 6 cycles of TAC (Arm A) or 4 cycles of AC given every 2 weeks, followed by 3 weekly doses of carboplatin and Abraxane® and one week of no treatment, repeated for 3 treatment cycles (ACAC, Arm B). Patients with HER-2

overexpressing tumors will also receive ACAC, but they will also receive trastuzumab during the carboplatin/Abraxane® phase of therapy. (Arm C).

Arm A	**Agent**	**Dose**	**Route**	**Schedule**
	Doxorubicin	50 mg/m^2	IVP	Q 3 weeks x 6
	Cyclophosphamide	500 mg/m^2	IV, 1 hour	Q 3 weeks x 6
	Docetaxel	75 mg/m^2	IV, 1 hour	Q 3 weeks x 6
	Filgrastim or pegfilgrastim	300 ug 6 mg	sc sc	days 2-10 day 2

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Arm B	**Agent**	**Dose**	**Route**	**Schedule**
	Doxorubicin	60 mg/m^2	IVP	Q 2 weeks x 4
	Cyclophosphamide	600 mg/m^2	IV, 1 hour	Q 2 weeks x 4
	Filgrastim or pegfilgrastim	300 ug 6 mg	sc sc	days 2-10 day 2
	⇩ Carboplatin	AUC 2	IV, 1 hour	weekly x 3, 1 week off
	(Abraxane®)	100 mg/m^2	IV, 30 min	weekly x 3, 1 week off
	Filgrastim	300 ug	sc	weeks$^+$ 2 & 3, days 9-12, 16-19

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Q 4 wks x 3

Arm C	**Agent**	**Dose**	**Route**	**Schedule**
	Doxorubicin	60 mg/m^2	IVP	Q 2 weeks x 4
	Cyclophosphamide	600 mg/m^2	IV, 1 hour	Q 2 weeks x 4
	Filgrastim or pegfilgrastim	300 ug 6 mg	sc sc	days 2-10 day 2
	⇩ Carboplatin	AUC 2	IV, 1 hour	weekly x 3, 1 week off
	(Abraxane®)	100 mg/m^2	IV, 30 min	weekly x 3, 1 week off
	Filgrastim	300 ug	sc	week$^+$ 2 & 3, days 9-12, 16-19
	Trastuzumab (A loading dose of 4 mg/kg will be administered)	2 mg/kg	IV	weekly x 12

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Q 4 wks x 3

7/15/08

[+] Patients on Arms B and C will receive filgrastim on days 9-12, and 16-19 with each cycle, regardless whether the ANC dropped below < 1000/μL. However, should there be a delay of the second weekly dose of Abraxane and carboplatin at any given cycle, G-CSF then would be administered for all subsequent cycles inclusive of the first week of therapy on Days 2-5.

7.6 Patients on Arms B and C will receive filgrastim on days 9-12, and 16-19 with each cycle, regardless whether the ANC dropped below < 1000/μL. However, should there be a delay of the second weekly dose of Abraxane and carboplatin at any given cycle, G-CSF then would be administered for all subsequent cycles inclusive of the first week of therapy on Days 2-5.

7/15/08

7.7 The use of erythropoietin growth factors is encouraged as per ASCO guidelines.

7.8 There will be no prophylactic administration of drugs to pre-empt cardiac, or neurotoxicity. If needed, symptomatic management of neuropathy, myalgias, or arthralgias are recommended as per the discretion of the treating physician, who is allowed to appropriate pain medications, anti-inflammatory agents, antidepressants, nutritional supplements, with mandatory careful documentation of the prescribed agents.

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7.9 Actual body weight will be used for patients whose weight is ≤ 30% above ideal. For all others, the weight used to calculate the dose will be topped off at 30% above their ideal body weight. To further clarify, this same rule will be applied when calculating the dose of carboplatin. Carboplatin AUC-2 will be calculated using the Calvert formula; however, the serum creatinine will be capped at no lower than 0 .7 mg/dl: patients with a serum creatinine of ≤ 0.7 mg/dl will have their dose calculated based on a creatinine value of 0.7 mg/dl, while patients with a serum creatinine of > 0.7 mg/dl will have their dose calculated based on the actual measured value.

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7.10 Ideal body weight formula will be used as follows: 50 kg + 2.3 times the number of inches over 5 ft. for males and 45.5 + 2.3 times the number of inches of 5 ft for females.

However, the dose of trastuzumab will be calculated based on actual body weight, since this was the methods used in the pivotal randomized trials by NSABP and the Intergroup.

7.11 Definitive surgical intervention (breast preservation or mastectomy) will be carried out preferably within 4 weeks after the last dose of chemotherapy. Patients with documented axillary nodal involvement (as documented earlier by ultrasound-guided, or mapping/sentinel node procedure) will undergo axillary node dissection. Those with negative sentinel node biopsy (negative, or with < 2 mm focal involvement either by H&E or by immunohistochemistry prior to neoadjuvant treatment) will not undergo formal axillary node dissection.

7.12 Following definitive surgery, patients are recommended to undergo local regional radiation therapy as per standard radiation therapy guidelines. Sites of relapse and relapse-free and overall survival will be documented during the follow-up period.

7.13 Patients with ER/PR receptor positive tumors are advised to receive adjuvant anti-estrogen therapy according to standard guidelines.

7.14 In addition to all patients participating in the Quality of Life study (see schedule of interviews in the Study Calendar Section) 20 patients each randomized to receive treatment either on Arm A (TAC), or B or C (ACAC) will be offered a more detailed assessment of neurotoxicity (See study calendar).

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7.15 Exploratory analysis of "Barriers to treatment". Among the patients with stage III breast cancer who have experienced a 3-month or greater delay in treatment, patients will be offered participation in qualitative interviews. A subgroup of up to 20 patients will be selected. Patients will be interviewed to explore the issues involved in the barriers and delay in seeking diagnosis once symptoms appear. Drs. Betty Ferrell and Marcia Grant who are experts in qualitative interviews will conduct the tape-recorded interviews. An interview guide will be used as provided in appendix C.

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8.0 TOXICITIES MONITORED AND DOSAGE MODIFICATIONS

8.1 Toxicities and potential complications

8.1.1 The side effects associated with the use of the individual drugs are listed under section 3.0. There will be no dose modifications for trastuzumab, cyclophosphamide or doxorubicin.

8.1.2 Trastuzumab:

8.1.2.1 Infusion-associated symptoms:

During the first infusion, a symptom complex of fever and/or chills may occur. These are usually mild-to-moderate and may be accompanied by nausea, vomiting, headache, dizziness, rigors, pain, hypotension, rash, and asthenia. These symptoms occur infrequently during subsequent infusions. Treat fever and chills as follows:

• Fever	• Treatment
Grade 1 (38°C - 39°C) Grade 2 (39.1°C - 40°C)	• Stop infusion and give antipyretics. Once temperature is < 38°C, resume infusion at slower rate.
• Grade 3/4 (>40°C for < 24 hours/> 24 hours) • Grade 4 (> 40°C for > 24 hours)	• Stop infusion immediately. • Give antipyretics. • Monitor patient for minimum of 1 hour • If temp drops to < 38°C within 3 hours, resume infusion at a slower rate • If fever does not resolve within 3 hours, inpatient monitoring is strongly recommended. • Subsequent administration is at the investigator's discretion.

Chills can be treated with acetaminophen and/or diphenhydramine hydrochloride. Meperidine may be used at the investigator's discretion.

8.1.2.2 Cardiac dysfunction and trastuzumab:

- During treatment, MUGA scan/echocardiogram will be performed at the discretion of the treating physician based on symptoms of congestive heart failure (CHF). The same procedure (MUGA scan/echocardiogram) must be used as was used prestudy (if done at prestudy).
- Trastuzumab therapy will be held if the patient develops signs and symptoms of CHF (i.e., dyspnea, tachycardia, cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, etc.)
- Trastuzumab therapy will be discontinued permanently if the ejection fractions fall below institutional lower limits of normal at any time during treatment.
- If trastuzumab therapy is discontinued, patient may continue treatment with ACAC, at the discretion of the treating physician.

8.1.3 Dose Modification for Docetaxel:

8.1.3.1 Neutropenia:

If ANC on day of chemotherapy is:

$\geq 1{,}000/mm^3$	Proceed with treatment as planned.
$< 1{,}000/mm^3$	Delay docetaxel treatment, continue G-CSF (if patient was initially given Neulesta, delay chemotherapy until recovery, and prescribe filgrastim for subsequent cycles), and recheck counts every 2-3 days per MD's discretion.

IF MORE THAN ONE WEEK DELAY:

$ANC \geq 1000/mm^3$	Treat with 25% dose reduction for docetaxel (for this and all subsequent cycles).
$ANC < 1000/mm^3$	Continue G-CSF and recheck counts in one additional week.

AFTER TWO WEEK DELAY:

$ANC \geq 1000/mm^3$	Treat with 25% dose reduction for docetaxel (for this and all subsequent cycles).
$ANC < 1000/mm^3$	Remove patient from protocol treatment.

8.1.3.2 Neutropenic fever:

If at any time, the patient experiences neutropenic fever (fever $\geq$ 101.5°F [38.5°C] with $ANC < 1{,}000/mm^3$), dose reduce docetaxel by 25% for this and all subsequent cycles.

8.1.3.3 Thrombocytopenia:

If platelet count on day of chemotherapy is:

$\geq$ 100,000/mm^3	Begin treatment as planned
< 100,000/mm^3	Delay start of cycle and recheck counts every 2-3 days.

AFTER ONE WEEK DELAY:

Platelets $\geq$ 100,000/mm^3	Treat with 25% dose reduction for docetaxel (for this and subsequent cycles).
Platelets < 100,000/mm^3	Recheck counts every 2-3 days.

AFTER TWO WEEK DELAY:

Platelets $\geq$ 100,000/mm^3	Treat with 25% dose reduction for docetaxel.
Platelets < 100,000/mm^3	Remove patient from protocol treatment.

If during any part of a treatment cycle the patient has a platelet count of < 10,000/mm^3 or requires a platelet transfusion, then the dose of docetaxel will be reduced 25% for this and all subsequent cycles.

Dose modifications for abnormal liver function:

Docetaxel treatment must be held for abnormal bilirubin; treatment may not be given until bilirubin returns to normal. Dose adjustments for docetaxel are as follows:

Bilirubin	Alkaline phosphatase	SGOT	Action
> ULN or	> 5 x ULN or	> 5 x ULN	Wait $\leq$ 3 weeks. If recovered*, reduce docetaxel dose by 25% for this and subsequent cycles.
$\leq$ ULN and	$\leq$ 5 x ULN and	1.6 x to $\leq$ 5 x ULN	Reduce docetaxel dose 25% for this and subsequent cycles.

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* - Bilirubin ≤ ULN and alkaline phosphatase ≤5 x ULN and SGOT ≤ 5 x ULN.

If docetaxel is held for greater than three weeks, no further docetaxel treatment will be given. While docetaxel is held, both cyclophosphamide and doxorubicin will be held.

