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CANCER AND LEUKEMIA GROUP B

CALGB 40101/CTSU 40101

CYCLOPHOSPHAMIDE AND DOXORUBICIN (CA X 4 CYCLES) VERSUS PACLITAXEL (4 CYCLES) AS ADJUVANT THERAPY FOR BREAST CANCER IN WOMEN WITH 0-3 POSITIVE AXILLARY LYMPH NODES: A PHASE III RANDOMIZED STUDY

All agents used in this trial are commercially available.

Optional Companion Study: 70301

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This study is supported by the NCI Cancer Trials Support Unit (CTSU).
Institutions not aligned with CALGB will participate through the CTSU mechanism as outlined below and detailed in the CTSU logistical appendix.

- The **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org
- Send completed **site registration documents** to the CTSU Regulatory Office. Refer to the CTSU logistical appendix for specific instructions and documents to be submitted.
- **Patient enrollments** will be conducted by the CTSU. Refer to the CTSU logistical appendix for specific instructions and forms to be submitted.
- Data management will be performed by the CALGB. **Case report forms** (with the exception of patient enrollment forms), **clinical reports, and transmittals** must be sent to CALGB unless otherwise directed by the protocol. Do not send study data or case report forms to the CTSU Data Operations.
- Data query and delinquency reports will be sent directly to the enrolling site by CALGB. (generally via email but may be sent via fax or postal mail). Please send query responses and delinquent data to CALGB and do not copy the CTSU Data Operations. Query responses should be sent to CALGB via postal mail (no transmittal form needs to accompany response). Each site should have a designated CTSU Administrator and Data Administrator and must keep their CTEP AMS account contact information current. This will ensure timely communication between the clinical site and the CALGB Statistical Center.

The pharmacogenetic component of this study is conducted as part of the NIH Pharmacogenetics Research Network, which is funded through a separate U01 mechanism (see http://www.nigms.nih.gov/phamacogentics/research_net.html for details).

CYCLOPHOSPHAMIDE AND DOXORUBICIN (CA X 4 CYCLES) VERSUS PACLITAXEL (4 CYCLES) AS ADJUVANT THERAPY FOR BREAST CANCER IN WOMEN WITH 0-3 POSITIVE AXILLARY LYMPH NODES: A PHASE III RANDOMIZED STUDY

All agents used in this trial are commercially available.

Patient Eligibility

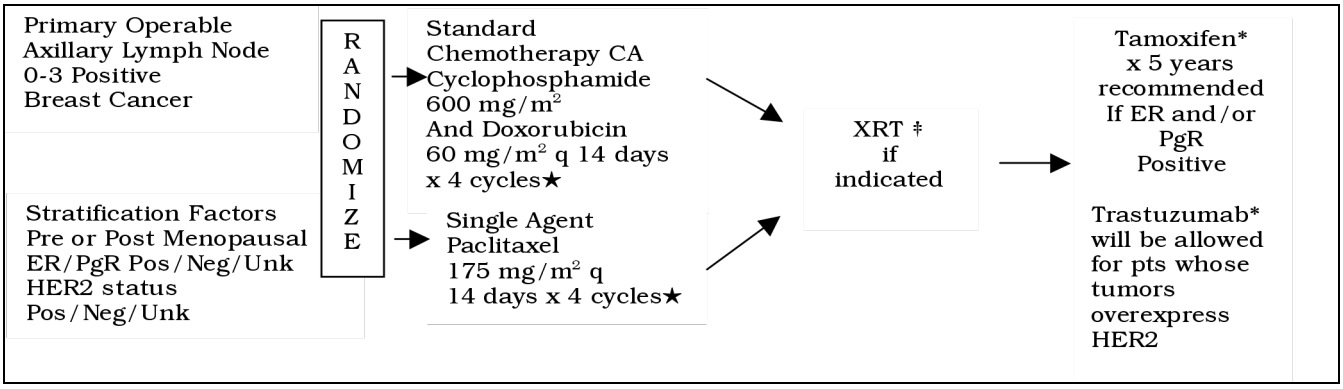
Histologically confirmed invasive carcinoma of the female breast with 0-3 positive axillary lymph nodes.
Node negative patients should have sufficiently "high risk" node-negative breast cancer to warrant chemotherapy (See Sections 5.1.1-5.1.2).
ER/PgR positive, negative or unknown.
HER2 positive, negative or unknown. It is recommended that patients with HER2 positive disease receive trastuzumab (See Sections 5.1.4 and 8.4)
Negative tumor margins for invasive cancer and DCIS; LCIS acceptable at the margin (See Section 5.1.5).
Multicentric breast cancer allowed if resected w/ neg margins and axillary nodes negative (See Sections 5.1.2 and 5.1.6).
Pts. must be registered $\leq$ 84 days from MRM or lumpectomy.
No previous trastuzumab, chemo or hormonal therapy except for tamoxifen (See Section 5.10).
No locally advanced or inflammatory breast cancer or involvement of dermal lymphatics (See Section 5.3).
Bilateral, synchronous breast cancers are eligible (See Section 5.4).
Disease free > 5 years for prior malignancies (See Section 5.5)
$\geq$ 18 years of age.
CTC Performance Status 0-1.
Non-pregnant and not nursing.
No concomitant exogenous hormone therapy (See Section 5.9).
Tamoxifen or another selective estrogen receptor modulator (SERM) for breast cancer prevention is allowed (See Section 5.10).
Adequate organ function; no active CHF; no MI < 6 months from the time of registration.
Patients may be enrolled in adjuvant bisphosphonate or adjuvant hormonal studies concurrently with protocol therapy (See Section 5.13)

Required Laboratory Values

ANC $\geq$1000/mm^3
Platelet Count $\geq$ 100,000/mm^3

Creatinine $\leq$ 2.0 mg/dl
Bilirubin $\leq$ 1.5 x ULN

Schema



★ Administration of filgrastim, sargramostim or pegfilgrastim is recommended (See Sections 8.2 and 8.3).

*Aromatase inhibitors may be substituted for tamoxifen in postmenopausal women. The use of tamoxifen or an aromatase inhibitor should be documented on the CALGB 40101 Follow-Up Form C-929. Trastuzumab will be allowed for patients whose tumors overexpress HER2 based on IHC 3+ staining, or FISH amplification receive trastuzumab as outlined in Section 8.4.

ǂ Patients who have undergone lumpectomy must receive XRT according to local institutional standards (see Section 8.7). Patients who have undergone mastectomy may receive chest wall and nodal XRT (See Section 8.7).

Patients should be followed for the occurrence of both first local and distant disease progression. After the occurrence of local progression, follow the patient for distant progression, secondary malignancy and survival with appropriate documentation. In the event of distant progression first, continue to follow the patient for local recurrence, secondary malignancy and survival. After both local and distant progression, follow the patient for secondary malignancy and survival. Follow all patients registered to this study, including those who do not receive any protocol treatment, for first local and first distant progression and survival for 15 years from study entry or until death, whichever occurs first (see Section 13.1).

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1.0 INTRODUCTION

1.1 Rationale

Chemotherapy has been shown to improve disease-free and overall survival for patients with primary breast cancer and negative axillary nodes [1, 2]. Both CMF (cyclophosphamide, methotrexate, 5-FU) and CA (cyclophosphamide, doxorubicin) are considered standard adjuvant therapies for these patients, though analyses of multiple chemotherapy trials suggests there may be a slight advantage to anthracycline-containing adjuvant regimens when compared to CMF. Though chemotherapy improves survival, many patients will still have a recurrence of their breast cancer in the form of distant metastatic disease, and therefore treatment regimens with improved efficacy and better toxicity profiles need to be developed. These goals could potentially be accomplished if we could better determine ideal duration of therapy as well as establish weekly taxane therapy as an equally efficacious regimen to which biological therapies could be added in the future.

Paclitaxel is an active drug in breast cancer, and has been suggested to have activity in the adjuvant setting in CALGB 9344 [3]. In addition, it has been shown to be effective, when compared to standard combination chemotherapy in both the neoadjuvant and metastatic settings [4, 5]. It is possible therefore, that paclitaxel will be equally as effective as CA in the adjuvant setting. If weekly paclitaxel was found to be equally efficacious, with possible anti-angiogenic effects (metronomic therapy), it might serve as a basis to which new biological agents might be added which would further improve the survival rates for patients with primary breast cancer. For patients with metastatic disease and tumors which overexpress HER2/neu, the addition of traztuzumab to paclitaxel has improved both time to tumor progression and overall survival. This concept is also currently being tested by the Intergroup in the adjuvant setting. Newer biological agents may prove equally or more effective when combined with weekly taxane therapy.

The optimal duration of adjuvant therapy is not defined. Though four cycles of CA has become a standard for patients with high-risk node-negative disease, it is possible that longer therapy will be more beneficial. NSABP B-15 has shown that 6 months of CMF is equally efficacious as 4 cycles of CA. However, the National Cancer Institute of Canada trial showed superiority of 6 cycles of CEF (cyclophosphamide, epirubicin, 5-FU) compared to 6 months of CMF [6, 7]. In addition, the USA Intergroup trial for patients with node-negative breast cancer showed superiority for 6 cycles of CAF (cyclophosphamide, doxorubicin, 5-FU) compared to CMF [8]. This raises the question of whether anthracycline-containing regimens of longer duration will be superior to 4 cycles of CA.

In addition, reduction in toxicity, while maintaining efficacy should also be a goal of adjuvant trials. The development of equally or more effective therapies with reduced toxicity will likely improve the quality of life of these patients, and it is possible that single-agent taxanes will have an improved toxicity profile when compared with CA.

Update #8: Closure of 6-Cycle Arms for CA and Paclitaxel

After the first 3,000 patients were entered on the trial, a decision was made to close the 6-cycle arms for both cyclophosphamide + doxorubicin (CA) and paclitaxel and to concentrate the study on the testing of equivalence between CA and paclitaxel for 4 cycles. This decision was based on the belief that the most important question in the study is the comparison of AC and paclitaxel, and the strong sense that accrual will improve dramatically if the two six cycle arms are eliminated from the randomization.

1.2 Paclitaxel as Adjuvant Therapy in Breast Cancer

Recent results from CALGB 9344 have suggested that the addition of paclitaxel to CA in patients with positive axillary nodes, improves both disease-free and overall survival, suggesting that paclitaxel is active in the adjuvant setting [3]. The benefit of the addition of paclitaxel was greatest in patients with ER negative tumors. NSABP B-28, which has been presented only in preliminary fashion at the NCI Consensus Conference in November 2000, suggested no advantage to the addition of paclitaxel to CA, though there was a numerical advantage in patients who did not receive tamoxifen [9]. At present, the utility of paclitaxel in this setting remains uncertain.

Docetaxel has been used in the adjuvant setting in a recent Intergroup trial for patients with 0-3 positive axillary nodes. In this trial CA x 4 was compared with AT (doxorubicin/docetaxel) x 4. The trial has met the accrual goal, and is closed to accrual, however data are not yet available.

1.3 Paclitaxel as a Single Agent in the Treatment of Breast Cancer – Demonstration of Efficacy

Paclitaxel has been compared to FAC (5-FU, doxorubicin, cyclophosphamide) in the neo-adjuvant setting and found to be as effective in anti-tumor effect, based on clinical and pathologic response of primary breast tumor. Patients were randomized to receive, pre-operatively, either 4 cycles of FAC or paclitaxel. Response was then assessed both clinically, and pathologically at time of primary surgery. In this situation, paclitaxel was as effective as FAC, as shown in the following table [4].

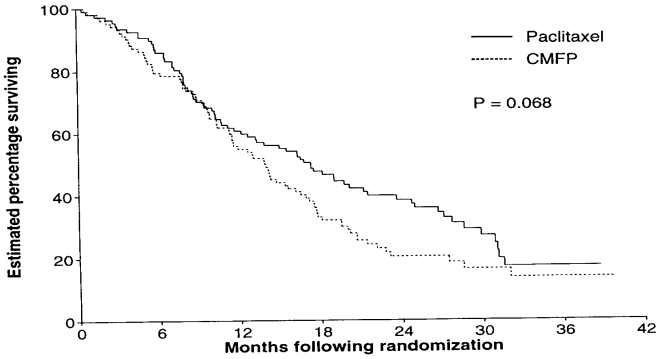
FAC v Paclitaxel as Neoadjuvant Therapy in pts with Operable Breast CA

	FAC	*Paclitaxel*
Clin CR	24%	27%
Clin PR	55%	53%
Path CR	18%	6%
Path in situ	5%	8%
Path min (<1cm)	12%	27%

Buzdar, MD Anderson, JCO 1999;17:3412

Since FAC is considered an acceptable standard adjuvant chemotherapy regimen, this raises the possibility of equivalent efficacy of single agent paclitaxel in the adjuvant setting.

When studied in the metastatic setting, paclitaxel is one of the most active agents currently available. When compared with CMF in a randomized trial as first line therapy for patients with metastatic disease, paclitaxel resulted in a similar response rate to CMFP, and significantly improved overall survival, as shown below [5].



In addition, many aspects of quality of life were better for patients receiving paclitaxel as compared to CMF. Paclitaxel resulted in significantly less bone marrow suppression, documented infection, mucositis, and nausea and vomiting. Alopecia, peripheral neuropathy, and myalgias and arthralgias were greater with paclitaxel. Overall quality of life appeared better for patients receiving paclitaxel.

Docetaxel has also been tested extensively in patients with metastatic breast cancer, and is clearly one of the more active agents in this setting [10]. It appears to be more active than doxorubicin, and is likely either equal or superior to paclitaxel in patients with metastatic disease [11-13]. Though many of the initial trials utilized q 3 week treatment, more recent trials have confirmed that weekly docetaxel is active and tolerable in patients with metastatic breast cancer [14].

Though it is difficult to extrapolate these data from the neo-adjuvant and metastatic settings to the adjuvant setting, the high level of anti-neoplastic activity for single-agent paclitaxel and docetaxel in advanced breast cancer suggests that there is a high likelihood that single agent paclitaxel will be at least as effective as adjuvant therapy for women with node-negative breast cancer. Though paclitaxel has not been directly compared with CA in the metastatic setting, as noted above, it has been favorably compared to FAC, which is a similar regimen. Also as noted, paclitaxel does compare favorably in the metastatic setting to CMF, and CMF has been shown to be equivalent to CA as adjuvant therapy in the NSABP study B-15 [6]. These data suggest there is a high likelihood that single agent paclitaxel will be at least as effective as CA when given as an adjuvant regimen.

1.4 Choice of Paclitaxel as the Investigational Treatment Arm

As noted above, paclitaxel has been shown to be at least as efficacious as standard adjuvant combination chemotherapy regimens in the neoadjuvant and metastatic settings. Paclitaxel was shown to be as efficacious as CA, it may be able to be administered with less toxicity [15-17]. Because of its biological properties, it might also serve as a better basis for the addition of biological therapies in the adjuvant setting.

1.5 Choice of Dose-Dense Scheduling in the Revision of this Protocol

Originally this protocol was designed using CA administered every 3 weeks, and weekly paclitaxel based on a number of studies, as noted previously. In December 2002, at the San Antonio Breast Cancer Symposium, initial results from CALGB 9741 were reported, showing an advantage to adjuvant chemotherapy administered every 2, rather than every 3 weeks. Not only was there an improvement in outcome, but toxicity was less in the treatment arms receiving therapy every 2 weeks, though these patients were required to use Neupogen. The dose-dense schedules,

administered every 2 weeks also resulted in a shorter treatment course with patients completing therapy more rapidly.

In addition, toxicity appeared very acceptable in patients treated on protocol CALGB 9741, with CA every 2 weeks, followed by paclitaxel administered every 2 weeks. Only 9% of patients had grade 4 neutropenia. In addition, 4% of patients had grade 3 sensory neuropathy, and 0% grade 4 sensory neuropathy. Nine percent of patients had grade 3 painful neuropathy, and < 1% grade 4 painful neuropathy. These toxicity rates compare very favorably with CA and paclitaxel administered every 3 weeks. In addition, no increase in leukemia or congestive heart failure was seen in patients treated with CA followed by paclitaxel every 2 weeks as compared to the q 3 week treatment groups.

Due to these findings of improved outcome, reduced toxicity, and reduced time on treatment, this protocol has been revised accordingly to use the dose-dense schedule. The concept of the original protocol remains unchanged. This will be a 2 x 2 factorial design testing equivalence of single agent paclitaxel with CA, and superiority of long versus short therapy. Duration symmetry will be maintained.

CA is now administered every 2 weeks, rather than every 3 weeks, with G-CSF support. Patients will receive either 4 or 6 cycles of therapy, now over 8 or 12 weeks, rather than 12 or 18 weeks as in the previous version of the protocol. For those patients randomized to the paclitaxel arm of the study, they will receive paclitaxel at a dose of 175 mg/m^2 every 2 weeks for 4 or 6 cycles of treatment, over 8 or 12 weeks, with G-CSF support. In this new treatment plan, duration of therapy and number of treatments are symmetric, and the comparisons of CA versus paclitaxel and short and long duration therapy will be straightforward.

Update #8: Revised Treatment Plan for CA and Paclitaxel

In protocol Update #8 the 6-cycle arms for CA and paclitaxel were closed to further accrual. Patients will be randomized with equal probability to receive either CA every 14 days x 4 cycles or paclitaxel every 14 days x 4 cycles.

1.6 Addition of Lymph Node Positive Patients to this Protocol

This protocol was originally designed to treat women with primary breast cancer and high-risk, node negative disease. At the North American Breast Intergroup Meeting, held at the December 2004 San Antonio Breast Cancer Symposium a consensus was reached that in addition to node-negative patients, patients with 1-3 positive lymph nodes would be appropriate to treat on CALGB 40101. As a result, CALGB 40101 has been modified to include patients with 1-3 positive axillary lymph nodes. The North American Breast Intergroup felt that including patients with 1-3 positive nodes was a reasonable alternative to current node-positive protocols for these low-risk, node-positive patients. A decision was also made not to alter the statistical section of the protocol at this time because, as of this date, the event rate for patients currently enrolled in CALGB 40101 has been lower than expected from previous node-negative studies. Inclusion of women with 1-3 positive nodes would be expected to have a slightly higher event rate than women with negative axillary nodes, and it is possible that the resulting event rate will more closely approximate that predicted in the statistics section of the protocol.

1.7 Use of Trastuzumab as a Component of this Protocol

At the American Society of Clinical Oncology (ASCO) meeting on May 16, 2005 the results of two North American cooperative group trials of adjuvant trastuzumab (Herceptin®), B-31 and N9831, showed a statistically significant improvement in both disease-free and overall survival when trastuzumab was added to chemotherapy for women with early stage HER2 positive breast cancer. At the same meeting the first interim analysis of the international HERA adjuvant trastuzumab trial was presented, and showed that the addition of trastuzumab following adjuvant

chemotherapy significantly increases disease-free survival for women with early-stage HER2 positive breast cancer.

The original 40101 study design did not prohibit the use of trastuzumab. As a result of these findings adjuvant trastuzumab will be allowed only in patients whose tumors are HER2 positive by either IHC 3+ staining or gene amplification by FISH. If there is concern about the accuracy of the test result, a confirmatory test is strongly advised. A fifty-two week course of trastuzumab will be permitted for all HER2 positive patients. For patients enrolled in the paclitaxel arms, trastuzumab may be administered concurrently with paclitaxel. The concurrent use of an anthracycline and trastuzumab is not acceptable.

1.8 Proposed Study Design

This study will be constructed as a 2x2 factorial study to compare paclitaxel as a single agent adjuvant therapy, with CA as standard treatment, for women with primary breast cancer with 0-3 positive lymph nodes. The study will be designed to test equivalence between these regimens, with disease-free survival as the primary end point.

In addition, the study will compare short- and long-term chemotherapy for both the CA and the paclitaxel arms of the study, comparing 8 weeks versus 12 weeks of therapy in each treatment arm.

In protocol Update #8 the study design was revised. This study was originally designed to compare short- and long-duration chemotherapy for both the CA and paclitaxel arms of the study, comparing 8 weeks and 12 weeks of therapy in each treatment arm. In protocol Update #8, after the first 3000 patients were accrued, the 12 week (6-cycle) arms for both the CA and paclitaxel arms were closed. Subsequent patients will be randomized with equal frequency to CA x 4 cycles or paclitaxel x 4 cycles.

2.0 OBJECTIVES

2.1 Primary Objectives

2.1.1 To determine the equivalence of paclitaxel given every two weeks with CA given every two weeks as adjuvant therapy for women with 0-3 positive axillary lymph nodes, for disease-free survival.

2.1.2 To determine if longer therapy, 12 weeks, is superior to shorter therapy, 8 weeks, of either CA or paclitaxel for disease-free survival for women with primary breast cancer with 0-3 positive axillary lymph nodes.

With the closure of the 6-cycle treatment arms the primary study objectives remain unchanged.

2.2 Secondary Objectives

2.2.1 To determine the equivalence of paclitaxel given every two weeks with CA given every two weeks, and the potential superiority of longer vs. shorter therapy, in relation to overall survival, local control (regardless of metastatic status) and time to distant metastases (regardless of local recurrence status).

2.2.2 To compare toxicities of short and long course CA and paclitaxel as adjuvant therapy for women with 0-3 positive axillary lymph node breast cancer.

2.2.3 To determine the effect of long and short course CA and paclitaxel on the induction of menopause for pre-menopausal patients.

2.2.4 To assess the discrepancy of myelosuppression among the common MDR1 haplotypes in the CA treatment arm.

2.2.5 To assess the effect of MDR1 haplotypes on DFS adjusted for treatment.

2.2.6 Exploratory analysis of the effect of CYP3A5, CYP2C8 and CYP2B6 polymorphisms on DFS and toxicity.

2.2.7 To identify genetic markers associated with the risk of developing neutropenia in adriamycin/cyclophosphamide-treated breast cancer patients.

2.2.8 To identify genetic markers associated with the risk of developing peripheral neuropathy in paclitaxel-treated breast cancer patients.

2.2.9 To identify genetic markers associated with differences in the efficacy of each chemotherapy regimen.

2.2.10 To examine genetic associations with other response and toxicity phenotypes that become apparent during future analysis of CALGB 40101 data. Potential examples include adriamycin/cyclophosphamide-induced menopause or cardiotoxicity.

2.2.11 To identify copy number variants associated with adriamycin/cyclophoshamide-induced neutropenia and paclitaxel-induced peripheral neuropathy.

3.0 INCLUSION OF WOMEN AND MINORITIES

Entry to this study is restricted to female patients. It will be opened to women of all ethnic backgrounds who meet eligibility criteria. Based on current data we do not expect differences in outcome by ethnic group, and therefore accrual targets will not be specific for ethnic groups.

4.0 ON-STUDY GUIDELINES

The following guidelines are to assist physicians in selecting patients for whom protocol therapy is safe and appropriate. Patients with surgically staged high-risk, primary, 0-3 node-positive breast cancer are eligible for this study. They must be deemed candidates for chemotherapy by their treating physician, and must meet the eligibility criteria in Section 5.0.

Physicians should recognize that the following may seriously increase the risk to the patient entering this protocol:

- Psychiatric illness which could compromise the ability of the patient to give informed consent.
- Medical conditions such as uncontrolled infection (including HIV), uncontrolled diabetes mellitus or cardiac disease which, in the opinion of the treating physician, would make this protocol unreasonably hazardous for the patient.
- Women of reproductive potential should agree to use an appropriate method of birth control throughout their participation in this study due to the teratogenic potential of the chemotherapy and radiation therapy utilized in this trial. Appropriate methods of birth control include double barrier method (diaphragm plus condom) or IUDs which do not contain hormones.

5.0 ELIGIBILITY CRITERIA

All questions regarding eligibility should be directed to the CALGB Study Chair. The following eligibility criteria may not be waived by the Study Chair, and will be reviewed at the time of audit. There will be no exceptions to eligibility without the authorization of the CALGB Group Chair or his designee.

5.1 Histologic Documentation: Patients must have histologically confirmed invasive carcinoma of the female breast, with 0-3 positive axillary lymph nodes (see Section 5.1.1.2).

5.1.1 Patients must have 0-3 positive axillary lymph nodes to be eligible for this study. Patients with node-negative breast cancer should have sufficiently “high risk”

disease to warrant chemotherapy. As general guidelines, node-negative patients with tumors of $\geq$ 1 cm or estrogen or progesterone receptor negative tumors of any size may be eligible. Ultimately though, the definition of "high risk" may be determined by the treating physician, and if the treating physician feels the patient warrants chemotherapy, the patient is eligible. For patients with 1-3 positive axillary nodes, the patient is eligible regardless of primary breast tumor characteristics, if in the opinion of the treating physician, chemotherapy is deemed potentially beneficial to the patient.

5.1.1.1 Definition of node-negative disease: If the patient has had a negative sentinel node biopsy, then no further axillary dissection is required, and the patient is determined to be node-negative. If an axillary dissection, without a sentinel node biopsy, is performed to determine nodal status, at least six axillary lymph nodes must be removed and analyzed and negative for the patient to be considered node-negative. Axillary nodes with single cells or tumor clusters $\leq$ 0.2 mm by either H&E or immunohistochemistry (IHC), will be considered node-negative. Lymph nodes positive for PCR with tumor cells/clusters $\leq$ 0.2 mm will be considered node-negative. Any axillary lymph node with tumor clusters > 0.2 mm will be considered positive (see below).

5.1.1.2 Definition of 1-3 positive axillary nodes: If the patient has a sentinel node biopsy and one of the sentinel nodes is positive, as defined by tumor clusters > 0.2 mm, an axillary dissection must be performed. A total of at least 6 axillary lymph nodes, including sentinel nodes plus the subsequent dissection, must be removed for the patient to be eligible. Of all the lymph nodes removed from both the sentinel node procedure and the axillary dissection, 1-3 must be positive for the patient to be eligible as a node-positive patient. If an axillary dissection is done without a sentinel node procedure, at least 6 lymph nodes must be removed and 1-3 nodes must be positive for the patient to be considered node-positive and eligible for this study.

5.1.2. Determination of involvement of axillary nodes with metastatic cancer will follow the revised TNM staging system [25]: axillary nodes with single cells with tumor clusters $\leq$ 0.2 mm, by either H&E or immunohistochemistry (IHC), will be considered negative axillary node. Lymph nodes positive for PCR with tumor cells/clusters < 0.2 mm will be considered negative axillary node. Any axillary lymph node with tumor clusters >0.2 mm will be considered to be positive.

5.1.3 Patients with estrogen-receptor and/or progesterone receptor negative, positive, or unknown tumors are eligible. ER and PgR assays should be performed by immunohistochemical methods according to the local institution's standard protocol.

5.1.4 Patients with HER2 positive, negative or unknown disease are eligible for this trial. Patients whose tumors are HER2 positive by either immunohistochemistry 3+ staining or demonstrate gene amplification by FISH may receive trastuzumab, as outlined in Section 8.4.

5.1.5 There must be negative tumor margins for invasive cancer and DCIS in the case of mastectomy or lumpectomy. LCIS is acceptable at the margin.

5.1.6 Patients with multi-centric breast cancer are eligible as long as all known disease is resected with negative margins, and have 0-3 positive axillary lymph nodes as defined in Section 5.1.2.

5.2 Prior Treatment:

5.2.1 Patients must be registered within 84 days of the last breast surgery. Patients must have undergone either modified radical mastectomy or lumpectomy. For patients undergoing sentinel node sampling or axillary dissection a simple mastectomy is acceptable. Lumpectomy patients must receive radiation therapy per Section 8.7. For patients treated with radiation therapy prior to chemotherapy, the patients should be registered on this study after the conclusion of radiation, with chemotherapy administration beginning within 7 days of registration.

All primary breast and axillary node surgery must be completed prior to enrollment on study.

5.2.2 No previous trastuzumab, chemotherapy or hormonal therapy for this malignancy, except for tamoxifen therapy as defined below in Section 5.10.

5.2.3 No previous anthracycline chemotherapy for any disease.

5.3 Patients with locally advanced breast cancer, inflammatory breast cancer or metastatic breast cancer are not eligible. Patients with involvement of dermal lymphatics on pathology are not eligible, even if there are no clinical signs of inflammatory cancer.

5.4 Patients with bilateral, synchronous invasive breast cancer are eligible as long as both primary tumors meet the criteria in Section 5.1. If a patient has an invasive cancer on one side that meets the eligibility criteria, and DCIS or LCIS on the contralateral side, the patient is eligible. DCIS or LCIS should be managed according to institutional guidelines.