AFTER ONE WEEK DELAY:

Platelets ≥ 100,000/mm^3	Treat with 25% dose reduction for docetaxel (for this and subsequent cycles).
Platelets < 100,000/mm^3	Recheck counts every 2-3 days.

AFTER TWO WEEK DELAY:

Platelets ≥ 100,000/mm^3	Treat with 25% dose reduction for docetaxel.
Platelets < 100,000/mm^3	Remove patient from protocol treatment.

If during any part of a treatment cycle the patient has a platelet count of < 10,000/mm^3 or requires a platelet transfusion, then the dose of docetaxel will be reduced 25% for this and all subsequent cycles.

8.1.3.4 Neuropathy (motor or sensory):

If Grade ≤ 2, proceed with full dose of drug(s) scheduled for that day.

If Grade ≥ 3, delay treatment with docetaxel until recovery to Grade ≤ 2.

If delay of > 3 weeks is required, remove patient from protocol treatment.

8.1.3.5 Stomatitis:

If Grade 1, proceed with full dose of drugs scheduled for that day.

If Grade ≥2, hold scheduled chemotherapy (docetaxel and doxorubicin and cyclophphosphamide) until recovery to Grade ≤ 1 is documented.

If delay of > 3 weeks is required, remove patient from protocol treatment.

8.1.3.6 Docetaxel Hypersensitivity Reactions:

Docetaxel premedications consit of dexamethason 8 mg orally, BID, given prior to, on the day, and after administration of chemotherapy. The dose can be adjusted if docetaxel is tolerated,

to 4 mg BID prior to, on the day, and the day after chemotherapy. Docetaxel treatment shall be discontinued for Grade 4 docetaxel hypersensitivity reactions. There are no docetaxel dose reductions for hypersensitivity reactions.

Hypersensitivity	Treatment Guidelines
Grade 1	• Consider decreasing the rate of infusion until recovery from symptoms; closely monitor patient. • Resume docetaxel infusion at the planned initial rate after symptoms resolve on that day of treatment.
Grade 2	• Interrupt docetaxel infusion immediately. • Give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV; monitor patient until symptoms resolve. • Resume docetaxel infusion after recovery of symptoms; depending on the physician's assessment of the patient, infusion should be resumed at a slower rate, then increased incrementally to the initial planned rate. • Depending on the intensity of the reaction observed, additional oral or IV premedication with an antihistamine should also be given for the **next cycle** of treatment, and the rate of infusion should be decreased initially and then increased back to the recommended 1-hour.
Grade 3	• Immediately discontinue docetaxel infusion. • Give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV and/or epinephrine as needed; monitor patient until resolution of symptoms. • Resume docetaxel infusion after recovery of symptoms on that day of treatment; depending on the physician's assessment of the patient, infusion should be resumed at a slower rate, then increased incrementally to the initial planned rate. • Depending on the intensity of the reaction observed, additional oral or IV premedication with an antihistamine should also be given for the **next cycle** of treatment, and the rate of infusion should be decreased initially and then increased back to the recommended 1-hour.
Grade 4	• NO FURTHER DOCETAXEL THERAPY.

8.1.3.7 Fluid retention due to docetaxel:

Treatment of Fluid Retention

No dose reduction for fluid retention is planned. Patients developing new onset of symptomatic edema, or other signs of increasing fluid retention, are to be treated with oral diuretics. Regimens that were found to be effective in the management of fluid retention due to docetaxel are listed below:

- Hydrochlorothiazide 25mg po qd up to tid. Potassium supplementation should be given as needed.
- Furosemide 40 mg po qd if edema is not responsive to hydrochlorothiazide. Potassium supplementation should be given as needed.
- If this regimen is ineffective after a two week trial, the patient may be treated with furosemide 20 mg po qd plus metolazone 2.5 mg po qd with potassium supplementation as needed.

Further therapy should be customized depending upon the clinical situation.

8.1.4 **Dose Modifications for Carboplatin and Abraxane®**

8.1.4.1 General Considerations

a. A repeat course of therapy may only be given when granulocytes $\geq$ 1,000/μl and platelets $\geq$ 100,000/μl. If treatment start is delayed more than three weeks for any toxicity, patient should be removed from protocol treatment.

b. Doses which have been reduced for toxicity will not be re-escalated.

c. Treatment may be delayed no more than 3 weeks.

d. If Grade 3 or 4 myelosuppression is experienced at the -2 dose level and does not recover in 2 weeks, remove the patient from protocol treatment.

e. If any toxicity occurs that requires dose reduction and the patient is already receiving the -2 dose level, the patient should be removed from protocol treatment.

8.1.4.2 Dose Levels

LEVEL	DRUG
Starting dose	Carboplatin AUC = 2, Day1
-1	Carboplatin AUC = 1.5, Day 1
-2	Carboplatin AUC = 1, Day 1

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Carboplatin AUC-2 will be calculated using the Calvert formula; however, the serum creatinine will be capped at no lower than 0 .7 mg/dl: patients with a serum creatinine of $\leq$ 0.7 mg/dl will have their

dose calculated based on a creatinine value of 0.7 mg/dl, while patients with a serum creatinine of > 0.7 mg/dl will have their dose calculated based on the actual measured value. Dose adjustments are to be made according to the system showing the greatest degree of toxicity (see Section 8.1 for information on the NCI-CTCAE).

8.1.4.3 Dose modification for hematologic toxicity will be based on blood counts obtained in preparation for the first day of new cycle of chemotherapy. A repeat course of therapy will only be given when granulocytes $\geq$ 1,000/μL and platelets $\geq$ 100,000/μL. If Day 1 is delayed less than 1 week, (CBC to be checked every 2-3 days) due to a granulocyte count of < 1,000/µL, the patient should receive the same chemotherapy dose that she received during the previous course, and start G-CSF. If the second or third weekly dose of the cycle is delayed between 1-2 weeks while the patient is already receiving G-CSF, or the patient experiences febrile neutropenia, the doses of both Abraxane® and carboplatin should be reduced one level. A dose delay of between 1-2 weeks of any cycle or weekly doses within any cycle due to platelets $\leq$ 100,000/µL will necessitate a reduction of carboplatin by one dose level. No more than 3 weeks of delay will be allowed, following a second reduction of carboplatin. Once platelets recover, patients can continue with Abraxane. Weekly doses of carboplatin and nab-paclitaxel will be allowed to be replaced as long as the delay is < 14 days. Following a second reduction of carboplatin, or need to stop carboplatin due to thrombocytopenia, patients could continue with Abraxane. Should there be a need of more than 2 dose reductions of Abraxane, the patient will be taken off study.

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8.1.4.3.1 Neulesta dose will be reduced to 3 mg given subcutaneously, if significant (grade 2 or greater musculoskeletal toxicity is experienced by the patient.

8.1.4.4 Adjustments for Renal Toxicity

Calculated Creatinine Clearance	Dose Level
Clcr < 25 ml/minute	Delay chemotherapy by a minimum of one week until Clcr $\geq$ 25 ml/minute then reduce carboplatin dose one level. Abraxane® will be administered on schedule, while carboplatin is on hold
Clcr $\geq$ 25 ml/minute	No chemotherapy delay or dose reduction required.

8.1.4.5 Adjustments for Hepatic Toxicity (for carboplatin and Abraxane®)

a.Bilirubin

Total Bilirubin	Dose Level
≤ institutional upper limit of normal	No delay or dose reduction required
> 2 - ≤ 3 x institutional upper limit of normal	Delay chemotherapy until bilirubin ≤ the institutional upper limit of normal, then reduce dose one level.
> 3 x institutional upper limit of normal	Delay chemotherapy until bilirubin ≤ the institutional upper limit of normal then reduce dose two levels.

SGOT or Alkaline Phosphatase

SGOT or Alkaline Phosphatase	Dose Level
≤ 2 x institutional upper limit of normal	No delay or dose reduction required
> 2 - ≤ 5 x institutional upper limit of normal	Delay in of both carboplatin and Abraxane® chemotherapy until enzymes ≤ 2 x institutional upper limit or normal then administer same dose as previous course.
> 5 - ≤ 20 x institutional upper limit of normal	Delay chemotherapy until enzymes ≤ 2 x institutional upper limit of normal, then reduce dose one level
> 20 x institutional upper limit of normal	Delay chemotherapy until enzymes ≤ 2 x institutional upper limit of normal, then reduce dose two levels.

8.1.4.6 Adjustments for Neuropathy-sensory

Degree of Numbness/other peripheral neuropathy	Dose Level
Grade 2	Delay chemotherapy until peripheral neuropathy resolves to < grade 2, then administer at the next lower dose level.
Grade 3 and Grade 4	Delay chemotherapy until peripheral neuropathy resolves to < grade 2, then reduce Abraxane® dose one level, and carboplatin 2 dose levels.
Grade 4	Delay chemotherapy until peripheral neuropathy resolves to grade 2, then reduce Abraxane ™ and carboplatin by 2 dose levels.

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8.1.4.7 Other Toxicities Not Listed Above

Hypersensitivity reactions are rare. If they do occur, minor symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia may require temporary interruption of the infusion. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema or generalized urticaria require immediate discontinuation of study drug administration and aggressive symptomatic therapy. Patients who develop severe hypersensitivity reactions to carboplatin and/or Abraxane® may be re-challenged following premedication that the institution would typically use for intravenous paclitaxel (intravenous steroid, antihistamines, and with using a slower infusion rate for carboplatin). If severe hypersensitivity reaction to Abraxane is seen do not re-challenge.

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For all other toxicities excluding Grade 3 hyperglycemia, anemia, SGPT elevation, 4 nausea and vomiting (which will be management symptomatically), reduce drug according to the following. If any toxicity occurs that required a dose reduction and the patient is already receiving the -2 Dose Level, the patient should be removed from protocol treatment.

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Grade 1-2	Delay chemotherapy until resolution of toxicity to Grade 0, then administer same dose level as previous course.
Grade 3	Contact Study Coordinator and delay chemotherapy until resolution of toxicity to Grade 0, then reduce dose one level.
Grade 4	Contact Study Coordinator and delay chemotherapy until resolution of toxicity to Grade 0, then reduce dose two levels.

8.1.5. Dose adjustment for Abraxane® (ABI-007).

Abraxane® should be withheld in patients who experience ≥ Grade 2 peripheral neuropathy. Treatment may be resumed at the next lower dose level (see Table 2) after the peripheral neuropathy improves to ≤ Grade 1. The time to resolution to Grade ≤ 1 should be the adverse event duration used for adverse event reporting. In those patients who experience Grade 4 peripheral neuropathy, study drug should be withheld, and treatment resumed at a reduction of 2 dose levels (Dose Level -2; see Table 2) in subsequent cycles after the peripheral neuropathy improves to ≤ Grade 1.

Table 2.

Dose Level	Abraxane® (mg/m^2)
0	100
-1	80
-2	65

Table 3.: Use of G-CSF and Dose reductions for Hematologic Toxicity

Adverse Event	Occurrence	Action to be Taken
ANC < 500 cells/mm^3 (nadir count) with neutropenic fever > 38° OR Delay of next cycle due to persistent neutropenia (ANC < 1000 cells/mm^3)	Any Occurrence	At the first occurrence of a hematological toxicity (as outlined in the Adverse Event column), the same dose is maintained and G-CSF is given as outlined below. In the event that a hematological toxicity re-occurs in the face of G-CSF, dose reduction to the next lower level will be required for subsequent cycles once ANC is ≥ 1,000 cells/mm^3.
Thrombocytopenia Grade 3 or Grade 4*	1st Occurrence	Dose reduction to next lower level
	Recurrence	Dose reduction to next lower level

*See NCI CTCAE Version 3.0 for definition of Grade 3 and Grade 4 events.

Hypersensitivity Reactions

Hypersensitivity reactions are rare. If they do occur, minor symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia may require temporary interruption of the infusion. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema or generalized urticaria require immediate discontinuation of study drug administration and aggressive symptomatic therapy. Patients who experience a severe hypersensitivity reactions to Abraxane should not be re-challenged.

Adjustments for Cardiac Toxicity

Clinical Signs	Action Required
Cardiac arrhythmia with evidence of AV nodal block (e.g., Mobitz type 1 or 2 or total heart block).	Discontinue chemotherapy and contact Study Coordinator.
Asymptomatic bradycardia	No delay or dose reduction required.

Other Toxicities
If toxicities are ≤ grade 2, manage symptomatically if possible, and retreat without dose reduction.

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If toxicities are ≥ grade 3, except for anemia, treatment should be withheld until resolution to ≤ grade 1 or baseline if baseline was greater than grade 1, then reinstituted, if medically appropriate, at the next lower dose level.

8.1.6 Up to two weeks of delay of any given dose of therapy is allowed once, and up to one week of dose delay of any given dose of therapy is allowed twice, in order to allow recovery to acceptable functional/toxicity grade level as per the list under eligibility criteria, before a patient is taken off study.

3/19/08

8.1.7 For patients randomized to receive treatment on Arm A filgrastim 300 µg between days 2-10, or pegfilgrastim 6 mg on day 2 will be given subcutaneously after each cycle. Filgrastim can be held if the ANC is on the rise from the nadir and is ≥ 1000µ/L for 3 consecutive days.

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8.1.8 Patients on Arms B and C are will receive filgrastim on days 9-12, and 16-19 with each cycle, regardless whether the ANC dropped below < 1000/µL. However, should there be a delay of the second weekly dose of Abraxane and carboplatin at any given cycle, G-CSF then would be administered for all subsequent cycles inclusive of the first week of therapy on Days 2-5.

8.2 The study will utilize the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0, publish date December 12th, 2003 for toxicity and Adverse Event Reporting. A copy of the CTCAE version 3.0 is given in Appendix B. Additionally, a copy of the CTCAE version 3.0 can also be downloaded from the CTEP home page http://ctep.cancer.gov/reporting/ctc.html

3/22/06

8.3 DATA AND SAFETY MONITORING

8.3.1 Definition of Risk Level

This is a Risk Level 3 study, as defined in the "Guidance, Policy and Procedures for Data and Safety Monitoring for In-House Trials at City of Hope", http://www.infosci.coh.org/gcrc/doc/dsmp.doc because it is a Phase II clinical trial where the risks are at least balanced by the potential benefit to subjects and the importance of the knowledge that may result.

8.3.2. Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the PI, at least two Collaborating Investigators, CRA, protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of any stopping rules for safety and efficacy.

03/22/06

Reporting of data to the DSMB will occur at intervals separated by no more than six months, groups of 17 patients treated, or after a treatment related death. This report (the PMT report) will include a summary of accrual, adverse events and treatment related mortality. This report is in addition to reports as specified in section 8.4

8.3.3 Adverse Events

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Reporting: Adverse events must be reported to the COH DSMB, IRB, and GCRC (if GCRC supported) according to definitions and guidelines described in section 8.4. SAEs will be monitored by the PMT. Less then serious adverse events will be reported only at the time of protocol continuation reports.

8.4 ADVERSE EVENT MANAGEMENT GUIDELINES

8.4.1 Background

These adverse event management guidelines are intended to ensure the safety of each patient while attempting to characterize the safety and tolerability of the test products. In agreeing to the provisions of this protocol, the investigator accepts all legal responsibilities for prompt notification of SAEs. The PI must submit the original and one copy of the report to the DSMB, American Biosciences (see below for address) and the IRB office.

3/22/06

All STEAEs (Serious Treatment Emergent Adverse Events**)** should be recorded on a MedWatch 3500 Form and submitted to the FDA as soon as possible. A copy of this MedWatch 3500 Form should be faxed within 24 hours to:

1) Abraxis BioScience, LLC
Drug Safety and Surveillance Department
4505 Emperor Blvd, Suite 400
Durham, NC 27703
Ph: 919-433-8515 (8am-5pm EST, normal business days and hours)
Ph: 919-606-1832 (24hr hotline)
Fax: 919-433-8402
E-mail: SAE-REPORTING@abraxisbio.com

and

Abraxis BioScience, LLC.
200 Somerset Corporate Blvd – Suite 8000
Bridgewater, NJ 08807
Office: 908-393-8248
Fax: 908-393-8304
Cell: 267-337-2720
E-mail: AbraxisMedAffairs@abraxisbio.com

For more detailed guidelines please access the following web site: http://www.cityofhope.org/resed/irb/hsguideline.htm. The PI/COH IRB in turn will be responsible for reporting the SAEs to the appropriate regulatory authorities, including the FDA.

Adverse events occurring during the study will be graded according to the NCI Common Toxicity Criteria Scale CTCAE version 3.0, where applicable. Adverse events that are not included on the toxicity scale will be designated as Grade 1 = mild, Grade 2 =moderate, Grade 3 = severe, Grade 4 = life-threatening, and Grade 5 = death. The investigator should evaluate all adverse events and should make an immediate effort to determine their etiology. Adverse events that are determined not to be possibly, probably, or definitely related to study drug may not require further evaluation but will need to be recorded on the CRFs. Study medications may be interrupted for an adverse event at the discretion of the investigator. Patients requiring toxicity management should be assessed and evaluated at least weekly as indicated by the severity of the event.

8.4.2 Definition of an Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient receiving a marketed pharmaceutical product or in a patient who is participating on a clinical trial who is receiving an investigational or non-investigational pharmaceutical agent. The AE does not necessarily have a causal relationship with the patient's treatment. Therefore, an adverse event can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered to be related to the medicinal product. In cancer clinical trials, many AEs are in fact related to progression of the patient's underlying malignancy.

An adverse event includes:
• an exacerbation of a pre-existing illness;
• an increase in frequency or intensity of a pre-existing episodic event or condition;
• a condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study;
• continuously persistent disease or symptoms that were present at Baseline and worsen following the start of the study.

An adverse event does not include:
• medical or surgical procedures (eg, surgery, endoscopy, tooth extraction, or transfusion); however, the condition that leads to the procedure is an adverse event.
• pre-existing diseases, conditions, or laboratory abnormalities present or detected at the start of the study that do not worsen;
• hospitalizations or procedures that are done for elective purposes not related to an untoward medical occurrence (eg, hospitalizations for cosmetic or elective surgery or social/convenience admissions);

• the disease being studied or signs/symptoms associated with the disease unless more severe than expected for the patient's condition;
• overdose of study drug without any clinical signs or symptoms.

8.4.3 Definition of a Serious Adverse Event (SAE)

Definition

A serious adverse event as defined by ICH is any adverse experience that at any dose meets any of the following conditions:

- results in death
- is life-threatening (The patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations; for example, important medical events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any adverse event is considered a serious adverse event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

Hospitalizations that do not meet this criteria are:

- reasons described in the protocol, e.g., drug administration, protocol-required testing
- social reason in the absence of an AE
- surgery or procedure planned prior to entry into the trial

"Serious" Versus "Severe" Adverse Events

There will be an acknowledged distinction between serious and severe AEs. Assessment of seriousness will be made solely by the serious criteria listed above. Severity of AEs will be graded according to the NCI Common Terminology Criteria for Adverse Events v3.0. Therefore, serious events will not be automatically considered severe. For example, a stroke that results in only a limited degree of disability may be considered a mild (not severe) stroke, but it would still meet serious criteria and thus, be captured as an SAE. Similarly, severe events may not always be serious. An example would be an episode of severe, transient nausea which persists for several hours. This would be classified as a "severe" episode of nausea, but if it did not require treatment, intervention, or somehow meet other serious criteria, it would not be considered an SAE.

Nonserious Adverse Events

Any adverse event that is not an SAE is, by default, a non-serious AE.

8.4.3.1 **Adverse Events Emerging Subsequent to Study Cessation**

For 30 days subsequent to study completion or withdrawal, new onset adverse events will be captured. Follow up of these events will follow the same procedure as described above for AEs observed during the study period.

8.4.4 Time Frame for considering an event to be a reportable AE or SAE

The investigator is responsible for recording, reporting and following all adverse events, regardless of causality, observed during the study period, starting with the registration of the patient and ending at the time the patient goes off study or 30 days after patient's last dose of study drug, whichever is later. The investigator should follow adverse events until they are resolved or stabilized, the patient is lost to follow-up, or the event is otherwise explained. Events occurring within 30 days prior to study drug administration should be recorded as pre-treatment signs and symptoms.

8.4.5 Lack of Efficacy is not considered an AE or SAE

"Lack of efficacy" (progressive disease) is not considered an adverse event. The signs and symptoms or clinical sequelae resulting from lack of efficacy should be reported if they fulfill the adverse event or SAE definitions.

8.4.6 Patient Reporting of AEs and SAEs

Patients are to be encouraged to call the site to report any unexpected symptoms or problems they encounter between office visits. These events should be considered in the same fashion as if they had been reported at a scheduled office visit. At each scheduled office visit, after the patient has had an opportunity to spontaneously mention any problems, the investigator should inquire about adverse events by asking the following standard questions:

• Have you had any (other) medical problems since your last clinic visit?
• Have you taken any new prescribed or over-the-counter medicines or herbal/vitamin preparations, other than those given to you in this study, since your last visit/assessment?
• Have any new procedures been performed since your last study visit?

8.4.7 Investigator Reporting of AEs and SAEs

The investigator or designee must completely and promptly record each adverse event in the source documentation and in the appropriate CRF, regardless of relationship to study drug as determined by the investigator. The Principal Investigator must assess AE/SAE causality for any patients treated at his/her site and for any patients treated under the direct care of his/her sub-investigators. The investigator should attempt, if possible, to establish a diagnosis based on the patient's signs and symptoms. When a diagnosis for the reported signs or symptoms is known, the investigator should report the diagnosis, not the symptoms, as the adverse event.

Clinically significant laboratory abnormalities present at the Baseline visit will be recorded as pre-treatment signs and symptoms. After study treatment administration, Grade 1 and Grade 2 laboratory abnormalities will not be recorded as adverse events unless considered clinically significant by the investigator. All Grade 3 and Grade 4 laboratory abnormalities will be recorded as adverse events. Grade 4 laboratory abnormalities will be reported as serious except in those cases when the abnormality is associated with a SAE that has already been reported. The investigator must follow all SAEs observed during the study, until these events have resolved or stabilized, the patient is lost to follow-up, or the events are otherwise explained.