5.5 Patients must be disease free from prior malignancies for > 5 years, except for curatively treated basal cell or squamous cell carcinoma of the skin or carcinoma-in-situ of the cervix. Patients with a history of invasive breast cancer, or DCIS are eligible if they have been disease free for > 5 years. Patients with a history of LCIS are eligible regardless of the interval from diagnosis.

5.6 Age $\geq$ 18 years of age.

5.7 CTC Performance Status 0-1.

5.8 Women must not be pregnant or nursing as the chemotherapy drugs used in this study may cause harm to a fetus or newborn.

5.9 Concomitant exogenous hormone therapy, including oral contraceptives, post-menopausal hormone replacement therapy, and raloxifene must be stopped before patients can be enrolled.

5.10 Patients may have received up to 4 weeks of tamoxifen therapy for this malignancy and still be eligible for this study. Patients who received tamoxifen or another selective estrogen receptor modulator (SERM) for prevention or for other indications (e.g. osteoporosis) are eligible. Tamoxifen therapy or other SERMs must be discontinued before the patient is enrolled on this study.

The use of bisphosphonates for the treatment of osteoporosis is permitted. The use of raloxifene is not permitted after enrollment on this study.

5.11 Patients must have adequate organ function including no active congestive heart failure, and no myocardial infarction < 6 months from time of registration.

5.12 Required Initial Laboratory Data:

ANC	≥1,000/mm^3
Platelet count	≥100,000/ mm^3
Creatinine	≤ 2.0 mg/dl
Bilirubin	≤ 1.5 x upper limits of institutional normal

5.13 Enrollment on Other Investigational Studies

5.13.1 Adjuvant Bisphosphonate Studies

Patients may be enrolled on adjuvant bisphosphonate studies. This should be noted on the appropriate forms. Patients may be enrolled concurrently or sequentially on 40101 and bisphosphonate trials.

5.13.2 Adjuvant Hormonal Studies

Patients may be enrolled on adjuvant hormonal studies approved by CALGB or CTSU, such as the SOFT and TEXT trials. Enrollment on these studies should be noted on the appropriate forms. Hormonal therapy will begin as outlined in the specific adjuvant hormonal trial. Specifically, patients enrolled on the TEXT trial should commence ovarian suppression concurrently with chemotherapy. In all of these trials, the specified adjuvant hormonal therapy will replace the standard tamoxifen or aromatase inhibitor recommended for after the conclusion of chemotherapy (see Section 8.4).

6.0 REGISTRATION/RANDOMIZATION, STRATIFICATION, DATA AND PATHOLOGY SUBMISSION

6.1 Randomization

6.1.1 Randomization Requirements

- **Informed Consent:** the patient must be aware of the neoplastic nature of her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Human protection committee approval of this protocol and a consent form is required.

6.1.2 CALGB Randomization Procedures

This study uses the CALGB on-line Patient Registration system. Randomization will be accepted only through CALGB Main Member/At Large institutions, selected affiliate institutions, and CCOPs using the on-line Patient Registration system. Randomization must occur prior to the initiation of therapy.

Confirm eligibility criteria (Section 5.0). Complete the Registration Worksheet. Access the on-line Patient Registration system via the patient registration icon on the CALGB Information Systems (IS) Application main menu. If the registering CRA requires assistance, he/she may consult the on-line help file located under the help menu of the CALGB IS Application. If further assistance is required, the registering CRA may call the CALGB Registrar (919-668-9396, Monday-Friday, 9 AM – 5 PM, Eastern Time; Registration Fax: 919-668-9397). Enter the following information:

Study
Name of group
Name of institution where patient is being treated
Name of treating physician
Name of responsible CRA

Other group patient ID #, if applicable
CALGB patient ID #, if applicable
Patient's initials (L, F, M)
Patient's Social Security #, date of birth, and hospital ID #
Patient's gender
Patient's race
Patient's performance status
Patient's height, in centimeters
Patient's weight, in kilograms
Type of insurance (method of payment)
Stratification factors
Patient's Postal Code, if applicable
Treatment start date
Date of signed consent
Companion studies, if applicable
Eligibility criteria met (no, yes)
Patient participating in the pharmacogenetic companion (no, yes)*

When the patient is registered, a patient identification number will be generated. Please write the number in your records. Registration to any mandatory or optional companion studies will be done at the same time as registration to the treatment study. Registration to both treatment and companion studies will not be completed if eligibility requirements are not met for all selected trials (treatment and companions).

6.1.3 Registration to companion studies

* If the patient answers "yes" to "My blood may be used for the purpose of learning about how certain genes influence the effectiveness and side effects of adjuvant breast cancer treatment…", then the patient has consented to participation in the pharmacogenetic companion study described in Section 9.0 and should be enrolled on CALGB 60202. Although it is preferable that patients are registered to 60202 at the same time they are registered to 40101, registration to 60202 may occur up to 60 days following registration to the treatment trial. For enrollment to 60202 after enrollment to 40101 has occurred, a revised CALGB 40101 Registration Worksheet, indicating that the patient has consented to late enrollment to 60202, must be faxed to the CALGB Registrar. See Section 6.4 for sample submission instructions.

CALGB 70301 is a separate companion protocol and is available on the CTSU menu. Patients are also encouraged to participate in CALGB 70301 the quality of life, employment and informal care cost analysis companion study. Registration should occur simultaneously with the CALGB registration/randomization to CALGB 40101. Registrations to CALGB 70301 may take place following registrations to CALGB 40101; however, the pre-treatment Quality of Life, Employment/Care Costs, and Peripheral Neuropathy questionnaires must be completed prior to the initiation of treatment on CALGB 40101.

After registration is complete the patient may be randomized. The patient is randomized according to the stratification factors indicated below, which must be entered to obtain a treatment assignment. For example, if age is a stratification factor the actual age is collected. If the stratification question is a "no, yes" question, please enter the value "1" for no and "2" for yes. Once the randomization is complete, note the patient's treatment assignment in your records.

The Main Member/At Large Institution and registering institution will receive a Confirmation of Randomization. Please check for errors. Submit corrections in writing to CALGB Statistical Center, Data Operations, Hock Plaza, 2424 Erwin Road, Suite 802, Durham, NC 27705.

6.1.4 Stratification

Menopausal Status: a) premenopausal: < 6 months since last menstrual period and no prior bilateral ovariectomy and not on estrogen replacement therapy.

b) postmenopausal: prior bilateral ovariectomy or > 12 months since last menstrual period with no prior hysterectomy.

If a. and b. are not applicable, then premenopausal is age < 50 years and postmenopausal is age ≥ 50 years.

ER/PgR Status: a) Tumor ER and/or PgR positive or unknown.

b) Tumor ER and PgR negative.

HER2 Status: a) HER-2 positive by immunohistochemistry 3+ staining or by gene amplificaton by FISH

b) HER-2 negative

c) HER-2 unknown

6.2 Data Submission: Forms should be submitted to the CALGB Statistical Center, Data Operations (SCDO) in compliance with the Data Submission schedule below. There are three options for submitting forms that use the Teleform barcode and cornerstones:

- the preferred method is to submit the forms electronically using the "Submit to CALGB" button located at the bottom of the last page of each form. Forms submitted electronically should not be submitted by fax or mail.
- the forms may be faxed to 919-416-4990. Please note that the four cornerstones and the form id ("bitmap") must appear on the form. Copies must be 100% of the original form size.
- the forms may be mailed to the CALGB Statistical Center, Data Operations, Hock Plaza, 2424 Erwin Rd, Suite 802, Durham, NC 27705. Please note that the four cornerstones and the form id ("bitmap") must appear on the form. Copies must be 100% of the original form size.

Amended data and supporting documentation (e.g., reports or flow sheets) should be submitted by fax (919-416-4990) or mail (CALGB Statistical Center, Data Operation, Hock Plaza, Suite 802, 2424 Erwin Road, Durham, NC 27705.

For the most up-to-date data forms, please visit the CALGB website at www.calgb.org.

Form		**Submission Schedule**
C-924	CALGB 40101 On-Study Form	Submit within one week of randomization.
	CALGB 40101 Pre-study Eligibility Checklist **All Operative and Pathology Reports**	
C-1443	CALGB:HER2/neu Testing Form	
C-490	CALGB Tracking Form (Tissue Blocks) **Pathology Reports**	Submit with paraffin block to CALGB PCO and a copy to the CALGB SCDO.
C-1077	CALGB: Blood Sample Tracking Form	Send original with serum sample (see section 6.4) to the CALGB PCO and a copy to the CALGB SCDO
C-925	CALGB 40101 Treatment Summary Form All Patients	Submit once at the end of all protocol chemotherapy for all patients.
C-926	CALGB 40101 Treatment Summary Subset Form	For the first 500 patients on each arm, submit at the end of every cycle of chemotherapy. Patients enrolled after 02/15/07 do not need to have this form submitted.
C-928	CALGB 40101 Adverse Event Form	Submit at the end of every cycle of chemo-therapy for all patients. Final submission required 6 wks after Day 1 of the last cycle of chemotherapy.
C-717	CALGB Adjuvant Radiotherapy Report Form	Submit at completion of radiation therapy (if applicable, see Section 8.7).
C-1444	CALGB: Trastuzumab Monitoring Form	Submit at the completion of trastuzumab therapy (if applicable, see Section 8.4)
C-929	CALGB: 40101 Follow-Up Form	For all patients, during active protocol treatment, only submit at any significant clinical event.[#] After completion or dis-continuation of protocol therapy, submit every 6 months for the first two years, then annually for 15 years from study entry or until death. In addition, submit at any significant clinical event.[#]
C-260	CALGB Remarks Addenda	Submit as needed to document significant clinical events during and after treatment.

Significant clinical event = relapse, disease progression, metastases, toxicity, significant non-protocol therapy, secondary malignancy, death.

All patients who are registered to this study, including those who do not receive any protocol treatment, must be followed for 15 years from study entry or death, whichever occurs first (see Section 13.1).

6.3 CALGB Pathology Submission

One paraffin embedded block with representative tumor, for all patients who have consented to tissue submission, should be labeled with the institutional surgical pathology number, and must be submitted within 3 months of registration in a properly-packaged sturdy box along with copies of the original ER/PgR and HER2/neu report, institutional pathology report and consultative pathology reports (if available). A completed CALGB Tracking Form for Tissue Blocks (C-490) should also be sent with the above tissue specimens and pathology reports to the following address:

The CALGB PCO
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967
path.calgb@osumc.edu

Please consider using a secure and temperature safe method of packaging your specimens. [Extreme heat precautions should be taken when packaging blocks.] Use of a shipping method that is traceable is recommended. If the above requirements cannot be met, please include a detailed explanatory letter.

A copy of the C-490 Form and all reports should also be sent to the CALGB Statistical Center, Data Operations, Hock Plaza, 2424 Erwin Road, Suite 802, Durham, NC 27705).

The CALGB has instituted special considerations for the small percentage (5%) of hospitals whose policy prohibits long-term storage of blocks, and the smaller percentage (4%) of hospitals whose policies prohibit release of any block. If, due to institutional policy, a block cannot be sent, please call the CALGB PCO at 614-293-7073 to obtain a protocol to cut the sections at your institution.

The goal of the CALGB PCO is to provide investigators with quality histology sections for their research while maintaining the integrity of the tissue. All paraffin blocks that are to be stored at the PCO will be vacuum packed to prevent oxidation and will be stored at 4ºC to minimize degradations of cellular antigens. For these reasons it is preferred that the PCO bank the block until correlative studies have been initiated and the study investigator requests thin sections. Please contact the PCO if additional assurances with your hospital pathology department are required.

6.4 CALGB Whole Blood Submission for Pharmacogenomic Companion (See Section 9.0)

All participating institutions must ask patients for their consent to participation in the pharmacogenetic studies described in Section 9.0, although patient participation is optional. All patients who consent to submission of blood samples are to be registered to CALGB 60202. Consent to participation to in the pharmacogenetic companion is indicated by the patient answering “yes” to the consent question “My blood may be used for the purpose of learning about how certain genes influence the effectiveness and side effects of adjuvant breast cancer treatment…”. For patients being registered to 60202 at the time of registration to 40101, a whole blood sample will be taken prior to beginning study treatment. For patients registered to 60202 within 60 days of registration to the 40101, the blood sample may be taken after consent is obtained.

Draw and refrigerate 5 to 10 ml whole blood in a tube that uses an EDTA anticoagulant. The blood should be kept refrigerated until shipped. Specimens should be shipped with a cool pack to:

CALGB PCO
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967

Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary. Ship on Monday-Friday by overnight service to assure receipt. If shipping on Friday, FedEx or UPS must be used at the air bill must be marked "For Saturday delivery." Do not ship specimens on Saturday. **Additional CALGB-PCO information can be found at http://www.pathology.med.ohio-state.edu/calgb/.**

This sample should be labeled with the CALGB patient ID number, protocol number, and date. A sample label is illustrated below:

CALGB Patient ID#: CALGB Protocol #: Date:

The CALGB: Blood Sample Tracking Form (C-1077) must accompany sample. A copy of the C-1077 form must be submitted to the Statistical Center, Data Operations.

7.0 REQUIRED DATA

Guidelines For Pre-Study Testing

To be completed within 16 DAYS before registration:

- All blood work and physical examination.

Tests & Observations	Prior to Registration	Day 1 of each cycle (every 2 weeks)	Post Treatment Follow up
History and Progress Notes	X	F	C
Physical Examination	X	F	C
Pulse, Blood Pressure	X		E
Height	X		
Weight/Body Surface Area	X	X	E
Performance Status	X	X	E
Drug Toxicity Assessment		F	E
Pelvic exam w/cytology	£		£
Eye exam	¶		¶
EKG	B	B	
MUGA/Echocardiogram	B	♠	♠
Laboratory Studies			
CBC/Diff/Platelets	A	A	D
Serum Creatinine, BUN	A	A	D
Serum Electrolytes	A	A	D
AST, ALT, Alk.Phos., Bilirubin	A	A	D
Whole blood for pharmacogenetics	★		
Staging			
ER/PgR; HER2	X		
Chest x-ray, PA & Lateral≠	X		PRN
Bone Scan≠	X*		
Mammogram≠	X		◆

A CBCs and serum chemistries must be performed either the day of or the day before each treatment with the following exception: the pre-study CBC and serum chemistries may be used for the first treatment of cycle 1.

B To be performed per institutional guidelines and clinical judgment of the treating physician.

C 4-6 weeks after last dose of study treatment, then q 6 months x the first 2 yrs, then annually until 15 years from study entry or death.

D To be performed on the basis of suspicious findings.

E 4-6 weeks after last dose of study treatment.

F To be performed within 72 hours of the initiation of each cycle.

£ Pelvic exam within 12 months prior to study entry is recommended. Yearly pelvic exams are recommended for those patients receiving tamoxifen, unless s/p hysterectomy.

¶ Eye exam recommended at baseline and every two years for those women receiving tamoxifen.

* To be performed only if there is a clinical suspicion of positivity.

≠ CXR and mammograms obtained as part of the original diagnosis, biopsy and surgical treatment will suffice and need not be repeated. CXR is not required if a CT scan of the chest was performed as part of the patient's workup and showed no evidence of metastatic disease.

◆ Yearly recommended.

★ Optional companion: 5-10ml of whole blood to be drawn <u>prior</u> to beginning study treatment (see Sections 6.4 and 9.0).

♠ It is recommended that patients receiving adjuvant trastuzumab have LVEF measured by MUGA or echocardiogram prior to initiation of trastuzumab, and repeated at 3, 6, 9 and 18 months after the initiation of trastuzumab.

8.0 TREATMENT PLAN

8.1 Chemotherapy

Patients will be randomized to receive either CA or paclitaxel. Treatment to begin within 7 days of registration.

Patients will also be randomized to duration of therapy, as below: CA x 4 cycles or 6 cycles, or paclitaxel for 4 or 6 cycles.

The 6-cycle arms for CA and paclitaxel were permanently closed to further accrual in Update #8.

8.2 Standard Chemotherapy Arm: Cyclophosphamide and Doxorubicin (CA)

The 6-cycle arms for CA and paclitaxel were permanently closed to further accrual in Update #8.

Cyclophosphamide	600 mg/m^2	IV per institutional guidelines	day 1
Doxorubicin	60 mg/m^2	IV bolus per institutional guidelines	day 1

Administered q 14 days x 4 cycles **or** 6 cycles, depending on randomization.

Needed to initiate each q 2 week cycle: ANC $\geq$ 1000/mm^3, platelets $\geq$ 100,000/mm^3. If these criteria are not met, delay until counts recover to this level. If a delay of > 16 days is required, permanently discontinue patient from study treatment and notify Study Chair.

Nadir counts not required.

It is recommended that filgrastim be administered on days 3-10 of each cycle, at approximately 5 mcg/kg, rounding off to either 300 or 480 mcg, whichever is closer to the actual calculated amount. Filgrastim is administered subcutaneously. Patients may have filgrastim discontinued prior to day 10 if neutrophil count has recovered to an acceptable range, as determined by the treating physician. A primary goal of filgrastim use in this circumstance is to allow treatment on schedule every 14 days, as well as to reduce the incidence of neutropenic fever, and dosing should reflect an attempt to meet these goals. Sargramostim (GM-CSF) 250-500 mcg/m^2 days 3-10 may be used in place of filgrastim. Pegfilgrastim (Neulasta) is permitted as a substitute for filgrastim or sargramostim. Pegfilgrastim should be administered at a dose of 6 mg subcutaneously, between 24-36 hours after the administration of chemotherapy. If pegfilgrastim is used, filgrastim or sargramostim should not be used for that cycle.

When filgrastim, sargramostim or pegfilgrastim is used, document on the CALGB: 40101 Treatment Summary Form (C-925). See Section 12.4.2 for guidelines regarding the use of these agents.

8.3 Single Agent Arm: Paclitaxel

The 6-cycle arms for CA and paclitaxel were permanently closed to further accrual in Update #8.

Paclitaxel	175 mg/m^2	IV over 3 hours, day 1

Administered every 14 days for 4 or 6 cycles, depending on randomization. Paclitaxel may be given over a longer period than 3 hours if the patient develops toxicities and the infusion is better tolerated when administered more slowly.

Needed to initiate each q 2 week cycle: ANC $\geq$ 1000/mm^3,platelets $\geq$ 100,000/mm^3. If these criteria are not met, delay until counts recover to this level. If a delay of > 16

days is required, permanently discontinue patient from study treatment and notify Study Chair.

Nadir counts are not required.

It is recommended that filgrastim be administered on days 3-10 of each cycle, at approximately 5 mcg/kg, rounding off to either 300 or 480 mcg, whichever is closer to the actual calculated amount. Filgrastim is administered subcutaneously. Patients may have filgrastim discontinued prior to day 10 if neutrophil count has recovered to an acceptable range, as determined by the treating physician. A primary goal of filgrastim use in this circumstance is to allow treatment on schedule every 14 days, as well as to reduce the incidence of neutropenic fever, and dosing should reflect an attempt to meet these goals. Sargramostim (GM-CSF) 250-500 mcg/m^2 days 3-10 may be used in place of filgrastim. Pegfilgrastim (Neulasta) is permitted as a substitute for filgrastim or sargramostim. Pegfilgrastim should be administered at a dose of 6 mg subcutaneously, between 24-36 hours after the administration of chemotherapy. If the treating physician feels that neutrophils will recover by 14 days without filgrastim, sargramostim, or pegfilgrastim then they may be omitted. If treatment cannot be administered on schedule for the next cycle, then one of these agents must be used in subsequent cycles. If pegfilgrastim is used, filgrastim or sargramostim should not be used for that cycle.

When filgrastim, sargramostim or pegfilgrastim is used, document on the CALGB: 40101 Treatment Summary Form (C-925). See Section 12.4.2 for guidelines regarding the use of these agents.

8.3.1 Premedication for Paclitaxel

The following premedication regimen is recommended for the initial dose of paclitaxel. If the patient does not experience an allergic reaction, the premedication regimen may be altered at the discretion of the treating physician.

Benadryl	12.5 - 50 mg IV	30-60 minutes pre-paclitaxel
Ranitidine	50 mg IV	30-60 minutes pre-paclitaxel
(can be replaced with cimetidine 300 mg, or famotidine 20 mg)		
Dexamethasone	10 mg IV	< 60 minutes pre-paclitaxel
	OR	
Dexamethasone	10 mg po	> 60 minutes pre-paclitaxel
	OR	
Dexamethasone	20 mg po	6 hrs and 12 hrs pre-paclitaxel

8.4 Trastuzumab

Adjuvant trastuzumab should be considered for patients whose tumors are HER2 positive by either IHC 3+ staining or gene amplification by FISH analysis. If there is concern about the accuracy of the test result, a confirmatory test is strongly advised.

Timing of Initiation of Trastuzumab

For patients treated on the CA arms, trastuzumab should not begin until at least 3 weeks after the last dose of CA. It is recommended that trastuzumab be initiated within 8 weeks of the last cycle of CA.

For patients treated on the paclitaxel arms, trastuzumab can be initiated concurrently with paclitaxel, or at the completion of paclitaxel.

For patients who have already completed chemotherapy, trastuzumab can be initiated up to 6 months from the completion of chemotherapy.

Cardiac Monitoring

Patients should have a cardiac ejection fraction, measured by MUGA or echocardiogram, above the lower limits of normal for the testing institution, **prior** to initiation of trastuzumab. It is recommended that the baseline ejection fraction be repeated if the estimate is 74% or greater as this may be a false elevation and could lead to erroneous determinations of trastuzumab toxicity during follow-up. It is recommended that repeat cardiac monitoring be performed at 3 months, 6 months, 9 months and 18 months after the initiation of trastuzumab. Please note that for patients treated on the CA arms, the initial cardiac monitoring should occur after the completion of CA chemotherapy and before the initiation of trastuzumab. If at any time there is a greater than 15% absolute reduction in the ejection fraction, then discontinuation of trastuzumab should be strongly considered. In addition, if the patient develops an ejection fraction below the lower limit of normal for the testing institution, even if this does not represent a 15% reduction in EF, or if the patient develops symptomatic congestive heart failure, it is recommended that trastuzumab be discontinued.

Dosing and Schedule of Trastuzumab

It is recommended that for patients receiving concurrent paclitaxel and trastuzumab, that trastuzumab be given on a weekly schedule. After the completion of paclitaxel, and for patients receiving trastuzumab after CA, trastuzumab can either be given on a weekly schedule, or may be given on an every 3 week schedule, as outlined below.

The concurrent use of an anthracycline and trastuzumab is **not** acceptable.

Weekly schedule

4 mg/kg IV loading dose, followed by weekly dose of 2 mg/kg.

Every 3 week schedule

8 mg/kg IV loading dose, followed by a dose of 6 mg/kg every 3 wks.

It is permissible to switch from weekly to every 3 week dosing and vice versa. When switching from weekly to every 3 weeks there is no need for the 8 mg/kg loading dose. When switching from every 3 week to weekly there is no need for the 4 mg/kg loading dose.

Duration of Trastuzumab Therapy

It is recommended that trastuzumab be administered over a period of 52 weeks. If it is necessary to miss doses for any reason, it is recommended they not be "made up" but that the total duration of trastuzumab therapy approximate 52 weeks.

8.5 Tamoxifen

Tamoxifen, 20 mg/day orally for 5 years, is recommended after the conclusion of chemotherapy and radiation therapy, for patients whose tumors are either estrogen or progesterone receptor positive, whether they are pre- or post-menopausal. Pre-menopausal patients and the treating physician have the option to enroll the patient in the SOFT and TEXT trials. The treatment specified by these protocols would replace the standard post chemotherapy tamoxifen (see Section 5.13.2).

Hormone therapy follows completion of chemotherapy, unless otherwise specified by a CALGB or CTSU-approved adjuvant hormone therapy trial (see Section 5.13.2). For patients receiving trastuzumab, hormonal therapy is given concurrently, once chemotherapy has been completed.

8.6 Aromatase Inhibitors

Aromatase inhibitors, administered for 5 years, may be substituted for tamoxifen in postmenopausal women or administered after 2-5 years of tamoxifen.

Hormone therapy follows completion of chemotherapy, unless otherwise specified by a CALGB or CTSU-approved adjuvant hormone therapy trial (see Section 5.13.2). For patients receiving trastuzumab, hormonal therapy is given concurrently, once chemotherapy has been completed.

8.7 Radiation Therapy

Patients treated with lumpectomy (breast conserving surgery) must receive radiation therapy. Patients may be treated according to local institutional guidelines, and may participate in radiation therapy clinical research trials. Patients may be treated with conventional, post-chemotherapy whole breast radiation, or partial breast radiation, administered by external beam or brachytherapy. Partial breast radiation may be delivered prior to chemotherapy or at the conclusion of chemotherapy.

If breast radiation is administered prior to chemotherapy, patients must be registered after the conclusion of radiotherapy, but within 84 days of the last breast surgery. In addition, at least 7 days must elapse between the conclusion of radiation therapy and the initiation of chemotherapy. Chemotherapy administration should begin within 7 days of registration.

Patients who undergo mastectomy may receive chest wall and nodal radiation therapy according to institutional guidelines and physician preference.

Details of radiation therapy should be entered on the CALGB: Adjuvant Radiotherapy Report Form (C-717).

9.0 PHARMACOGENOMIC STUDIES

9.1 Rationale

Candidate gene, pathway analyses and whole genome scans are common approaches for the identification of germline polymorphisms that contribute to a given phenotype. Germline DNA collection was embedded in CALGB 40101 for investigation of genetic associations with response and toxicity in women treated with adriamycin/cyclophosphamide or paclitaxel. A candidate gene approach focusing on drug metabolizing enzymes and transporters was originally proposed and will be carried out. In addition, a genome-wide single nucleotide polymorphism (SNP) and copy number variant (CNV) scan will also be performed.

9.1.1 Candidate Gene Approach: Drug Metabolism and Transport Pharmacogenomics in Breast Cancer

Variable factors contribute to the success of chemotherapy, including the functional activity of drug metabolizing enzymes and transport proteins. Interindividual variation in drug metabolism and transport can alter plasma and intracellular levels of drugs and their metabolites and therefore affect drug efficacy and/or toxicity. One mechanism accounting for these interindividual differences is genetic variation in the genes encoding drug metabolizing enzymes and transporters. Germ line polymorphisms have been well documented for numerous enzymes involved in drug metabolism, including the cytochrome P450 (CYP) enzymes, thiopurine methytransferases, UDP-glucuronosyl transferases and dihydropyrimidine dehydrogenase [26]. More recently, functionally significant genetic polymorphisms in drug transporters have been identified and characterized, most notably in the multidrug resistance transporter P-glycoprotein [27, 28].

CALGB 40101 offers an excellent population to assess the role of genetic polymorphisms in drug metabolism and transport genes in drug response and toxicity. Cyclophosphamide is bioactivated by CYP2B6 and CYP3A4 to 4-hydroxycyclophosphamide which is further metabolized to the active phosphoramide mustard [29]. CYP3A4 is also the major enzyme involved in metabolism of cyclophosphamide to inactive metabolites. The importance of CYP2B enzymes in generating cytotoxic concentrations of the phosphoramide mustard is illustrated by the increased exposure to 4-hydroxycyclophosphamide following implantation of cyclophosphamide-impregnanted polymer into human tumor xenografts that have viral-induced CYP2B activity [30]. Paclitaxel is largely metabolized by CYP3A4/3A5 although CYP2C8 is also implicated [31, 32]. Genetic polymorphisms in *CYP3A4/5* and/or *CYP2C8* could alter the clearance of cyclophosphamide and paclitaxel, therefore affecting the concentration-response profile. Both doxorubicin and paclitaxel are substrates for P-glycoprotein and altered function of P-glycoprotein is associated with altered plasma levels of adriamycin [33]. Increased survival and decreased relapse were recently associated with one of two major *MDR1* haplotypes (TTT at nts 1236/2677/3435) in a study of 280 acute myeloid leukemia patients [37]. This is consistent with decreased function of P-glycoprotein in carriers of the *MDR1* TTT haplotype and increased intracellular levels of the cytotoxic agents mitoxantrone, etoposide and daunorubicin. A similar relationship between *MDR1* haplotype and CA toxicity will be explored in the current study [27,28]. In addition, the effect of *MDR1* haplotype on DFS will be examined and an exploratory analysis will also be made to assess the effect of genetic polymorphisms in *CYP3A5*, CYP2C8 and *CYP2B6* on drug response and/or toxicity [34, 35, 36].

Additional candidate genes may be included once evidence is found for their importance in determining the drug levels of the study drugs. It is anticipated that the results of this study will lead to further hypothesis-driven research into the importance of genetic variation in determining drug response. A long-term goal of these studies is to use pharmacogenetic information to appropriately dose patients at the initiation of treatment with cyclophosphamide, adriamycin and paxlitaxel thereby optimizing response and minimizing toxicity.