8.4.7.1 Safety Reporting Requirements and Timelines

The Sponsor-Investigator will utilize the Abraxis SAE Completion Form for the reporting of adverse events and follow up information to those events.

All serious adverse events regardless of severity or relationship must be reported to Abraxis BioScience, LLC and Abraxis Oncology within 24 hours of the investigational staff's knowledge.

Abraxis BioScience, LLC.
Drug Safety and Surveillance Department
4505 Emperor Blvd, Suite 400
Durham, NC 27703
Ph: 919-433-8515 (8am-5pm EST, normal business days and hours)
Ph: 919-606-1832 (24hr hotline)
Fax: 919-433-8402
E-mail: SAE-REPORTING@abraxisbio.com

Abraxis BioScience, LLC.
200 Somerset Corporate Blvd – Suite 8000
Bridgewater, NJ 08807
Office: 908-393-8248
Fax: 908-393-8304
Cell: 267-337-2720
E-mail: AbraxisMedAffairs@abraxisbio.com

In addition, the Sponsor-Investigator will adhere to the safety reporting requirements and timelines described in the Clinical Trial Agreement with Abraxis BioScience, LLC.

The Sponsor-Investigator will provide full and timely cooperation with any requests from Abraxis, governing IRB, institution, or regulatory agency with any requests regarding reports of individual reports of adverse events.

8.4.8 Additional Investigator Responsibilities on Follow-up of SAEs

The investigator and supporting personnel responsible for patient care should institute any supplemental investigations of SAEs based on their clinical judgment of likely causative factors. This may include extra clinical laboratory tests, physical examinations or consulting an appropriate specialist. ABI may also request the investigator to conduct supplemental assessments. The results of any additional assessments conducted must be reported to ABI. If a patient dies during participation in the study and an autopsy is performed, a copy of the report must be submitted to ABI. If a patient dies during the follow-up period for the SAE, the event causing the death will be reported as part of that SAE. The relationship between the SAE and the death should be specifically addressed.

8.4.9 Sponsor Notification of Post-Study SAEs

The investigator should notify DSMB, IRB, ABI of any death or SAE occurring after a patient has withdrawn from the study, when such death or SAE may reasonably be related to the medication used in the study. However, investigators are not obligated to actively seek adverse events in former study participants.

9.0 Study Calendar

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•	Prior to chemo Rx	On day of chemoRx Prior to treatment	Within 24 hours after chemoRx	Midway through chemoRx #	Within 3 weeks prior to surgery	Surgery	Q 6 months	Yearly
H&P including standard neurological evaluation	X%	X			X		X	X
CBC, ANC, platelet count	X%	X@			X			X
Comprehensive chem., Mg,	X%	X@			X			X
PT/PTT	X%				X			
Blood samples for buffy coat/DNA extraction, proteomics (circulating tumor cells)	X+	X+		X+	X+		X+	
• Urinalysis	X%							
Core biopsies, node biopsy, procurement of fresh frozen and preservative embedded tissue, microdissection	X***			X*		X§		
Creatinine Clearance - Calculated or measured	X%							
Mammogram/US	X				Xa			X
MRI of breast and PET as feasible	Xa				Xa			
CT chest + abdomen	Xa							
Bone scan	X							
MUGA scan or Echocardiogram/EKG	X						XX	
Detailed neurological evaluation (see 11.3 and appendix D)†	X			X	X		XX	
Urine pregnancy test	X%							
QOL questionnaire	X			X	X		XX	

a As per the indications for breast MRI in the American Society of Breast Surgeons and American Society of Breast Diseases guidelines/recommendations.

* Optional

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** Every 3 months, for the first year

*** In order to be eligible, patients do need to agree to undergo core biopsy and clip placement as indicated prior to initiation of neoadjuvant therapy.

\# Midway thru chemo: Arm A – after 3rd cycle of TAC / Arm B – after AC

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X+ See section 11.2.1.2 for specific tubes to be drawn.

† For those pts. participating in the separate detailed neuropathy/neuropathic pain assessment subset evaluation.

02/07/08

§ An attempt will be made to procure fresh surgical specimen tissue of the residual primary tumor at the time of definitive operative procedure for the purpose of generating cell lines or xenographts.

% Pretreatment laboratory parameters must be performed within 1 week of Cycle 1 Day 1

@ May be done done up to 72 hours prior to treatment

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

10.1 Patients will be evaluated for tumor response with the primary endpoint being pathological complete response from invasive cancer at the time of definitive surgery. Pathological response is defined as complete resolution of invasive tumor both in breast and (if applicable) in dissected axillary lymph nodes. PCR rates will be compared between the two primary treatment arms (A and B) and will be descriptive for patients on arm C.

10.2 Toxicities (CTCAE version 3.0) and QOL outcome will be compared between Arms A and B, and will be descriptive for patients on Arm C.

10.3 Relapse-free and overall survival data will be collected but will not constitute endpoints. Overall survival is defined as the time from first day of treatment to time of death due to any cause. If a patient is still alive, survival time is censored at the time of last follow-up. Relapse-free survival is defined as the time from first day of treatment to the first observation of disease progression or death due to any cause. If a patient has not progressed or died, progression-free survival is censored at the time of last follow-up.

10.4 Biological/molecular parameters will be assessed in an exploratory manner, and in an attempt to generate hypotheses for future validation studies.

11. 0 SPECIAL INSTRUCTIONS

Tissue and body fluid samples will be collected under this IRB approved clinical protocol in accordance with the IRB-approved HIPPA compliant and signed consent form.

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11.1 Tissue Specimen Processing and Designation

11.1.1 Core and other biopsy specimens (lumpectomy/mastectomy specimens/lymph nodes) will be fresh frozen according to standard procedure by the Department of Anatomic Pathology. Core biopsies (estimated 12 specimens from biopsy), and larger tissue samples from biopsy or resections of subsequent post-chemotherapy specimens will be stored both fresh frozen, and, in case of abundance, specimens will be embedded. H&E and immunohistochemical analysis will be carried out by Dr. Wilczynski.

An attempt will be made to obtain core biopsy specimens halfway through the neoadjuvant chemotherapy course, but this biopsy will be optional and patients may elect to opt out.

Specimens will undergo LCM in batches every 4-8 weeks, pending the speed of accrual, under the supervision of Dr. Wilczynski.

All specimens will be logged in and out using a master logbook, and stored according to standards developed by the Department of Anatomic Pathology. A unique patient identifier (specific for this study) will be assigned to each patient and subclassification (for example: 1prestromaS: patient #1, pre-chemotherapy stroma specimen for the Sommer lab; 1capostL: patient # 1, post-chemotherapy cancer specimen for the Lee lab, etc).

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11.1.2 Sample Design
Core biopsies of primary tumors (an estimated 11 (up to 12-14 if feasible) specimens from biopsy) and larger tissue samples from biopsy or resections of subsequent post-chemotherapy specimens will be procured in the following preservatives:

3 (or more if feasible) specimens in formalin for Anatomic Pathology
3 specimens in EDTA/ETOH for the translational research laboratory and/or Dr. Sommer's laboratory for further distribution

2 specimens in RNAlater to the translational research lab for further distribution
3 specimens fresh frozen to the translational research lab for further distribution

Core and other biopsy specimens will be fresh frozen according to standard procedure by the Department of Anatomic Pathology, Tissue collected in RNAlater (RNAlater is stores at room temperature) will be submerged in ten times the volume of the tissue and the will be less than 0.5 cm in at least one dimension. The tissue will be submerged immediately after it is extracted from the patient and the vial kept upright so the tissue stays submerged in transit. ETOH preserved, frozen and RNAlater samples will be delivered to the translational research laboratory for further distribution.

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11.1.2.1 Mutational Analysis: Sommer Laboratory

Following laser capture microdissetion (LCM) cells procured from core biopsy specimens (2 each pre- and midcourse during neoadjuvant therapy) will be separated containing representative cells of both tumor and host (non-malignant breast tissue) and will be provided for Bi-PAP mutational analysis for the Sommer laboratory. Each core biopsy is expected to weigh 150 milligrams; hence a minimum of about 200-300 malignant and "host" cells each per each core biopsy is expected to be available for analysis.
The Sommer lab will also receive post-treatment residual tumor and normal tissue from the segmentectomy/mastectomy specimens.

11.1.2.2 Proteomics: Lee Laboratory

Core and large biopsy specimens will be batched for laser capture microdissetion (LCM) and cells procured from 2 core biopsies (pre- and midcourse during neoadjuvant therapy) containing representative cells of both tumor and host (non-malignant breast tissue) will be provided for the Lee laboratory for proteomics analysis.
The Lee lab will also receive post-treatment residual LCM tumor and normal tissue from the segmentectomy/mastectomy specimens.

11.1.2.3 RT-PCR and DNA microarray analysis

Two core biopsy samples of the procured pre-treatment, mid-cycle, and from the post-chemotherapy lumpectomy or mastectomy will be used for RNA extraction and subsequent RT-PCR and possible microarray analysis. RNA will be extracted from multiple sections by Qiagene RNA extraction kits (Qiagene Inc., Valencia, CA).

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To confirm overexpression or underexpression of candidate genes, RT-PCR will be performed using an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Real-time PCR has been shown to be a reliable and accurate for quantitative determination of specific RNA expression in tissue samples. Briefly,

RNA is isolated as described above and reverse transcribed to cDNA. Each cDNA sample as well as 3 none-template controls will be run in triplicate. Using labeled oligonucleotide probes, florescent signals are generated at each PCR cycle by the 5' nuclease activity of AmliTaq Gold DNA polymerse. The standard curve of a housekeeping gene such as GAPDH, B-actin, or 18S will be used as normalization..

Gene expression analysis will be performed at a micro array core facility. The integrity of the starting total RNA will be evaluated on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). single-stranded, then double-stranded cDNA will be synthesized from the polyA template to generate biotin-tagged cRNA that will be hydribized on an Affymetrix HG:U133A 2.0 array (Affymetrix, Inc., Santa Clara, CA). Gene expression patterns from the microarray will be analyzed by the Department of Bioinformatics or designees. Fresh frozen specimens will be provided for DNA analysis to assess copy numbers/SNP in Dr. Michael Press' laboratory at USC and will be coordinated by and in collaboration with Dr. Yen's laboratory, and with collaboration from the COH pathology core, spearheaded by Dr. Sean Lau.

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Gene expression analysis pending sufficient quantity of specimens will also be carried out in collaboration with Agendia Corporation under the recently City of Hope-approved material transfer agreement (MTA). This goal is in line with the original objectives and goal of this study. The statistical analysis of this collaboration will be carried out in collaboration with the study statistician Paul Frankel, PhD and the designated bioinformatics personnel (to be named) at Agendia, at the NCI of the Netherlands.