9.1.2 Genome-Wide Approach: SNP Scan of DNA

Most pharmacogenetic analyses have taken a candidate gene approach that utilizes biological data to guide the selection of drug response genes in a pathway as described above. This approach is somewhat limited by our knowledge of the mechanisms underlying the phenotypes. In the case of drug response phenotypes, most candidate gene studies have focused on drug metabolizing enzymes and transporters, thus limiting the chance of discovering causal SNPs not involved in mediating drug levels [39,40]. In contrast, a genome-wide approach collects SNP data across the entire human genome and has significant power to detect common variants that confer a modest risk for a complex phenotype [41]. Genome-wide studies capitalize on the large number of SNPs (more than 10 million available in dbSNP) that have been localized and validated across the genome, a majority of which have resulted from the HapMap project [42]. This valuable collection of publicly available, validated SNPs has provided the framework for performing genome-wide association studies. Recent technological advancements in genotyping platforms have also enabled the development of genome-wide associations. Searching the whole genome in an association study requires genotyping of anywhere between 105 to 106 markers across the genome [43-46]. Until recently, this approach was fiscally prohibitive and impractical. However, new gene chip platforms from Affymetrix and Illumina have made large scale genotyping feasible and cost effective. The Illumina HumanHap550 chip that will be used in this study has the capacity to genotype over 555,000 SNPs simultaneously. In addition, there are 4,300 SNPs in regions of copy number variations (CNVs), thus allowing for the detection of CNVs as well.

This new capability represents a paradigm shift in the number of genotypes that can be evaluated in any given individual with one genotyping assay and provides a platform for the identification of novel genes involved in the response to and toxicity associated with adriamycin/cyclophosphamide and paclitaxel.

An increasing number of reports of significant findings from genome-wide association studies in cancer are being published. To date, these have all focused on SNPs associated with risk of developing cancer, and include studies in prostate [47-51], colorectal [52-54], lung [55] and breast cancer [56-58]. The success of these studies illustrates the power and validity of this approach for identifying genetic causes of disease. To date, there are no published reports of genome-wide association analyses in cancer pharmacogenetics. The relatively large size of CALGB 40101 and robust response and toxicity phenotype data make it an ideal sample set for whole genome analysis. The identification of SNPs that contribute to response and toxicity of the three widely used drugs studied in CALGB 40101 will lead to additional studies to understand the mechanism for these associations and to investigate the application of genetic information for the optimization of breast cancer therapy.

9.2 Objectives

9.2.1 Primary Objective

9.2.1.1 To assess the discrepancy of myelosuppression among the common MDR1 haplotypes in the CA treatment arm.

9.2.1.2 To identify genetic markers associated with the risk of developing neutropenia in adriamycin/cyclophosphamide-treated breast cancer patients**.**

9.2.1.3 To identify genetic markers associated with the risk of developing peripheral neuropathy in paclitaxel-treated breast cancer patients**.**

9.2.2 Secondary Objectives

9.2.2.1 To assess the effect of *MDR1* haplotypes on DFS adjusted for treatment.

9.2.2.2 Exploratory analysis of the effect of *CYP3A5, CYP2C8* and *CYP2B6* polymorphisms on DFS and toxicity.

9.2.2.3 To identify genetic markers associated with differences in the efficacy of each chemotherapy regimen.

9.2.2.4 To examine genetic associations with other response and toxicity phenotypes that become apparent during future analysis of CALGB 40101 data. Potential examples include adriamycin/cyclophosphamide-induced menopause or cardiotoxicity.

9.2.2.5 To identify copy number variants associated with adriamycin/ cyclophoshamide-induced neutropenia and paclitaxel-induced peripheral neuropathy.

9.3 Eligibility Criteria

This study is an embedded companion (CALGB 60202) within CALGB 40101. All patients enrolling in CALGB 40101 are eligible for this study, and all patients who consent to blood submission will be enrolled on CALGB 60202. Although it is preferable that patients are registered to 60202 at the same time they are registered to 40101, registration to 60202 may occur up to 60 days following registration to the treatment trial.

9.4 Study Design/Methods

Patients will be recruited to this companion protocol by the physician enrolling them on CALGB 40101. Patients who have consented will be enrolled to this optional companion study at the time of registration to 40101. A single 5 to 10 ml blood sample will be collected prior to beginning the study treatment. Samples will be shipped to the CALGB Pathology Coordinating Office (PCO) at The Ohio State University. Genomic DNA will be extracted using a commercially available kit from Qiagen. The concentration and quality of DNA will be quantified by ultraviolet spectroscopy. All DNA samples will be stored at the PCO until they are distributed to the appropriate laboratory for analysis.

The typical yield of DNA from the CALGB 40101 samples is 12 mg (range of 5-20 mg). We will send up to 5 mg to the RIKEN SNP Research Center for whole genome analysis, leaving on average 7 mg for planned candidate gene analyses. A minimum of 2.5 mg of DNA from each subject will remain at the PCO for future genotyping, but typically this will be ≥7 mg. Phenotypic data will be extracted from the CALGB database by the CALGB statistical group.

9.4.1 Individual Candidate Gene

Aliquots of DNA will be sent to Drs. Kroetz and Relling for the candidate gene analysis. Genotyping for MDR1 will be performed by primer extension analysis at the Genetics Core Facility at the University of California San Francisco. Genotyping for CYP3A4/CYP3A5, CYP2C8 and CYP2B6 will be carried out at St. Jude Children's Research Hospital. Haplotypes will be assigned to all individuals homozygous at all variant sites and those single site heterozygotes. For the remaining individuals, haplotypes will be statistically inferred using the program PHASE [38].

The genotype/haplotype for MDR1, CYP3A5, CYP2C8 and CYP2B6 will be correlated with disease-free survival and toxicity data collected in CALGB 40101 as described in Section 15.6.

9.4.2 Genome-Wide SNP Scan of Germline DNA

Aliquots of DNA will be sent to the Riken Institute for the whole-genome analysis. For the whole-genome analysis, the genotyping will be performed at the laboratory of Dr. Yusuke Nakamura and Dr. Hitoshi Zembutsu at the Riken SNP Research Center and the University of Tokyo Human Genome Center, Japan. Each Illumina HumanHap550 chip requires 750 ng of DNA and additional sample will be used for repeat assays where necessary. Considering that a typical candidate gene analysis of a single SNP requires 1-5 ng of DNA, there will be sufficient DNA remaining at the PCO to perform >500 SNP genotyping assays (and on average >1400 assays). In the Nakamura/Zembutsu laboratory, the current plan is that each DNA sample will be analyzed by two platforms. The first platform is the Illumina HumanHap550 SNP chip for genome-wide screening and the analysis will be performed according to the recommended Illumina protocol. The second platform that will be used by the RIKEN investigators is the combination of Invader assays with multiplex-PCR for target SNP genotyping. More than 7,000 variants in 267 possible drug-related genes will be genotyped using pre-established assays developed in Dr. Nakamura's lab [59]. The number of variants to be genotyped with the Invader assays might be less than 7,000 due to redundancy with the coverage in the Illumina platform.

Illumina's HumanHap550 Genotyping BeadChip enables whole-genome genotyping of over 555,000 SNP loci efficiently and accurately on a single BeadChip. The HumanHap550 BeadChip is powered by the Infinium(tm) II assay, which uses a single-tube, whole-genome amplification method that does not

require PCR and enables intelligent SNP selection using tagSNPs. TagSNPs are loci that can serve as proxies for many other SNPs. The use of tagSNPs greatly improves the power of association studies, as the same information and power from a larger number of SNPs can be gathered by genotyping only a subset of loci. TagSNPs on the HumanHap550 BeadChip were selected from the recently completed International HapMap Project.

10.0 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

All questions regarding treatment or dose modifications should be directed to the CALGB Study Chair.

10.1 Standard Chemotherapy (CA): The principle goal is to attempt to administer full doses of therapy on schedule.

10.1.1 Hematologic Toxicity

The following will be required to initiate any cycle of CA.

ANC $\geq$ 1000/mm^3, platelet count $\geq$ 100,000/mm^3.

If counts are below these levels, delay initiation of cycle until counts recover to these levels. If a delay of > 16 days is required, permanently discontinue patient from study treatment and notify Study Chair.

There will be no dose reductions for hematologic toxicity. Doses will not be reduced based on low nadir blood counts. Nadir blood counts are not required.

10.1.2 Febrile Neutropenia

Filgrastim, sargramostim or pegfilgrastim is recommended for all cycles of chemotherapy as described in Sections 8.2 and 8.3. If they are not used and febrile neutropenia (defined as a temperature > 38.5°C [101°F] sustained for more than one hour) concomitant with an ANC < 500/mm^3, a colony stimulating factor preparation is strongly recommended for all subsequent cycles. There will be no dose reduction for febrile neutropenia.

When filgrastim, sargramostim or pegfilgrastim is used, document on the CALGB: 40101 Treatment Summary Form (C-925).

10.1.3 Gastrointestinal Toxicity

10.1.3.1 Mucositis

If grade 3 or 4 mucositis occurs, the subsequent cycle of therapy should not be initiated until mucositis has resolved to grade 1 or 0. Subsequent cycles of therapy should have doxorubicin dose reduced by 25%.

10.1.3.2 Diarrhea

If grade 3 or 4 diarrhea occurs, the subsequent cycle of therapy should not be initiated until diarrhea has resolved to grade 1 or 0. Subsequent cycles of therapy should have doxorubicin dose reduced by 25%.

10.1.4 Bladder Toxicity

If gross hematuria occurs, without other cause such as urinary tract infection, therapy should be held until hematuria resolves. If therapy is delayed more than 2 weeks, patient should be withdrawn from study. If hematuria resolves, and patient resumes therapy, additional hydration should be prescribed.

10.1.5 Hepatic Dysfunction

The LFTs are to be checked every two weeks, and the results should be available before proceeding with that week's treatment. No dose modifications will be made for grade 1 elevation of AST, ALT, alkaline phosphatase, or bilirubin. If any of the LFTs reach grade 2, CA should be held one week. If the abnormal test returns to grade 0 or 1, CA should be continued at full dose. If the abnormal test does not return to grade 0 or 1 in one week, but remains at grade 2, continue CA with a 20% dose reduction.

If LFT grade 3 or 4 toxicity has occurred, CA should be permanently discontinued.

10.1.6 Other Grade 3 or Grade 4 Toxicities

Any patient experiencing a grade 3 or 4 toxicity, other than those described above, must be discussed with the CALGB Study Chair.

10.2 Paclitaxel

10.2.1 Anaphylaxis/Hypersensitivity

10.2.1.1 Mild Symptoms (Grade 1) (mild flushing, rash, pruritis): Complete infusion, observation in treatment area. No treatment required.

10.2.1.2 Moderate symptoms (Grade 2) (moderate rash, flushing, mild dyspnea, chest discomfort): Stop infusion. Give intravenous diphenhydramine 25 mg and intravenous dexamethasone 10 mg. Resume paclitaxel infusion after recovery of symptoms, at a slower rate, 10 ml/hour for 15 minutes, then 25 ml/hour for 15 minutes, then, if no further symptoms, at full dose rate until infusion is complete.

If moderate or severe symptoms recur after rechallenge, stop paclitaxel infusion, and report as an adverse event. Patient may be rechallenged after premedication with dexamethasone 8 mg po or IV q 6 hrs x 4 doses (moderate symptoms) or 20 mg po or IV q 6 hrs x 4 doses (severe symptoms) and diphenhydramine 25 mg po or IV q 6 hrs x 4 doses (moderate or severe symptoms) [18-20]. The paclitaxel should be administered at a slower rate, 10 ml/hour for 15 minutes, then 25 ml/hour for 15 minutes, then, if no further symptoms, at full dose rate until infusion is complete.

10.2.1.3 Severe life-threatening symptoms (Grade 3) (hypotension requiring pressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria): Stop paclitaxel infusion. Give intravenous diphenhydramine and dexamethasone as above. Add epinephrine or bronchodilators if indicated and report episode as an adverse event. Patient may be rechallenged after premedication with dexamethasone 20 mg po or IV q 6 hrs x 4 doses and diphenhydramine 25 mg po or IV q 6 hrs x 4 doses [18-20]. The paclitaxel should be administered at a slower rate, 10 ml/hour for 15 minutes, then 25 ml/hour for 15 minutes, then, if no further symptoms, at full dose rate until infusion is complete.

10.2.2 Cardiac Arrhythmias: For *symptomatic*, EKG-documented arrhythmias, stop paclitaxel, and manage arrhythmia according to standard practice. Protocol treatment will be discontinued and the episode reported as an adverse event. For asymptomatic sinus bradycardia or tachycardia, no intervention is necessary.

For sinus bradycardia or tachycardia associated with hypersensitivity reaction, treat as above in Section 10.2.1.

10.2.3 Hematologic Toxicity

The following will be required to initiate any cycle of paclitaxel.

ANC $\geq$ 1000/mm^3, platelet count $\geq$ 100,000/ mm^3.

If counts are below these levels, delay initiation of cycle until counts recover to these levels. If a delay of > 16 days is required, permanently discontinue patient from study treatment and notify Study Chair.

There will be no dose reductions for hematologic toxicity. Doses will not be reduced based on low nadir blood counts. Nadir blood counts are not required.

10.2.4 Neurologic Toxicity

There will be no dose modifications for grade 1 or 2 neurotoxicity. If the patient is experiencing significant distress from grade 2 neurotoxicity, and the treating physician is uncomfortable with continued treatment at full dose, the dose of Paclitaxel should be reduced by 20%, and noted on Treatment Summary form C-925.

If patients develop grade 3 neurotoxicity, treatment should be withheld until toxicity has improved to at least grade 2, at which time Paclitaxel should be administered at 140 mg/m^2. If grade 3 toxocity occurs again, treatment should again be held until toxicity improves to at least grade 2, and the Paclitaxel dose should be reduced to 105 mg/m2. If grade 3 toxicity occurs again, treatment should be held again until toxicity improves to at least grade 2, and Paclitaxel should be administered at a dose of 80 mg/m^2. If grade 3 toxicity occurs again, the patient should not receive further Paclitaxel. Further treatment will be at the discretion of the treating physician.

Dose reductions for Paclitaxel are:

Full dose	175 mg/m^2
First dose reduction	140 mg/m^2
Second dose reduction	105 mg/m^2
Third dose reduction	80 mg/m^2

10.2.5 Gastrointestinal Toxicity

10.2.5.1 Mucositis: If grade 2 mucositis is present on the day of any treatment, the treatment should be delayed until the mucositis has resolved to a grade 1 or 0 and then resume paclitaxel at 175 mg/m^2.

If grade 3 or 4 mucositis occurs, delay treatment until mucositis has resolved to grade 1 or 0, then the dose of paclitaxel should be reduced to 140 mg/m^2. If grade 3 or 4 mucositis recurs, treatment should be delayed until mucositis has resolved to grade 1 or 0, and the dose of paclitaxel should be reduced to 105 mg/m^2. If mucositis causes a delay of > 16 days, the patient should be permanently discontinued from protocol therapy. Once the paclitaxel dose has been decreased it should not be re-escalated.

10.2.5.2 Diarrhea: If grade 2 diarrhea is present on the day of any treatment, the treatment should be delayed until the diarrhea has resolved to a grade 1 or 0 and then resume paclitaxel at 175 mg/m^2.

If grade 3 or 4 diarrhea occurs, delay treatment until the diarrhea has resolved to grade 1 or 0, then the dose of paclitaxel should be reduced to 140 mg/m^2. If grade 3 or 4 diarrhea recurs, treatment should be delayed until diarrhea has resolved to grade 1 or 0, and the dose of paclitaxel should be reduced to 105 mg/m^2. If diarrhea causes a delay of > 16 days, the patient should be permanently discontinued from protocol therapy. Once the paclitaxel dose has been decreased it should not be re-escalated.

10.2.6 Hepatic Dysfunction

The LFTs are to be checked every two weeks, and the results should be available before proceeding with that week's treatment. No dose modifications will be made for grade 1 elevation of AST, ALT, alkaline phosphatase, or bilirubin. If any of the LFTs reach grade 2, paclitaxel should be held one week. If the abnormal test returns to grade 0 or 1, paclitaxel should be continued at full dose. If the abnormal test does not return to grade 0 or 1 in one week, but remains at grade 2, continue paclitaxel at 140 mg/m^2. If the abnormal test result returns to grade 0 or 1, return to full dose, 175 mg/m^2.

If LFT grade 3 or 4 toxicity has occurred, paclitaxel should be permanently discontinued.

10.2.7 Other Grade 3 or Grade 4 Toxicities

Any patient experiencing a grade 3 or 4 toxicity, other than those described above, must be discussed with the CALGB Study Chair.

10.3 Dose Modification for Obese Patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, **all dosing is to be determined solely by (1) the patient's BSA as calculated from actual weight or (2) actual weight without any modification unless explicitly described in the protocol**. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. **Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.** Physicians who are uncomfortable with administering chemotherapy dose based on actual body weight should not enroll obese patients on CALGB protocols.

11.0 Drug Formulation, Availability, and Preparation

11.1 Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

11.2 Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

11.3 The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

11.4 Doxorubicin (Adriamycin RDF/PFS, Rubex, Doxorubicin Hydrochloride)

Availability

Commercially available as lyophilized powder for reconstitution in 10, 20, 50, 100, 150 mg vials. Also available are 2 mg/ml solution for injection in 10, 20, 50, and 200 mg vials of doxorubicin in solution. Please refer to the FDA-approved package insert for complete product information.

Preparation

Reconstitute the vials with 5, 10, 25, 50 or 75 ml, respectively, of Sodium Chloride for Injection, USP.

Storage and Stability

Intact vials of doxorubicin should be stored in the refrigerator. Intact vials of powder for reconstitution should be stored at room temperature. Reconstituted solutions are stable for 7 days at room temperature and 15 days under refrigeration when protected from light. Commercially available solutions labeled as such are intended to be multidose vials.

Administration

Intravenously, either peripherally as a bolus injection or through a central venous line. Avoid extravasation as severe local tissue necrosis may result.

Toxicities

Hematologic: Leukopenia (dose-limiting), also thrombocytopenia and anemia. Nadir 10-14 days, recovery in 21 days.

Dermatologic: Reversible usually complete alopecia; increased sensitivity to sunlight; hyperpigmentation of skin and nailbeds.

Gastrointestinal: Nausea and vomiting, sometimes severe; anorexia, diarrhea; mucositis (stomatitis and esophagitis).

Cardiovascular: Arrhythmias, ECG changes; rarely sudden death. Cardiomyopathy may occur and is related to total cumulative lifetime dose. The risk for cardiomyopathy increases with total doses > 450 mg/m^2.

Other: Red discoloration of urine x 24–48 hours; vesicant if extravasated.

11.5 Cyclophosphamide (Cytoxan, Neosar)

Availability

Commercially available as powder for injection in 100, 200, 500 mg, 1 gm and 2 gm vials. Please refer to the FDA-approved package insert of complete product information.

Preparation

Reconstitute 100 mg, 200 mg, 500 mg, 1 gram and 2 gram vials with 5, 10, 25, 50 or 100 ml of sterile water for injection for a final concentration of 20 mg/ml. Vigorous shaking and/or gentle warming may be necessary. Bacteriostatic water (paraben preserved only) may also be used for reconstitution.

Storage and Stability

Store intact vials of powder at room temperature (15° to 30°C). Reconstituted lyophilized cyclophosphamide is chemically and physically stable for 24 hours at room temperature or for 6 days in the refrigerator (2° to 8°C). It does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions.

Administration

Intravenous injection.

Toxicities

Hematologic: Leukopenia, with nadirs about 8-14 days after administration and recovery in 18-25 days and thrombocytopenia.

Dermatologic: Temporary alopecia.

Gastrointestinal: Nausea and vomiting.

GU: Hemorrhagic cystitis (onset of cystitis may be delayed from 24 hours to several weeks). Patients should be well hydrated before, during and after treatment.

Other: Amenorrhea, may be long-term, possible irreversible sterility in both sexes; rarely, anaphylaxis; teratogenic; may cause secondary neoplasms.

11.6 Paclitaxel (Taxol)

Availability

Paclitaxel is commercially available in 30 mg/5 mL, 100 mg/16.7 mL and 300 mg/50 mL multidose vials containing a clear colorless to slightly yellow viscous solution. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor® EL (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP. Please refer to the FDA-approved package insert of complete product information.

Preparation

Paclitaxel must be diluted prior to administration with 0.9% sodium chloride Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP; or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL. Paclitaxel should be prepared and stored in glass, polypropylene, or polyolefin containers due to leaching of DEHP [di-(2ethylhexyl)phthalate] plasticizer from polyvinyl chloride (PVC) containers. Non-PVC containing tubing and connectors such as the IV administration sets (polyethylene or polyolefin) used to infuse parenteral nitroglycerin should be used. In-line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 micron (e.g. IVEX-2®) into the IV fluid pathway distal to the infusion pump. The Chemo Dispensing Pin™ device or similar devices with spikes should **not** be used with vials of paclitaxel since they can cause the stopper to collapse resulting in loss of sterile integrity of the paclitaxel solution.

Storage and Stability

Intact vials should be stored between 20° - 25°C (68° - 77°F) in the original package to protect from light, and remain stable until the expiration date on the label. Neither freezing nor refrigeration adversely affects stability. Upon refrigeration components in the paclitaxel vial may precipitate, but will redissolve upon reaching room temperature with little or no agitation. There is no impact on product quality under these circumstances. If the solution remains cloudy or if an insoluble precipitate is noted, the vial should be discarded. Solutions for infusion prepared as recommended

are stable at ambient temperature (approximately 25°C) and lighting conditions for up to 27 hours.

Administration

Paclitaxel will be administered as an IV infusion using an in-line 0.22 micron filter. Please see Section 8.3.1 for premedication regimen.

Toxicities

Myelosuppression, liver function test abnormalities (elevated SGOT, SGPT, bilirubin, alkaline phosphatase), nausea, vomiting, diarrhea, mucositis, peripheral neuropathy, transient asymptomatic bradycardia and much less frequently, arrhythmias, hypotension, hypersensitivity/anaphylaxis reactions (dyspnea, tachycardia, rash, urticaria, hypotension or hypertension), myalgias, arthralgias, and alopecia.

12.0 ANCILLARY THERAPY

12.1 Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on the C-260 form.

12.2 Treatment with hormones or other chemotherapeutic agents may not be administered except for steroids given for adrenal failure; hormones administered for non-disease-related conditions (e.g., insulin for diabetes, synthroid for hypothyroidism); and intermittent use of dexamethasone as an antiemetic and premedication for paclitaxel.

12.3 The use of dexrazoxane will not be permitted.

12.4 CALGB Policy Concerning the Use of Growth Factors

12.4.1 Erythropoetin (EPO)

The use of EPO is permitted at the discretion of the treating physician.

12.4.2 Filgrastim (G-CSF) and Related Agents

Filgrastim, sargramostim, or pegfilgrastim is strongly recommended for all cycles of therapy in order to achieve treatment on schedule, every 2 weeks, as well as to reduce incidence of neutropenic fever, as noted in sections 8.2, 8.3. and 10.1.2. Filgrastim or sargramostim will be obtained from commercial sources and is recommended to be administered on days 3-10, though it can be discontinued sooner if there is acceptable neutrophil recovery as determined by the treating physician. Pegfilgrastim will be obtained from commercial sources and should be administered between 24-36 hours after chemotherapy. These agents can be omitted from paclitaxel cycles if the treating physician feels that neutrophil recovery will occur in time to initiate the subsequent cycle on schedule. If there is a delay in treatment for any cycle, they are required for all subsequent cycles.

13.0 CRITERIA FOR EVALUATION

13.1 The primary endpoint of this study is duration of disease-free survival (DFS). Objective progression is defined as the appearance of local (chest wall, axillary, supraclavicular nodes) or distant metastases. For patients after lumpectomy, a recurrence in the same breast should be reported on appropriate forms and Remarks Addenda C-260. For patients who develop contralateral breast cancer, the treating physician should make a determination as to whether this represents a new primary or metastatic disease.

Patients should be followed for the occurrence of both first local and first distant disease progression. After the occurrence of local progression, follow the patient for distant progression, secondary malignancy and survival with appropriate documentation. In the event of distant progression first, continue to follow the patient for local recurrence, secondary malignancy and survival. After both local and distant progression, follow the patient for secondary malignancy and survival. Follow all patients for all major clinical endpoints, namely, first local, first distant progression and survival for 15 years from study entry or death, whichever occurs first. For patients who go on to receive non-protocol therapy, discontinue follow-up for toxicity, but continue to follow for local and distant disease progression, survival and secondary malignancy.

The following in and of themselves do not constitute progression; however, they should lead to restaging to detect other sites of possible metastases:

- A single new lesion on a bone scan without evidence of lytic disease on x-ray. Multiple new lesions or increased isotope uptake associated with new symptoms, however, are more likely due to metastases. Document with x-rays and clinical description.
- The appearance of a new primary (non-metastatic) cancer in the contralateral breast.

13.2 Secondary endpoints that will be recorded include overall survival and toxicity. Endpoints for the pharmacogenetic study include myelosuppression among the common MDR1 haplotypes and CYP3A5, CYP2C8 and CYP2B6 polymorphisms.

14.0 Removal of patients from protocol therapy

14.1 Disease Progression: If patient progresses locally or distantly, protocol treatment should be permanently discontinued. Contralateral breast cancer that is deemed to be a second breast cancer (Section 13.1) is not considered disease progression. However, if deemed to be metastatic disease, then it is considered disease progression. Follow patient as described in Section 13.1.

14.2 Extraordinary Medical Circumstances: If, at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair.
- Document the reason(s) for discontinuation of therapy on the CALGB: 40101 Treatment Summary Form (C-925).
- Follow the patient as indicated in Section 13.1.

15.0 STATISTICAL CONSIDERATIONS

15.1 Study Design

The design is a 2x2 factorial. The factors are chemotherapy agent and treatment length. Cyclophosphamide plus doxorubicin (CA) is the standard agent; the experimental agent is paclitaxel (T). The standard length is shorter (4 cycles) treatment duration; the experimental length is longer (6 cycles) duration. The trial is designed to show: (1) equivalence of the experimental agent T with the standard agent combination CA; and (2) superiority of longer versus shorter treatment duration. Patients will be randomized with equal probability to one of the four possible treatment arms. Prior to randomization, patients will be stratified by menopausal status (pre vs post), ER/PgR status (either positive or unknown vs both negative) and HER-2 status (positive, negative or unknown).

Update #8: Closure of 6-Cycle Arms for CA and Paclitaxel

The statistical aspects of this trial are unchanged. The AC versus T comparison continues. The 4- vs 6-cycle comparison will be based on only the concomitantly randomized patients. The AC vs T comparison will be as specified in the original protocol.

15.2 End Points:

The primary end point is duration of disease-free survival (DFS), which will be measured from study entry until first relapse, either local or distant, or death due to any cause, whichever occurs first. Surviving patients who are disease-free will be censored at the date last known to be free from disease. Secondary end points are overall survival (OS), local control, time to distant metastases and toxicity. OS will be measured from study entry until death due to any cause. Survivors will be censored at the date of last follow-up. Local control and distant metastasis will be calculated as the cumulative incidence of first local relapse and first distant metastasis, respectively.

15.3 Sample Size:

The target accrual of patients onto this study is 4646 at an anticipated monthly rate of 160 patients. Assuming a 2% dropout rate (not treatment-related), gives a total of 4556 treated patients at 157 patients per month. This will require approximately 29 months of patient accession.

15.4 Power Considerations:

The primary study end point upon which power is based is DFS. Power calculations were designed to detect effects in the marginal distributions of the two study factors, agent and length, and assume no interaction between the two factors. Calculations also assume exponential DFS and a total of 4556 treated patients accrued over 29 months and followed for four years after accrual termination for a total study time of 6.4 years.

Regarding the first factor, chemotherapeutic agent, we assume that the 5-year DFS of CA therapy is 88%. Regarding the other factor, length, we assume a 5-year DFS of 84.7% for the shorter duration. These assumptions are based upon results of SWOG 8897, in which an 18-week CA regimen was used to treat a similar but not identical patient population.

The following table shows the hypothesized 5-year DFS estimates.

Superiority: Length	Equivalence: Agent		Marginal
	CA	T	
Short (4 cycles)			84.7%
Long (6 cycles)			88%
Marginal	88%	84.7%	

Although the cell percentages are left unspecified, we assume no interaction.