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Additional collaborators to assess RPT-CR and protein expression, bioimmunohistochemistry will include Michael Press Laboratory at USC as well as Response Genetics and the Danenberg Laboratory with whom the PI and the City of Hope signed a material transfer agreement (MTA). Standard operating procedures according to the City of Hope standards are in place to keep samples deidentified for the outside investigators.

11.1.2.4 **Clarke Lab Breast Tumor Dissociation Protocol**

Max Diehn, modified by Neethan Lobo, October 2006

Specimen (either mastectomy or large segmentectomy) removed in OR using sterile instruments sent for inking by pathologist. Tissue sections are cut with sterile instruments and placed into 50-ml conical tubes containing RPMI/10% HICS/Ceftazidime/PSA on wet ice. Samples are kept at 4^{o}C and shipped same day using overnight shipping to:

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Dalong Qian
Stanford University

Institute for Stem Cell and Regenerative Medicine
1050 Arastradero Road
Palo Alto, CA 94304
Lab phone: (650) 724-0574
Cell: (734) 330-3585

Total time on ice will therefore be less than 24 hours total.

Keep cells on ice or at 4°C for entire protocol

1) Make sure to clean and autoclave all surgical instruments before processing tissue. We commonly employ the following equipment: razor blades or scalpels, tissue forceps, surgical scissors. Tools may be cleaned during the dissection by rinsing with 10% bleach followed by 70% EtOH.
2) Put 5ml of Medium 199/H_2CO_3/Earl's/25mM HEPES/pH 7.3 supplemented with PSA into 10mm or 15mm plastic petri dish (cells will not adhere as much as with tissue culture dishes). Transfer tumor piece(s) to petri dish with tissue forceps.

We routinely perform two separate procedures for human tumors: xenotransplantation and flow cytometry. This protocol will mainly deal with processing the tumor tissue for flow cytometry.

3) **Xenotransplantation**: Using "L"-shaped forceps, cut 1-2mm cubes. Transfer all pieces to a sterile eppendorf containing medium and keep on ice. Generally, we implant 2 pieces per mouse at different sites. We use a trocar to deliver the tissue into the upper fat pads of NOD/SCID mice. We also place an estrogen pellet into the back of the mouse if dealing with an ER+ breast tumor. All tumor chunks are coated with matrigel before engraftment.

The following is based on our procedures for processing human breast tumors. Other tumor types may require alternate dissociation enzymes, digestion times, etc.

4) **If leaving tissue overnight**: Put entire piece in RPMI w/ 10% HICS and ceftazidime and Pen/Strep/Ampho and leave on ice O/N. In the morning, wash 1x with cold PBS, then proceed as follows.
5) **Make single cell suspension**: If specimen contains a significant amount of adipose tissue, trim this first. Then cut tissue into slivers by holding with forceps and sliding prongs around razor blade. Next mince using slicing motion. Once adequately disaggregated, bring total volume to 27 ml with media and add 3 ml of 2000 unit/ml collagenase type 3 (10x) [Can adjust total vol based on size of specimen]. Add DNase to the digestion (5 Kunitz units/ml). Scrape cells off bottom of plate with razor blade before placing into incubator (37°C, 5% CO_2) and mechanically dissociate by pipetting every 20 minutes. Incubate for 2-4 hours, or until tissue is disaggregated. Often we need to spike the digestion with an

additional 1.5 ml Collagenase Type III at ~1.5 hrs to achieve complete dissociation.

6) **Neutralizing:** Add 10 mls of neutralizing media (RPMI 1640/10% HICS/PSA) per 20 mls of digestion media. Mix by pipetting.
7) **Filtering**: First pipette media over nylon mesh held over 50ml conical. May need to change nylon mesh several times due to clogs. Next pipette through 70 µm filter into a fresh 50ml conical. Tilt filter slightly to increase flow speed if it slows down. Finally, pipette through 40 µm filter into a fresh 50ml conical. Pellet cells for 5 min at 1000 RPM at 4°C.
8) **RBC lysis:** Resuspend pellet in 5 ml of ACK buffer and incubate on ice for 2 minutes. Bring up volume to 50 ml with HBSS w/ 2% HICS to stop lysis. Pellet cells for 5 min at 1000 RPM at 4°C.
    a. Optional: can repeat 40 µm filtering to remove debris from RBC lysis if desired.
9) **Count cells:** Bring up cells in an appropriate volume of HBSS w/ 2% HICS. Cells are now ready for staining.

Optional: Freezing Tumor Samples

1) Resuspend cells in 10% DMSO/80% DMEM/10%FBS at a concentration of 13 million cells/ml in cyrovials.
2) Place in freezing chamber O/N in -80C.

Transfer to liquid nitrogen for long term storage.

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Once City of Hope capabilities allow, cell line development and stem cell line identification will be carried out in the laboratories of Shiuan Chen, Jack Shively, Yun Yen, John Rossi and other COH collaborators as feasible.

11.2 Blood Specimen Processing and Designation

11.2.1 Blood Samples

11.2.1.1 All blood specimens will be drawn using the following vacutainer tubes:

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Tubes #1-4 – 7 ml EDTA (purple-top) tube
Tube #5 – One 7 ml Paxgene tube
Tube #6-9 – Four - 10ml tubes containing EDTA anticoagulant and Catches preservative

11.2.1.2 Timing:

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Pre-chemotherapy:	Tubes #: 1-9
Mid-chemotherapy:	Tubes #: 1-9
Prior to definitive surgery:	Tubes #: 1-9
Every 3 months x 4:	Tubes #: 1-5
Every 6 months x 1 year :	Tubes #: 6-9

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11.2.1.3 Specimen Handling:

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All EDTA tubes (purple top) will be kept on ice. After centrifugation at 1100 rpm for 10 minutes at 4 degrees Celsius, the plasma will be transferred to Eppendorf tubes and centrifuged at 14000 rpm for 10 minutes at 4 degrees Celsius to separate the WBC fraction. The plasma will be divided into 500 microliter aliquots and stored at -80 degrees Celsius. The WBC will be washed with a red cell lysis buffer, pelleted and stored at -80 degrees Celsius.

The EDTA tubes will be delivered either to the translational research lab within 1 hour of procurement and centrifuged to separate the buffy coat and plasma fractions.

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Tube #5 is a PAXgene tube for stabilization of whole blood and subsequent isolation of cellular RNA. After incubation for 2 hours at ambient temperature, the tube will be stored in a loose wire rack at -20 degrees Celsius for up to one year. The RNA will be extracted following the protocol in the PAXgene Blood RNA Kit sold by QIAGEN. The RNA will be analyzed by real-time PCR or micro array. This tube will be procured for RNA extraction from PMN cells in Dr. Yen's laboratory.

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Tubes # 6-9. will be procured and shipped in order to quantitate, isolate, and characterize circulating tumor cells to the Bruce laboratory.

After venipuncture, the initial 1/2 cc of blood will be discarded (or drawn into any pediatric tube, which is then discarded) to not contaminate the subsequent blood sample with skin epithelial cells.

To test for the presence of tumor-like cells, the blood will be labeled with fluorescent molecules marking for Cytokeratin (CK), CD-45 and DAPI antibodies with subsequent processing for markers for ER, PR, HER2, EGFR and ERCC-1 or other suitable markers.
Blood will be collected in the 10ml tubes containing EDTA anticoagulant and Cytochex preservative. These Rare Cell tubes are made by Streck and will be supplied by PARC/Bruce Laboratory. The blood will be shipped at room temperature using overnight delivery in Styrofoam containers provided by PARC and samples will be marked as customary with an anonymized patient ID by the specialist taking the blood. PARC personnel will have medical information relevant to the study, but the patient will be anonymous.

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Contact information for PARC/Bruce Lab:

07/15/08

Bruce Lab
PARC (Palo Alto Research Center)
3333 Coyote Hill Road
Palo Alto, CA 94304
Phone 650 812 4000

11.2.2 Specimen Tracking:

11.2.2.1 The date of blood draw will be recorded. For tracking purposes, the following will also be recorded upon receipt to the processing and receiving laboratories:

a. Specimen ID number (all specimens will be anonymized by assigning a unique identifier, similarly to the process described under "Tissue specimen handling", with coding provided by Biostatistics.

b. Number and types of tubes received and date of receipt.

c. Refrigerator location of stored cell pellet for DNA extraction and date of initial storage.

d. Number of aliquots of plasma and serum (5-6 aliquots per tube expected), freezer location(s) stored, and date(s) of initial storage.

11.2.2.2 Liquid nitrogen tank location of stored PBMC and date of initial storage will be tracked.

3/22/06

11.2.2.3 Upon transfer of specimens to the Sommer, Lee, Yen, Synold and any other designated labs, tracking will include:

a. Specimen ID number

b. Lab to which specimen was sent

c. Date of transfer

3/22/06

11.2.2.4 Upon return of data from the designated labs, the following will be recorded:

a. Specimen ID number

b. Date of run and run number (all specimens will be run in replicate for at least two runs in order to evaluate intra-and inter-essay reliability)

c. Type of assay

d. Lab performing assay

e. Assay results

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11.3 Assessment of peripheral neuropathy

Right peroneal nerve motor conduction studies are performed with the stimulating electrode at the ankle and the recording electrode 8 cm distally over the extensor digitorum brevis muscle. For sensory conductions, the right sural nerve is stimulated antidromically in the posterior lower leg and recorded 14 cm distally at the lateral malleolus. Quantitative sensory testing of vibratory and cold detection thresholds will be performed with the Case IV system one-time-period 4, 2, 1 Stepping Algorithm (145). The point of stimulation is immediately distal to the base of the nail of the right large toe for vibration and the dorsal surface of the right foot for cold. The relative changes in nerve conduction and quantitative sensory tests will be recorded and expressed as the logarithm to the base 2 of ratios of post chemotherapy/baseline determinations. (A logarithm ratio of 0 indicates no change, -1 indicates the value was reduced by half, +1 indicates an increase by a factor of 2, -2 indicates a reduction by a factor of 4, etc.)

Peripheral neuropathy scores will be obtained by a modification of the method by Chaudhry et al (146). As shown in Appendix, scores of 0-3 are given for each of 7 categories: (1) sensory symptoms, (2) pin sensory loss, (3) vibration sensory loss, (4) strength, (5) deep tendon reflexes, (6) sural sensory nerve action potential (SNAP) amplitude, and (7) peroneal nerve compound muscle action potential (CMAP) amplitude. Percentage reduction of SNAP and CMAP will be determined on the basis of the lower limit of the laboratory normal. Sum of peripheral neuropathy composite scores could range from 0 (no impairment) to 21 maximum impairment on this scale).

12.0 STATISTICAL CONSIDERATIONS

04/26/07

03/19/08

This is a randomized phase II study of neoadjuvant chemotherapy regimens in patients with stages I-III breast cancer. The primary objectives include comparison of toxicity and efficacy and Quality of Life endpoint. Efficacy will be defined by comparing the percentage of patients whose definitive surgical specimens following neoadjuvant therapy are scored by our pathology collaborators as either Symmans scores 0 or 1 versus all others, between TAC versus ACAC in patients with HER-2 negative breast cancer, and establishment of toxicity profile and efficacy with ACAC and trastuzumab in patients with HER-2 overexpressing breast cancer.