Equivalence of CA and T: For T to be considered equivalent to the standard CA, a confidence interval of the hazard ratio of T to CA should be wholly to the left of 1.3, corresponding to a 30% increase in hazard rate. If the 5-year DFS for CA is 88% then an increase of 30% in hazard rate for T corresponds to 5-year DFS of 84.7%. The null hypothesis is that the hazard ratio of T to CA exceeds 1.3. The alternative hypothesis is that the two hazard rates are equivalent.

To assess the equivalence of CA with T, analyses of DFS will be conducted on a semiannual basis to coincide with the semiannual meetings of the DSMB. The first formal analysis will be undertaken and reported to the DSMB when 54 events have occurred. Fifty-four (54) events is equivalent to 10% of the expected number of events of 567 under the alternative hypothesis. At the current projections, 54 events will occur 18 months after start of accrual. From these projections we anticipate up to 10 interim looks and one final analysis.

Interim analyses will use only the lower stopping boundary to allow early stopping if T is worse than CA (i.e., accept the null hypothesis); we will not stop early for equivalence. We will construct confidence bounds on the observed log HR using Z(alpha)'s obtained from the Lan-DeMets spending function (O'Brien-Fleming). We will conclude that there is evidence to terminate the trial early if 0 is not included in the confidence interval.

Based on the interim analysis schedule above and assuming an overall 1-sided significance level of 0.05, the overall power is 89% to reject the null hypothesis and declare equivalence. The power estimate was obtained from simulations under the study parameters described above with interim analyses beginning at 18 months and performed every 6 months for up to 10 interim looks.

Superiority of 6 cycles over 4 cycles: The null hypothesis is that the hazards of both 6- and 4-cycle regimens are equal. The alternative hypothesis is a hazard ratio of 0.77, corresponding to a decrease of 23% in hazard due to longer duration of chemotherapy. If the 5-year DFS for 4 cycles is 84.7% then a decrease of 23% in hazard rate for 6 cycles corresponds to an increase in 5-year DFS to 88%. Assuming a 2-sided significance level of 0.05, there is 90.9% power to detect such an increase at the final analysis conducted 6.4 years after study activation.

To assess the superiority of 6 cycles over 4 cycles, DFS will be analyzed on the same schedule of interim testing used for the equivalence trial. As in the equivalence trial, formal monitoring will begin at 18 months after study activation and will continue at 6-month intervals for up to 10 interim looks and one final analysis.

We will use logrank tests stratified by menopausal status, receptor status and HER-2 status with 2-sided bounds constructed from the O'Brien-Fleming approach [21]. We will use the Lan-DeMets [22]spending function and truncate alpha levels at 0.001 for each interim analysis. The use of these interim analysis boundaries has a negligible effect on Type I and II error rates of the logrank test [23].

15.5 Data Analysis:

The final data analysis is planned at 6.4 years after study activation. The primary analysis will use proportional hazards models to calculate the hazard ratios and standard errors of the two main effects, agent (CA vs T) and length (6 vs 4 cycles). In addition, standard pretreatment variables of recognized prognostic importance (such as, tumor size, menopausal status and hormone-receptor status) will be considered as covariates in these models.

Although the study is not powered to assess the interaction between agent and length regarding DFS, we will include an interaction term in the main effects model. We will report from this model the p-value of the interaction term, considering <0.05 to be of statistical significance, and graphically display the relationship between agent and length using Kaplan-Meier curves of the 4 treatment arms.

The implications of a significant interaction term will depend on the strength and nature of the interaction and regimen-related toxicity. For instance, a negative interaction might suggest that single-agent T is less toxic but as effective as double-agent CA when both are given as 6 cycle regimens. In this case, the preferred regimen might be the one which uses only one agent, namely, T x 6. If the schedule of CA did not matter, then the shorter duration CA might be preferable. In the case of a positive interaction, the 4-cycle CA and 6-cycle T arms might be equally effective, but the 6-cycle CA regimen might be the most beneficial of all 4 regimens. The pros and cons of the longer treatment duration and extra drug would need to be weighed to decide between the CA x 4 and T x 6; however, due to the substantial benefits of the CA x 6 arm in this example it would be reasonable to advise in favor of CA at the longer duration.

A significant interaction term may also indicate the need to conduct a follow-up clinical trial to address the question further.

In secondary analyses we will calculate OS distributions using the Kaplan-Meier method and give logrank statistics when comparing two or more groups. We will test the difference between two or more groups in local control and distant metastases using the method of Pepe and Mori [24]. In view of previous trials showing a possible interaction between hormone-receptor status and use of paclitaxel, we will compare the benefits of the two types of chemotherapy within patient subsets of hormone-receptor positive (either ER and/or PgR positive or unknown) and negative. In addition, we will construct Kaplan-Meier graphs by treatment arm and by whether patients received the assigned dose.

Although there is no evidence to suggest that clinical outcome will differ by ethnicity and there is insufficient power to detect small or moderate effects, we will report these results by ethnicity in a secondary analysis.

Toxicity by treatment arm will be tabulated by type and severity.

15.6 Pharmacogenomic Correlative Studies

15.6.1 Candidate Gene Approach: Drug Metabolism and Transport Pharmacogenomics in Breast Cancer

Primary Objective

The primary objective of the pharmacogenomic correlative study is the assessment of the discrepancy of myelosuppression among the *MDR1* haplotype pairs (corresponding to nts 1236, 2677, and 3435): CGC/CGC, CGC/TTT and TTT/TTT. We will quantify myelosuppression by considering grade 4 granulocytopenia toxicity rates. In particular, we hypothesize a monotonic trend in myelosuppression among

these three groups. Since patients with the TTT/TTT haplotype are to have the highest plasma and intracellular levels the TTT haplotype would be associated with increased toxicity. A haplotype dose effect is predicted such that toxicity is lowest in the CGC/CGC group and highest in the TTT/TTT.

Although both adriamycin and paclitaxel are substrates of P-glycoprotein (the protein product of *MDR1*) only the plasma levels of adriamycin have been correlated with P-glycoprotein function. Co-administration of PSC 833 with adriamycin and paclitaxel increases plasma levels of these drugs and the effect on adriamycin is largely attributed to modulation of P-glycoprotein function (Advani et al. 2001). In contrast, the effects of PSC 833 on paclitaxel plasma levels are attributed to inhibition of CYP3A4. Therefore, we hypothesize that *MDR1* haplotype will influence hematological toxicity in the CA arm of CALGB 40101 and as such this analysis will be restricted to the CA arm.

Based on previous accrual from other studies, we expect to have approximately 80% of the patients receiving study treatment to consent to pharmacogenomic genotyping. Based on this, it would be reasonable to expect about 3600 samples available for the pharmacogenomic analyses, which corresponds to 1800 samples for each of the treatment arms.

The hypothesized frequencies for the two major *MDR1* haplotypes (genotypes at *MDR1* nts 1236, 2677 and 3435) are based on variant identification in a population of 100 Caucasians [60] and are tabulated in Table 1. Based on this, the expected prevalence rates for the three pairs of interest are 0.13 (CGC/CGC), 0.29 (CGC/TTT) and 0.16 (TTT/TTT) respectively. So it would be reasonable to expect n_1=234, n_2=522 and n_3=288 samples available for each group.

Table 1

1236	**2677**	**3435**	**Relative Frequency**
C	G	C	36%
T	T	T	40%
C	G	T	12%
Others	….	….	12%

Denoting the toxicity rates for these 3 pairs GCG/GCG, CGC/TTT and TTT/TTT by π_1, π_2 and π_3 respectively, the hypotheses of interest can be presented canonically as testing H_0: $\pi_1=\pi_2=\pi_3=\pi$ (for some unknown $\pi \varepsilon (0,1)$) versus H_1: $\pi_1 \le \pi_2 \le \pi_3$, where at least one of the two inequalities is strict. We will limit our focus to local alternatives where the log-odds ratio of any two consecutive pairs is constant. More specifically, we will assume that $\log((\pi_2(1-\pi_2))/(\pi_1\ (1-\pi_1)))= \log((\pi_3(1-\pi_3))/(\pi_2\ (1-\pi_2)))=\theta > 0$. The expected toxicity rates are based on the recently published results from CALGB 9741 [61]. In regimen III of this protocol patients received the dose-dense schedule of cyclophosphamide and doxorubicin that will be used in the present study. All patients on regimen III also received concurrent filgrastim treatment, which is highly recommended in 40101 so the toxicity rate is expected to be similar in these two studies. The incidence of grade 4 granulocytopenia on CALGB 9741 was 9%. We are assuming that the CCC/CCC haplotype group will have significantly decreased toxicity which we expect to be 5%. The power of the one-sided test, at the α=0.05 level of significance, is illustrated in Table 2 for various choices of $\theta \varepsilon \{0.40,0.45,0.50\}$ with π_1=0.05.

Table 2

π_1	θ	(π_1, π_2, π_3)	(n_1, n_2, n_3)	α	**Power**
0.05	0.40	(0.050,0.073,0.105)	(234,522,288)	0.05	0.73
0.05	0.45	(0.050,0.076,0.115)	(234,522,288)	0.05	0.83
0.05	0.50	(0.050,0.080,0.125)	(234,522,288)	0.05	0.90

Secondary Objectives:

Of further interest, is the effect of the *MDR1* haplotypes on DFS as defined in Section 13.1. This analysis will be carried out in the context of the standard Cox regression model of the form $\lambda_{ij} = \lambda_0 \exp[\alpha_i + \beta_j + (\alpha\beta)_{ij}]$ where i={1 (=TTT/TTT), 2 (=CGC/CGC)} and j={1 (=CA), 2 (=Taxol)}. This model will allow us to assess a potential interaction between the treatment and haplotype factor with respect to DFS and to assess the discrepancy in DFS between the CGC/CGC and TTT/TTT pairs adjusting for the treatment effect.

Analysis of the effect of *CYP3A5* and *CYP2B6* polymorphisms on DFS and on grade 4 neutropenia will be carried out using standard cox regression and logsitic regression methods. Further exploratory analysis may be carried out in this regard by employing classification tree based methods such as recursive partitioning.

It is preferable that registration to this companion study take place at the same time that patients are registered to the 40101 treatment trial, however, patients will be allowed to enroll to 60202 up to 60 days following registration to 40101.

Although not anticipated, there may be differences in the trends for "early" registrants (i.e., those registered to the companion 60202 at the same time of registering to the treatment trial 40101) versus "late" registrants (i.e. those registered to the companion 60202 within 60 days or registering to the treatment trial 40101). We will assess any potential discrepancy in the trends by comparing the results of the stratified ("early" versus "late") to that of the unstratified analysis.

15.6.2 Genome-Wide SNP Scan of Germline DNA

15.6.2.1 Primary Objectives:

The primary clinical endpoints for this study are grade 3-4 neutropenia in the adriamycin/cyclophosphamide arm (for objective 2.2.7), and grade 2 or greater peripheral neuropathy in the paclitaxel arm (for objective 2.2.8).

For each hypothesis, the primary statistical objective is the identification of specific SNPs and/or copy number variations that are associated with the primary clinical outcome of interest.

15.6.2.2 Pre-processing

For pre-processing (QC and genotype calls) the Illumina chips, we will use the commercial program Bead Studio developed by Illumina. Although, Illumina does not provide a Linux port of Bead Studio, one can run the software on VMWARE running on a Linux host. A two CPU dual core (four cores) AMD Opteron Socket F workstation with 16GB of RAM will be available for this purpose (the statistical analyses will be carried out on a Linux server with 8 dual core Opteron Socket F CPUs (16 cores) with 64GB of RAM [expandable to 128GB if needed]).

15.6.2.3 Analyses to Assess Genotyping Quality and Population Stratification

Initial quality studies will be conducted to identify SNPs that have generated sufficiently poor quality genotype data that they should be removed from analyses. Call rate, patterns of missing data, and departures from Hardy-Weinberg equilibrium (HWE) assessed using an exact test will all be scrutinized to identify markers that will not be used in analysis. In general, SNPs with call rates <95% and those with highly significant departures from HWE ($p < 10^{-7}$) will not be included in analyses. Non-random patterns of missing data are sometimes encountered in data generated on high-throughput genotyping platforms;

the most common non-random missing data problem is that heterozygous genotypes are more likely to be assigned as missing than either homozygous genotype. We will perform analyses using blind duplicates as well as analyses assessing the relationship between heterozygous call rates and missing data to identify any SNPs in which data are clearly not missing at random. Depending on the number and degree of difficulty observed, we will either remove problematic SNPs from analysis, or assign quality scores to reflect the extent of the non-random missing data.

Additional preliminary quality control analyses will be conducted to insure that the sample does not include duplicated samples or closely related individuals. These analyses can be rapidly conducted using PLINK [62]. Duplicated samples (or unrecognized identical twins) will be reduced to a single sample for further analyses. Although we do not expect to have closely related individuals included in this sample, only one member of any set of first-degree relatives will be included in subsequent analysis. For each sample, we will also generate a gender call based on the SNPs on the X chromosome and study the missingness patterns for the SNP on the Y and XY chromosomes in order to convincingly determine that all samples are from female patients.

Population structure that is not appropriately recognized and accommodated can lead to both false positive and false negative results in association studies. We will conduct studies using structure [63] to estimate ancestry proportions using 10,000 SNPs chosen for having no pairwise LD with unrelated individuals from the HapMap CEU, YRI and CHB+JPT samples used to model the ancestral populations. Substantial previous research has shown this to be a rapid and effective approach to defining historical geographic ancestry. Although self-identified race/ethnicity is usually highly correlated with estimated historical geographic ancestry, there are often a few individuals who appear to be misclassified with self-defined labels, and it is the genetically defined ancestry that is critical to correctly accommodate to insure robust results from association studies. Each individual will then have estimates of European, African and Asian ancestry. For individuals with high ancestry proportion for a single group (> 98%), we will conduct further analyses with eigenstrat [64] using all SNPs to determine whether there are additional important sources of variation among individuals leading to detectable stratification by allele frequencies (reflecting, for example, differences in ethnic make-up within individuals of European descent from different U.S. cities from which subjects for the trial were obtained). Primary analyses, described below, will be conducted within groups defined by historical geographic ancestry. Secondary analyses will be conducted using logistic regression with ancestry proportions (and any additional stratification identified using eigenstrat) as covariates.