This will be a randomized Phase II trial, with two strata.

Stratum 1: Patients with HER-2/neu- tumors.

In the stratum of patients that have her2/neu negative tumors, patients will be randomized to either Arm A (TAC) or Arm B (ACAC). A Simon optimum two-stage design will be used to evaluate the activity of the treatment in terms of pathological complete response (pCR) rate following neoadjuvant chemotherapy in each of the two parallel arms. If a response rate in an arm is promising, that arm can be considered for further study as neoadjuvant chemotherapy. If both arms are deemed promising, the preferred arm, based on a superior pCR rate, will be chosen for future evaluation providing toxicity and safety are acceptable.

Using a two-stage Simon optimum design, the initial stage of accrual will consist of 34 patients, 17 randomly assigned to each arm. Accrual will continue without interruption until all of the first 34 patients have been assessed for their neoadjuvant response following definitive surgery. That interim analysis is expected to occur approximately 9 months into the study. If, at that point, less than 4 subjects have a pCR, on an arm, then accrual to that arm will be stopped. If 4 or more experience a pCR in the first 17 patients on an arm, an

additional 20 patients will be accrued during the second stage to that arm, for a total of 37 patients on that arm. As long as both arms remain open, patients will be randomly assigned to an arm. In either arm, if at least 11 patients obtain a pCR out of the total of 37 evaluable patients, the regimen will be regarded worthy of further testing. Otherwise, further testing of the regimen corresponding to that arm would not be warranted. This two-stage design has at least 90% power to detect a true response rate of at least 40% in each arm. It yields at least a 90% probability of a negative result if the true response rate is no more than 20% with at least a 55% probability of early stopping under the discouraging alternative. With 37 evaluable patients in each arm, the 95% confidence interval for the response rate in an arm will have a half-width of ±16% or less. If neither arm is closed during the interim analysis, the total accrued on this stratum will be 74 patients.

If both arms are deemed worthy of further study, the arm with the superior rate of pathological complete responses will be selected for future evaluation, providing other characteristics such as toxicity and safety are equal. Such a selection rule has less than a 20% chance of choosing the inferior arm (~80% power), if the inferior arm has a true response rate of 30% and the superior arm has a true response rate of 40%.

Stratum 2: Patients with HER-2 positive tumors.

Patients with HER-2 positive tumors will not be randomized due to the imperative to offer Herceptin®. Due to concerns of potentially higher likelihood of cardiac toxicities if combined with TAC (Arm A), all HER-2 + patients will be accrued to Arm C (ACAC [Arm B] + Herceptin®).

A Simon optimum two-stage design will also be used to evaluate the activity of the treatment for these strata in terms of pathological complete response (pCR) rate following neoadjuvant chemotherapy. The initial stage of accrual will consist of 15 patients. Accrual will continue without interruption until all of the first 15 patients have been assessed for their neoadjuvant response following definitive surgery. That interim analysis is expected to occur approximately 9 months into the study. If, at that point, less than 6 subjects have a pCR, then accrual to that arm will stop. If 6 or more experience a pCR in the first 15 patients, an additional 17 patients will be accrued during the second stage to that arm. If at least 13 patients obtain a pCR out of the total of 32 evaluable patients, the regimen will be regarded worthy of further testing. Otherwise, further testing of the regimen corresponding to that arm would not be warranted. This two-stage design has at least 80% power to detect a true response rate of at least 50%. It yields at least a 90% probability of a negative result if the true response rate is no more than 30% with at least a 72% probability of early negative stopping under the discouraging response rate. With 32 evaluable patients, the 95 % confidence interval for the response rate in an arm will have a half-width of ±18% or less.

Additional 8 patients (up to 40 total evaluable) in Arm C have been added to increase the precision on the response rate and provide more opportunity for biopsy studies.

Total evaluable patients to be accrued: Stratum 1 (Arms A and B): 74 patients' Stratum 2 (Arm C): 40 patients. With current rate of accrual, the study will have been completed by the end of June 2009 at the latest.

.In any arm (on either stratum), if responses occur in patients that are accrued beyond the interim analysis point, and the arm was scheduled for closure based on the interim analysis, a stochastic curtailment procedure will be employed to assess whether an amendment to re-open that arm is warranted.

Total Patients Accrued: Stratum 1: 74 pts. Stratum 2: 40 patients. Total 114 patients. Expected time till completion of accrual: June/09

Correlative Statistics:

Neoadjuvant chemotherapy provides researchers with a unique and valuable opportunity for correlative studies to explore many of the most critical questions in chemotherapy and every effort will be made to fully utilize the valuable resources obtained during the study. Mutations in DNA by Bi-PAP, protein expression by immunohistochemistry and mass spectrometry, gene expression by RT-PCR quantification, and DNA polymorphisms and array cluster analysis all on a variety of source material will create a considerable database.

Standard descriptive methods will be used to summarize the baseline levels and the changes after treatment of the correlative assays. This will allow us to examine whether observed patterns are consistent with the hypothesized patterns. Patients who experience an objective response will be compared to those who did not – in terms of these correlates - and estimates of variation will be useful for planning further clinical research. In addition, these analytical measurements will be analyzed ensemble to seek correlational structure, and for use in exploratory models of response, survival, tumor characteristics, patient characteristics, and toxicity. Formal testing of these comparisons is not planned, and any resultant reports will discuss the multiple comparisons issue. The analyses of the correlates will be exploratory and descriptive and will be used to better understand the effect of the various treatment arms on the subjects.

Secondary objectives include quality of life comparisons among the treatment groups, and exploratory analysis of DNA, RNA, and proteomics profile of tumors and blood samples before, during, and after neoadjuvant therapy in order to identify prognostic, predictive, and target-identifying molecular markers for future validation studies.

Racial subgroups will be summarized. No differences in outcome due to race are anticipated. Exploratory tests for interaction of regimen and race will be done, if accrual warrants. No subgroup analyses (other than per stated stratification) are planned.

Quality of Life Comparisons:

The main quality of life tool will be version 4 of the FACT-Taxane questionnaire, although FACT-B version 4 data will also be collected. The QOL questionnaires will be administered at baseline, midway through the neoadjuvant therapy (3 months), at completion of neoadjuvant chemotherapy (6 months), and at nine months and at one year. The primary comparison will be to examine the change from baseline to completion of neoadjuvant chemotherapy in the FACT-Taxane QOL scale and to compare those changes between the two different neoadjuvant therapies, TAC and ACAC. Herceptin is not thought to influence Taxane related toxicities and therefore the HER-2+ patients will not be considered separately for the QOL endpoint. As a result, there will be 74 patients treated with ACAC and 37 with TAC. For each patient, the FACT-Taxane score difference from baseline to end of treatment will be recorded. A two-group comparison t-test will be used to evaluate the effect of neoadjuvant chemotherapy regimen. With these patient numbers, there is 83% power to

detect an effect size of 0.6 with a two-sided type I error of 5%. In case the patient stops the neoadjuvant chemotherapy prior to the 6 month QOL assessment, an attempt will be made to collect QOL at the time of drop out and last observation carried forward (LOCF) analysis will be used for missing data imputation if the reason for drop out was related to toxicity. Otherwise, that patient will be excluded from the QOL calculation. The FACT-B QOL data will also be reported and tabulated by neoadjuvant regimen according to the subscales in an exploratory fashion, and additional analysis of the other QOL time points will also be exploratory.

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The primary objective of the peripheral neuropathy aspect of this trial is to evaluate and compare the severity of neuropathy in the two arms of the study: TAC (group A) and ACAC (group B, without trastuzumab) by objective measures (nerve conduction, quantitative sensory tests, neuropathy composite score). Twenty subjects with stage II or IIIa disease from each group will be assessed prior to initiation of protocol chemotherapy (baseline), at completion of chemotherapy prior to surgery (18-20 weeks), and at 6 months. At each of these 3 time points in a single 60-90 minute appointment, subjects will undergo the following: a neurological examination focused on sensation, muscle weakness, and deep tendon reflexes; quantitative sensory tests of cold detection threshold (CDT) and vibration detection sensation threshold (VDT); and neurophysiological tests of peroneal nerve compound muscle action potential (CMAP) and sural nerve sensory nerve action potential (SNAP). Anticipated results are as follows: (1) change from baseline will be greater in group B than group A at the time point just after completion of chemotherapy; (2) change from baseline will be greater in group B than group A at six months and both groups will improve after the completion of chemotherapy; (3) nerve damage will be detected in both groups at both time points after chemotherapy (i.e., compared to baseline, there will be a decrease in peroneal CMAP amplitude, decrease in sural SNAP amplitude, increase in CDT, increase in VDT, and increase in neuropathy composite score).

A second objective is to correlate objective measures of neuropathy (nerve conduction, quantitative sensory tests, and neuropathy composite score) with questionnaire/quality of life measures. In the 40 subjects, 5 objective measures (peroneal CMAP amplitude, sural SNAP amplitude, CDT, VDT, neuropathy composite score) repeated at 3 time points (baseline, 18-20weeks, 6 months) will be compared to quality of life measurements at these time points. The hypothesis is that objective measurements of neuropathy will correlate negatively with quality of life measurements.

The statistical power calculation is based on the first consideration that group B will show more nerve damage, using the composite score, than group A. A sample size of 20 in each group will have 80% power to detect a probability of 0.727 that the nerve damage is greater in a group B subject than in group A subject, using a Wilcoxon (Mann-Whitney) rank-sum test with 0.05 one-sided significance level. Secondary considerations will be evaluated in an exploratory manner, and no correction for multiple comparisons will be employed.

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Barriers to treatment:

Patients with evidence of clinical, or image-documented (mammography, ultrasound, etc) abnormality of the breast with subsequent proof of malignancy will be interviewed to identify reasons for delay of treatment if 3 or more months passed by between the onset of symptoms and initiation of enrollment on this treatment study. We estimate that approximately 15-20%

of patients with locally advanced stage III disease may qualify for the interviewing process (See appendix C). An exploratory analysis as to the cause of delay will be carried out and may serve as the starting point for a formal prospective trial.

Microarray Analysis:

The cRNA product will be fragmented and hybridized to Affymetrix U133 2.0 arrays. These arrays contain greater than 54,000 probe sets allowing analysis of the expression level of more than 47,000 transcripts and variants, which include approximately 38,500 well-characterized human genes. Hybridization and data acquisition will be performed at the City of Hope Microarray Core Facility. Experimental information and data acquisition will be performed using Affymetrix GeneChip Operating Software (GCOS). Gene expression values will be extracted from probe level data using Plier algorithm implemented in Affymetrix GeneChip RNA Expression Analysis Software (GREX). Prior to statistical analysis, systemic variations, associated with procedures such as IVT labeling, hybridization and scanning will be adjusted to a comparable level between arrays within the study. The background correction, normalization, and other preprocessing steps will be performed in R using the affy library (150,151).

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To assess the validity and reproducibility of experiments we will process replicate samples independently for a subset of subjects and determine the correlation between gene expression levels on replicate arrays. This will include M vs. A plots among replicates and other diagnostics for faulty chip data. In addition we will determine whether replicate samples cluster tightly on hierarchical clustering analysis. We will also use Q-RT-PCR to independently determine the level of gene expression for selected genes in a subset of patients and will determine the correlation with microarray results. In addition we will correlate the relative changes in expression as determined by RT-PCR and microarrays.

The formal statistical analysis will follow the regression framework developed by Zhao et al. (152). Let $Y_l=(Y_{1l}, Y_{2l}, \ldots, Y_{Jl})$ denote the gene expression profiles on array l, where Y_{jl} denotes the expression of the jth gene on array l ($j=1,2\ldots J$; $l=1,2\ldots,L$), and J is total number of genes on an array and L is total number of samples. Let $d_l=0$ or 1 denote whether a response is achieved. We are mainly interested in analyzing gene expression profiles at baseline to find the genes whose profiles are different between the responding patients and non-responding patients. To adjust other unmatched but important clinical variables (such as medical history or medications), we include them as covariates in the following regression model:

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$y_{jl}=\delta_l+\lambda_l(\alpha_j+\chi_j d_l+\beta_j' x_l)+\xi_{jl}$, where (δ_l, λ_l) are additive and multiplicative heterogeneity factors that need to be adjusted for as part of the normalization, x_l is a vector of clinical covariates to be adjusted for in the analysis, $(\alpha_j, \chi_j, \beta_j')$ are the regression coefficients. In particular, χ_j quantifies the difference of the jth gene between responding and non-responding patients. To avoid making a distributional assumption about ξ_{jl}, we will calculate the robust standard errors (SE) using estimating equations theory (153). We will carry out statistical inference by hypothesis testing for each gene. The null hypothesis (H_o) is that there is no differential expression for jth gene between the responding patients and non-responding patients. Statistically, H_o: $\chi_j=0$, where $j=1,2\ldots J$. We will test the null hypothesis by calculating the Z-score: $Z_j=\chi_j/SE_j$, where SE_j is the estimated standard error. The analysis results in a vector of Z-scores of length J. The greater the Z-score is, the more likely this gene differentially expresses. After we set up a threshold for type I error rate (e.g. 0.05), we will select the genes that are differentially expressed between t-MDS and non t-MDS

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samples. Recognizing the concern with multiple comparisons, we will adapt a modified Bonferroni's correction proposed by Hochberg (154).

The statistical power of detecting differentially expressed genes is defined as the percentage of truly differentially expressed genes being discovered using statistical method among all the genes spotted on the array. The power of detecting the differentially expressed genes between the responsive and non-responsive patients can potentially be affected by multiple factors such as the variance of gene expression levels, the differences of expression levels of positive genes between the two groups, the proportion of truly differentially expressed genes among all genes under investigation, the correlations of expression levels among genes, and

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the tolerance for false discovery rate (FDR)(155). The Affymetrix technology used in this project is far superior to its earlier technology with much reduced technical variation. We will include additional longitudinal observations, and hence data become even more richer, resulting higher power. The above primary endpoint calculations were based on the assumed Phase II response rate, but we expect to be able to detect consistent and reproducible genes responsible for the genetic etiology for response among the various patient subgroups.

13.0 REGISTRATION GUIDELINE

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. 62468.

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

14.1 Data Collection Forms and Submission Schedule

All data will be collected using COH Biostatistics Information Tracking System (BITS) data collection forms. Copies of the completed forms will be submitted to City of Hope Department of Biostatistics for entry and stored in a secure location. The original data collection forms will reside at the originating institution in secure location.

14.1.1 The data manager will complete the Eligibility Checklist Worksheet at the time of registration:

14.1.2 Within two weeks of registration, the data manager will complete the On-Study Form (Form OS).

14.1.3 Within four weeks of completion of each course of treatment, the data manager must complete the following:

14.1.3.1 Treatment and Adverse Event Form

14.1.3.2 Supplemental Data Form (if applicable)

14.1.3.3 Flow Sheets (These are to be submitted along with each treatment form)

14.1.4 Each time a patient is evaluated for response and/or new follow-up information is obtained the data manager will complete the Response/Off-Study/Follow-Up Form.

14.1.5 Protocol deviations should be reported in a timely manner. (See http://www.infosci.coh.org/prot_office/cprmc/pd.asp)

14.2 An abbreviated Clinical Data Update System (CDUS) report will be generated quarterly.

14.3 Tracking log for tissue/blood samples /assignment of identifiers, log in/out for procured tissue specimens will be maintained by the individual processing and receiving centers (Anatomic Pathology, Pharmacology Core and Yen laboratory, and the receiving Sommer, Lee, Yen, Synold laboratories, and other end users, as material for processing becomes available (microarray, immunohistochemical analysis) with the master tracking sheet maintained in the Department of Biostatistics and shared with Informatics.

14.4 Analysis will be carried out in a joint effort between the Departments of Biostatistics and Informatics.

15.0 MINORITIES AND WOMEN STATEMENT

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 Update								
		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%)

Accrual Goal for Women and Minorities on this Study								
		By Sex		By Ethnicity				
Site	Accrual Goal	Female	Male	White	Hispanic	Black	Asian	Other
Breast	90	90 (100%)	0 (0%)	54 (60%)	15 (17%)	6 (6.5%)	9 (10%)	6 (6.5%)

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 All patients will have signed an informed consent for participation in research activities in accord with all institutional, NCI and Federal regulations, and will have been given a copy of the Experimental Subject's Bill of Rights.

17.0 PATHOLOGY REVIEW

All patients will have malignancy confirmed by review of their biopsy specimens by the Division of Pathology of the City of Hope National Medical Center,

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APPENDIX A

Common Terminology Criteria for Adverse Events (CTCAE) version 3

CTEP home page http://ctep.cancer.gov/reporting/ctc.html

APPENDIX B

QUALITY OF LIFE QUESTIONNAIRES

FACT-Taxane (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you <u>during the past 7 days.</u>**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please check this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-Taxane (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-Taxane (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
NTX 1	I have numbness or tingling in my hands	0	1	2	3	4
NTX 2	I have numbness or tingling in my feet	0	1	2	3	4
NTX 3	I feel discomfort in my hands	0	1	2	3	4
NTX 4	I feel discomfort in my feet	0	1	2	3	4
NTX 5	I have joint pain or muscle cramps	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
NTX 6	I have trouble hearing	0	1	2	3	4
NTX 7	I get a ringing or buzzing in my ears	0	1	2	3	4
NTX 8	I have trouble buttoning buttons	0	1	2	3	4
NTX 9	I have trouble feeling the shape of small objects when they are in my hand	0	1	2	3	4
An6	I have trouble walking	0	1	2	3	4
Tax1	I feel bloated	0	1	2	3	4
Tax2	My hands are swollen	0	1	2	3	4
Tax3	My legs or feet are swollen	0	1	2	3	4
Tax4	I have pain in my fingertips	0	1	2	3	4
Tax5	I am bothered by the way my hands or nails look	0	1	2	3	4

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please check this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-B (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-B (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
B1	I have been short of breath	0	1	2	3	4
B2	I am self-conscious about the way I dress	0	1	2	3	4
B3	One or both of my arms are swollen or tender	0	1	2	3	4
B4	I feel sexually attractive	0	1	2	3	4
B5	I am bothered by hair loss	0	1	2	3	4
B6	I worry that other members of my family might someday get the same illness I have	0	1	2	3	4
B7	I worry about the effect of stress on my illness	0	1	2	3	4
B8	I am bothered by a change in weight	0	1	2	3	4
B9	I am able to feel like a woman	0	1	2	3	4
P2	I have certain parts of my body where I experience significant pain	0	1	2	3	4

APPENDIX C

Interview Guide for Barriers of Care
in Locally Advanced Breast Cancer

Delays in Care in Locally Advanced Breast Cancer
Interview Guide **

We are interested in knowing the issues involved in women who experienced a delay in seeking a diagnosis or care after experiencing breast symptoms.

1. Can you tell me about the first time from when you had symptoms of your breast until when you first got medical care by an oncologist?

2. Were there are are there fears or beliefs that might have influenced your decisions in seeking treatment by an oncologist?

3. Were there delays in getting appropriate diagnostic studies and treatment related to your health care providers?

4. Are there any financial issues (health plans, insurance) that delayed getting treatment by an oncologist?

** A meeting was convened with City of Hope Staff, George Somlo, M.D., Betty Ferrell, PhD, Gloria Juarez, RN, PhD, Virginia Sun, RN, MSN, Connie Munoz, MSW, Nellie Garcia, MSW and Annette Mercurio, to discuss barriers to women seeking diagnoses and care after experiencing breast symptoms. Questions for the interview guide were derived based on the clinical experiences of the researchers and review of the literature.

APPENDIX D

Neuropathic Pain Symptom Inventory

Date:

First name: Last name:

Sex:

Age:

You are suffering from pain due to injury or disease of the nervous system. This pain may be of several types. You may have spontaneous pain, i.e. pain in the absence of any stimulation, which may be long-lasting or occur as brief attacks. You may also have pain provoked or increased by brushing, pressure, or contact with cold in the painful area. You may feel one or several types of pain. This questionnaire has been developed to help your doctor to better evaluate and treat various types of pain you feel.

We wish to know if you feel spontaneous pain, that is pain without any stimulation. For each of the following questions, please select the number that best describes your *average spontaneous pain severity during the past 24 h.* Select the number 0 if you have not felt such pain (circle one number only).

Q1. Does your pain feel like burning?

No burning	0	1	2	3	4	5	6	7	8	9	10	Worst Burning imaginable

Q2. Does your pain feel like squeezing?

No squeezing	0	1	2	3	4	5	6	7	8	9	10	Worst squeezing imaginable

Q3. Does your pain feel like pressure?

No pressure	0	1	2	3	4	5	6	7	8	9	10	Worst pressure imaginable

Q4. *During the past 24 h*, your spontaneous pain has been present:
Select the response that best describes your case.

Permanently	/__/
Between 8 and 12 h	/__/
Between 4 and 7 h	/__/
Between 1 and 3 h	/__/
Less than 1 h	/__/

We wish to know if you have brief attacks of pain. For each of the following questions, please select the number that best describes the *average severity of your painful attacks during the past 24 h.* Select the number 0 if you have not felt such pain (circle one number only).

Q5. Does your pain feel like electric shocks?

No electric shocks	0 1 2 3 4 5 6 7 8 9 10	Worst electric shocks imaginable

Q6. Does your pain feel like stabbing?

No stabbing	0 1 2 3 4 5 6 7 8 9 10	Worst stabbing imaginable

Q7. *During the past 24 h,* how many of these pain attacks have you had? Select the response that best describes you case

More that 20	/__/
Between 11 and 20	/__/
Between 6 and 10	/__/
Between 1 and 5	/__/
No pain attack	/__/

We wish to know if you feel pain provoked or increased by brushing, pressure, contact with cold or warmth on the painful area. For each of the following questions, please select the number that best describes the *average severity of your provoked pain during the past 24 h.* Select the number 0 if you have not felt such pain (circle one number only).

Q8. Is your pain provoked or increased by brushing on the painful area?

No pain	0 1 2 3 4 5 6 7 8 9 10	Worst pain Imaginable

Q9. Is your pain provoked or increased by pressure on the painful area?

No pain	0 1 2 3 4 5 6 7 8 9 10	Worst pain imaginable

Q10. Is your pain provoked or increased by contact with something cold on the painful area?

No pain	**0 1 2 3 4 5 6 7 8 9 10**	**Worst pain Imaginable**

We wish to know if your feel abnormal sensations *in the painful area.* For each of the following questions, please select the number that best describes the *average severity of your abnormal sensations during the past 24 h.* Select the number 0 if you have not felt such sensation (circle one number only).

Q11. Do you feel pins and needles?

No pins and needles	**0 1 2 3 4 5 6 7 8 9 10**	**Worst pins and needles imaginable**

Q12. Do you feel tingling?

No tingling	**0 1 2 3 4 5 6 7 8 9 10**	**Worst tingling imaginable.**

Results

	Total intensity score	Subscores	
		Burning (superficial) Spontaneous pain:	
1.	**Q1 =**	**Q1 =**	**/10**
		Pressing (deep) spontaneous pain:	
2.	**(Q2 + Q3) =**	**(Q2 + Q3)/2 =**	**/10**
		Paroxysmal pain:	
3.	**(Q8 + Q9 + Q10) =**	**(Q5 + Q6)/2=**	**/10**
		Evoked pain:	
4.	**(Q8 + Q9 + Q10) =**	**(Q8+Q9+Q10)/3=**	**/10**
		Paresthesia/dysesthesia:	
5.	**(Q11 + Q12) =**	**(Q11 + Q12)/2 =**	**/10**

(1 + 2 + 3 + 4 + 5) = /100

APPENDIX E

ABRAXIS SAE REPORTING FORM

Abraxis Oncology	CONFIDENTIAL	Protocol ABX_____
Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

Fax form to: SAE Coordinator at 919-433-8402
***and* Abraxis Oncology at 908-393-8304**

Email: SAE-REPORTING@abraxisbio.com *and*
AbraxisMedAffairs@abraxisbio.com
Tel: 919-433-8515 or 919-606-1832 (24 hours)

1. Report Status: ☐ Initial ☐ Follow-up # ☐☐☐

2. Date of this Report: ☐☐ DD ☐☐☐ MMM ☐☐☐☐ YYYY

3. SAE occurred in: ☐ Cycles 1-4 ☐ Cycles 5-8 ☐ Cycles 9-18

4. Date of Birth
☐☐ DD ☐☐☐ MMM ☐☐☐☐ YYYY

5. Gender
☐ Male
☒ Female

6. Height and Weight
☐☐☐.☐ cm ☐☐☐.☐ kg

7. Race

☐ Asian
☐ Black, of African Heritage
☐ Native Hawaiian or Other Pacific Islander
☐ North American Indian or Alaska Native
☐ White, Non-Hispanic and Non-Latino
☐ White, Hispanic or Latino
☐ Other, specify: _____

8. Reason for reporting (Check all that apply.)

☐ Death* (* Please attach death certificate)
☐ Life-threatening (Immediately Life Threatening)
☐ Required/prolonged hospitalization on
☐☐ DD ☐☐☐ MMM ☐☐☐☐ YYYY
☐ Persistent or significant disability/incapacity
☐ Congenital anomaly/birth defect
☐ Important medical event, *
Specify: _____

** An important medical event is an event that requires medical or surgical intervention to prevent one of the other outcomes listed above*

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

9. Diagnosis/Adverse Event Term Is this event cardiac in nature? Yes ☐ No ☐

10. Onset Date ☐☐ ☐☐☐ ☐☐☐☐ (DD MMM YYYY)

11. Resolution Date ☐☐ ☐☐☐ ☐☐☐☐ (DD MMM YYYY)

12. Outcome (Check one.)

☐ Resolved ☐ Ongoing ☐ This event ongoing at the time of death

☐ Resolved with sequelae ☐ Death (Check only for event that was primary cause of death.)

IN CASE OF DEATH

13. Date of Death ☐☐ ☐☐☐ ☐☐☐☐ (DD MMM YYYY)

14. Was an autopsy performed?

☐ Yes (Provide findings.)

☐ No

15. Is death certificate attached?

☐ Yes

☐ No

☐ Not Available

16. Describe Event: *(Please include presenting signs and symptoms, relevant tests, procedures, treatment and dates)*

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

17. Relevant Tests/Lab Data
If this event is cardiac in nature, please provide this patient's baseline and most recent echocardiogram results (including dates).

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

18. Treatment of the Event

Name of Medication (Generic)	Total Daily Dose	Route	Start Date (DD/MMM/YYYY)	Stop Date (DD/MMM/YYYY)	Indication

Procedures and/or Surgeries to treat event:

19. Relevant concomitant medications taken within 2 weeks prior to onset of the event (including procedural medications)

Name of Medication (Generic)	Total Daily Dose	Route	Start Date (DD/MMM/YYYY)	Stop Date (DD/MMM/YYYY)	Indication

20. Relevant Medical History

_____ _____ _____

_____ _____ _____

_____ _____ _____

Note: Attach additional sheet, if extra space is needed,

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

Abraxane (ABI-007)

21a. Relationship to Abraxane (Check one.)

☐ Not Related ☐ Possibly Related ☐ Probably Related ☐ Definitely Related ☐ Not Applicable

22a. Investigator: Comments/Rationale regarding relationship

23a. Abraxane Lot #:

24a. Abraxane Expiration Date :

25a. Date of First Dose Abraxane

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

26a. Abraxane Initial Dose (Calculated Total Dose) (mg)

☐☐☐

27a. Total Number Doses of Abraxane Received

☐☐

28a. Date of Last Dose Abraxane Prior to SAE

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

29a. Last Dose Abraxane Prior to SAE (mg)

☐☐☐

Action Taken Abraxane

30a. Changes to Abraxane due to <u>THIS</u> event (Check all that apply):

☐ None

☐ Dose reduced on __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY) to _____ mg/m^2

☐ Dose delayed on __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY) and Restarted on __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)

☐ Dose interrupted on __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)

☐ Permanently Discontinued on __ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)

31a. Did event abate after stopping or reducing Abraxane?

☐ Yes ☐ No ☐ Not Applicable

32a. If interrupted, did event reappear after reintroduction of Abraxane?

☐ Yes ☐ No ☐ Not Applicable

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

Other Agent in Combination (*specify*):_____

(Complete sections 21b-32b in reference to the specified agent noted)

21b. Relationship to agent (Check one.)

☐ Not Related ☐ Possibly Related ☐ Probably Related ☐ Definitely Related ☐ Not Applicable

22b. Investigator: Comments/Rationale regarding relationship to agent

23b. Lot #:

24b. Expiration Date :

25b. Date of First Dose

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

26b. Initial Dose (Calculated Total Dose)

☐☐☐

27b. Total Number Doses Received

☐☐

28b. Date of Last Dose Prior to SAE

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

29b. Last Dose Prior to SAE (mg)

☐☐☐

Action Taken

30b. Changes to agent due to THIS event (Check all that apply):

☐ None

☐ Dose reduced on __ __ / __ __ __ / __ __ __ __ to _____ mg/kg
DD MMM YYYY

☐ Dose delayed on __ __ / __ __ __ / __ __ __ __ and Restarted on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY DD MMM YYYY

☐ Dose interrupted on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY

☐ Permanently Discontinued on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY

31b. Did event abate after stopping or reducing the agent?

☐ Yes ☐ No ☐ Not Applicable

32b. If interrupted, did event reappear after reintroduction of agent?

☐ Yes ☐ No ☐ Not Applicable

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

Other Agent in Combination (*specify*):_____

(Complete sections 21c-32c in reference to the specified agent noted)

21c. Relationship to agent (Check one.)

☐ Not Related ☐ Possibly Related ☐ Probably Related ☐ Definitely Related ☐ Not Applicable

22c. Investigator: Comments/Rationale regarding relationship to agent

23c. Lot #:

24c. Expiration Date :

25c. Date of First Dose

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

26c. Initial Dose (Calculated Total Dose)

☐☐☐

27c. Total Number Doses Received

☐☐

28c. Date of Last Dose Prior to SAE

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

29c. Last Dose Prior to SAE (mg)

☐☐☐

Action Taken

30c. Changes to agent due to THIS event (Check all that apply):

☐ None

☐ Dose reduced on __ __ / __ __ __ / __ __ __ __ to _____ mg/kg
DD MMM YYYY

☐ Dose delayed on __ __ / __ __ __ / __ __ __ __ and Restarted on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY DD MMM YYYY

☐ Dose interrupted on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY

☐ Permanently Discontinued on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY

31c. Did event abate after stopping or reducing the agent?

☐ Yes ☐ No ☐ Not Applicable

32c. If interrupted, did event reappear after reintroduction of agent?

☐ Yes ☐ No ☐ Not Applicable

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

Other Agent in Combination (*specify*):_____

(Complete sections 21d-32d in reference to the specified agent noted)

21d. Relationship to agent (Check one.)

☐ Not Related ☐ Possibly Related ☐ Probably Related ☐ Definitely Related ☐ Not Applicable

22d. Investigator: Comments/Rationale regarding relationship to agent

23d. Lot #:

24d. Expiration Date :

25d. Date of First Dose

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

26d. Initial Dose (Calculated Total Dose)

☐☐☐

27d. Total Number Doses Received

☐☐

28d. Date of Last Dose Prior to SAE

☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

29d. Last Dose Prior to SAE (mg)

☐☐☐

Action Taken

30d. Changes to agent due to THIS event (Check all that apply):

☐ None

☐ Dose reduced on __ __ / __ __ __ / __ __ __ __ to _____ mg/kg
DD MMM YYYY

☐ Dose delayed on __ __ / __ __ __ / __ __ __ __ and Restarted on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY DD MMM YYYY

☐ Dose interrupted on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY

☐ Permanently Discontinued on __ __ / __ __ __ / __ __ __ __
DD MMM YYYY

31d. Did event abate after stopping or reducing the agent?

☐ Yes ☐ No ☐ Not Applicable

32d. If interrupted, did event reappear after reintroduction of agent?

☐ Yes ☐ No ☐ Not Applicable

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report

Reporter Information

33. Reporter's Name: **Address:**	**34. Health Professional** ☐ Yes ☐ No	**35. Phone Number**

36. (Print) Investigator's Name:

37. (Print) Investigator's Address:

38. Investigator's Signature: _____

39. Date: ☐☐ ☐☐☐ ☐☐☐☐
DD MMM YYYY

Serious Adverse Event Report

Investigator Name	Patient Number	Patient Initials
_____	☐☐☐☐	☐☐☐

Serious Adverse Event Report