15.6.2.4 Feature Discovery

The association between the genotype call (say AA, AB or BB) for each autosomal SNP and the clinical outcome [for example, adverse event (AE) or no AE] will be investigated within the framework of 2 by 3 contingency table for each of the major ancestry groups. Fisher's exact test (i.e., randomized conditional counterpart to Fisher's test for 2x3 tables) [65] will be used for carrying out inference on these tables. A feature (SNP) will be considered significant if the corresponding nominal unadjusted two-sided P-value is less than 0.05/K, where K is number of features which pass the pre-processing step. Needless to say, this approach may be conservative. It does however guarantee strict type I error control. The

type I error rate will not be adjusted across Objectives 1 and 2 as these are considered to be separate objectives.

For the sake of discussion, let B denote the risk allele with an assumed relative allelic frequency of q. Under the Hardy-Weinberg equilibrium assumption, the genotypes AA, AB or BB will have relative genotypic frequencies of (1-q2), 2q(1-q) and q2, respectively. Let Z denote the binary clinical outcome (Z=1 if the AE event occurs or =0 otherwise) and define the probability of an AE occurrence given the copies of the risk allele on the genotype, to be denoted by T, as

$$p_j=P[Z=1 \mid T=j], \qquad (1)$$

for j=0,1,2. The relationship between the event probability p=P[Z=1] in the general population is then expressible as

$$p=(1-q)^2p_0+2q(1-q)p_1+q^2p_2 \qquad (2)$$

The effect size in the context of genome-wide association (GWA) studies is typically quantified using the genotype relative risk (GRR) whose definition depends on the disease model. Under the recessive disease model $p_0=p_1$ and so

$$p=(1-q^2)p_0+q^2p_2, \qquad (3)$$

and

$$GRR=\frac{p_2}{p_1}=\frac{p_2}{p_0} \qquad (4)$$

while under the dominant disease model $p_1=p_2$ and so

$$p=(1-q)^2p_0+(2-q^2)p1, \qquad (5)$$

and

$$GRR=\frac{p_2}{p_0}=\frac{p_1}{p_0}. \qquad (6)$$

Finally, under the multiplicative disease mode, GRR=1/p0=p2/p1 and so

$$p=(1-q)^2GRR^{-1}+2q(1-q)+q^2GRR.$$

Grade 3-4 neutropenia has a prevalence of about 27% in the adriamycin/cyclophoshamide arm. Grade 2 or greater peripheral neuropathy has a prevalence of about 22% in the paclitaxel arm. There are currently over 1000 samples from each arm with consent banked at the CALGB Pathology Coordinating Office. This number is expected to increase as the main clinical study (CALGB 40101) is still accruing.

The power, at the two sided 0.05/500000 level (i.e., assume K=500,000 autosomal SNP markers pass through the pre-processing step), is illustrated in tables 1 (for objective 2.2.7) and 2 (for objective 2.2.8).

Recessive	q	0.5	0.18	0.31	0.44	0.57	0.70
	GRR	**5.20**	**2.30**	**2.10**	**2.10**	**2.30**	**3.00**
Dominant	q	0.28	0.40	0.53	0.65	0.78	0.90
	GRR	**7.30**	**3.20**	**2.40**	**2.10**	**2.10**	**2.30**
Multiplicative	q	0.05	0.22	0.39	0.55	0.72	0.89
	GRR	**5.20**	**2.00**	**1.70**	**1.70**	**1.70**	**2.10**

Table 1: Power illustration for objective 2.2.7: The minimum Genotype Relative Risk (GRR) detectable with a power of 0.9, at the two-sided level of 0.05/500,000 for a range of relative allele frequencies (q) assuming the event (grade 3-4 neutropenia) probability is P[D=1]=0.27 under recessive, dominant and multiplicative models assuming HWE. The sample size used in the illustration is based on N0=730 (controls) and N1=270 (cases) for a total of N=1000 patients.

Recessive	q	0.05	0.18	0.31	0.44	0.57	0.70
	GRR	**6.10**	**2.50**	**2.30**	**2.30**	**2.70**	**3.50**
Dominant	q	0.28	0.40	0.53	0.65	0.78	0.90
	GRR	**8.70**	**3.70**	**2.70**	**2.40**	**2.30**	**2.60**
Multiplicative	q	0.05	0.22	0.39	0.55	0.72	0.89
	GRR	**5.90**	**2.10**	**1.90**	**1.80**	**1.90**	**2.30**

Table 2: Power illustration for objective 2.2.8: The minimum Genotype Relative Risk (GRR) detectable with a power of 0.9, at the two-sided level of 0.05/500,000 for a range of relative allele frequencies (q) assuming the event (grade 3-4 neuropathy) probability is 0.22 under recessive, dominant and multiplicative models assuming HWE. The sample size used in the illustration is based on N0=780 (controls) and N1=220 (cases) for a total of N=1000 patients.

15.6.2.5 Submission of Molecular Data

The laboratory of Dr. Yusuke Nakamura will submit the Illumina *.idat image files using secure means to the CALGB Statistical Center. The lab will also submit a table along with this transmission, which at the minimum will provide the following information for each sample received from the repository.

- The lab ID number provided by the repository.
- The experimental ID, a concatenation of the plate, well and replicate information, generated by the lab.
- The idat file names (the file string name will contain the Lab ID).
- The md5sum signature of the idat files to ensure data integrity.
- The date the specimen was received from the repository.
- The date the sample was analyzed by the RIKEN laboratory.

Additionally, the lab will also provide the complete results from any quality control measures carried out. If a sample had to be redone (e.g., defective or poor quality array), the lab will provide all replicate idat files and add an appropriate column to the supplementary table. The molecular data generated for this aim may not be shared with other investigators or used for any analysis not specified in the protocol until a formal approval from the CALGB Statistical Center is obtained.

15.6.2.6 Other Secondary Objectives (2.2.9, 2.2.10 and 2.2.11)

Logistic regression models and conditional inference trees (or more generally conditional random forests) will be used to construct multi-variable models based on the SNPs identified as interesting. These models also allow for inclusion of other potentially relevant clinical and demographic variables.

The Illumina HumanHap550 contains 4,300 SNPs in regions with common copy number variants (CNVs). Given the complex structure of CNVs, it is not always clear how to define the genotype of a CNV. Instead of categorizing copy numbers into genotypes, we will estimate relative genomic abundance probe intensities. This approach allows for the consideration of other CNVs beyond deletions, including duplications and combinations of both. For notational brevity, we shall refer to these as CNV markers.

For each objective, the association between each CNV marker and the clinical AE endpoint, will be assessed using the Wilcoxon two-sample test. The family-wise error rate will be controlled at the 0.05 level using permutation resampling (based on B=10,000 replicates).

Regression methods, as in the case of the SNP markers, will be employed to construct multivariable models based on the CNV markers.

Secondary clinical endpoints are disease-free and overall survival. CALGB has not yet reached its accrual goals and there is no preliminary data yet on these endpoints.

In addition to conducting analyses on all features directly assessed on the high-throughput platform used in these studies, we will also interrogate all additional HapMap SNPs that are not in strong pairwise LD with any genotyped SNP but for which there is sufficient multi-locus LD to SNPs on the high-throughput platform. TUNA (Testing UNtyped Alleles) is a robust approach for conducting such analyses that provides inexpensive in silico follow up to the initial analysis and allows us to more efficiently design any follow up genotyping studies [66,67]. For example, use of Illumina HumanHap300 enables direct testing of 270K-450K SNPs, and indirect testing of 750K-1.5M additional SNPs (i.e. these SNPs are so highly correlated with SNPs that are directly tested for association that testing them would provide little additional information). The ranges given above bracket the expectations for different human populations, with European populations at the high end of the range, and populations of recent African descent at the lower end. Use of TUNA enables interrogation of an additional 100K-250K SNPs that are neither on the platform nor highly correlated with any individual SNP on the platform. Note that use of TUNA will facilitate comparisons to genome-wide association studies on potentially related phenotypes (e.g. clinical trials of the same or related drugs) conducted using other high-throughput platforms or candidate gene studies utilizing SNPs not directly genotyped on the high-throughput platform chosen for our studies.

15.6.2.7 Statistical Software

The R statistical environment [68] and Bioconductor [69] packages will be used for all of the primary statistical analyses relating features to phenotypes. Specialized statistical genetics software, including PLINK [62], structure [63], eigenstrat [64], and TUNA [66,67] will be used for some of the quality or secondary analyses, and R will be used for logistic regression analyses allowing for ancestry covariates.

16.0 ADVERSE EVENT REPORTING (AER)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. CALGB investigators are required to notify the CALGB Central Office, the Study Chair, and their Institutional Review Board if a patient has a reportable serious adverse event defined by the guidelines below. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting beginning April 1, 2010. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

All reactions in a "reportable" category must be reported using the NCI Adverse Event Expedited Reporting System (AdEERS).

CALGB requires investigators to route all adverse event reports (AERs) through the Central Office for CALGB-coordinated studies.

Reporting requirements for CALGB 40101: AdEERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of Treatment

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization	Unexpected without Hospitalization	Expected with Hospitalization	Expected without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hrs; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**

AdEERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 4 and Grade 5 unexpected events

AdEERS 10 calendar day report:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions (see below).

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND or a Non-CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Additional Instructions or Exclusions to AdEERS Expedited Reporting Requirements for Phase 2 and 3 Trials:

- All adverse events reported via AdEERS (i.e., serious adverse events) should also be forwarded to your local IRB.

- The drugs in this study are commercially available, but they are being used in an investigational setting (e.g. dose dense). The study is IND exempt, however the expedited reporting requirements in the table above apply to this study.

- Grade 3/4 myelosuppression and hospitalization resulting from grade 3/4 myelosuppression do not require AdEERS, but should be submitted as part of study results.

- A list of agent specific expected adverse events can be found in Section 11.0 (Drug Formulation, Availability and Preparation). Additional information regarding expected adverse events can be obtained from the package insert and the Physicians' Desk Reference®.
- To report cases of secondary AML/MDS, use the NCI/CTEP Secondary AML/MDS Report Form. New primary malignancies should be reported using the CALGB C-929 form.

- AdEERS reports are to be submitted electronically (http://ctep.info.nih.gov/reporting/adeers.html) to the CALGB Central Office(calgb@uchicago.edu). Faxed (312-345-0117) copies of the AdEERS paper template (downloadable from the AdEERS web page) will also be accepted, but electronic submission is preferred.

- The reporting of adverse events described in the table above is in addition to and does not supplant the reporting of adverse events as part of the report of the results of the clinical trial, e.g., study summary forms or cooperative group data reporting forms (see Section 6.2 for required CALGB forms).

17.0 REFERENCES

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18.0 MODEL CONSENT FORM:

CYCLOPHOSPHAMIDE AND DOXORUBICIN (CA X 4 CYCLES) VERSUS PACLITAXEL (4 CYCLES) AS ADJUVANT THERAPY FOR BREAST CANCER IN WOMEN WITH 0-3 POSITIVE AXILLARY LYMPH NODES: A PHASE III RANDOMIZED STUDY

(RANDOMIZATION TO TREATMENT GROUPS 2 AND 4 IS NO LONGER AVAILABLE, EFFECTIVE 12/15/07)

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision. Discuss it with your friends and family.

You are being asked to take part in this study because you have had breast cancer that has been removed by surgery, with either no lymph nodes or as many as 3 lymph nodes under your arm involved with cancer.

WHY IS THIS STUDY BEING DONE?

You have recently had surgery to remove your breast cancer. Your doctor has determined that adjuvant chemotherapy (adjuvant means "in addition" to the surgery) is advisable for your stage of breast cancer. Previous clinical trials have shown that use of chemotherapy reduces the likelihood that the cancer will come back in other parts of the body, such as the lungs, liver, bone, or elsewhere. A commonly used chemotherapy treatment, which has been a standard treatment for many patients with your type of cancer, is the combination of cyclophosphamide and doxorubicin (CA), given through a vein every 2 or 3 weeks for 4 treatments.

The purpose of this study is to compare the effectiveness of the standard adjuvant chemotherapy CA with the chemotherapy drug paclitaxel. In addition, we will learn more about the side effects of each treatment, and compare them with each other, in order to measure the effectiveness and tolerability of the treatments.

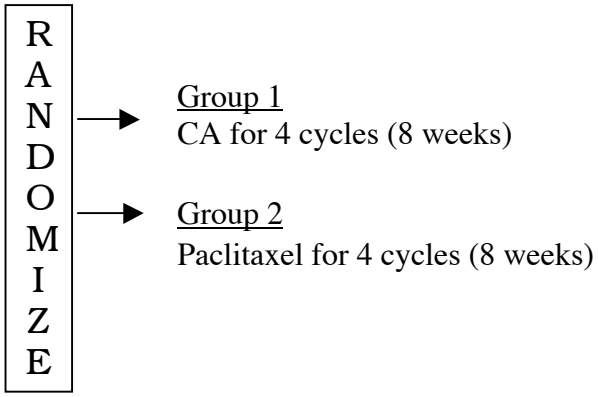
Cyclophosphamide and doxorubicin have been approved by the Food and Drug Administration of the United States (FDA) for the treatment of breast cancer. Paclitaxel given after combination chemotherapy has been approved for the adjuvant treatment of breast cancer that had spread to the lymph nodes. Paclitaxel is also approved for the treatment of breast cancer that has grown or that has spread to other parts of the body after previous chemotherapy. The use of paclitaxel as an adjuvant treatment for breast cancer that has not spread to the lymph nodes, as used in this study, are considered to be investigational or research.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 4646 women will take part in this study.

WHAT IS INVOLVED IN THE STUDY?

If you agree to participate, you will be "randomized" into one of the study treatment groups described below. Randomization means that you are assigned to a group by chance. The treatment group you are assigned to is chosen by a computer. Neither you nor your study doctor will choose which group you will be in. You will have an equal chance of being assigned to either of the 2 groups.



Group 1

If you are assigned to this treatment group, you will receive standard CA combination chemotherapy as an outpatient in the clinic. You will be given cyclophosphamide through a vein, followed by doxorubicin through a vein on day 1, every 14 days. This 14 day period is called a treatment cycle. You will receive 4 cycles of this treatment.

Group 2

If you are assigned to this treatment group, you will be given medications before the paclitaxel that have been shown to prevent allergic reactions, which can be severe. You will then be given the drug paclitaxel as an outpatient in the clinic. You will be given the paclitaxel through a vein, over 3 hours on day 1, every 14 days. This 14 day period is called a treatment cycle. You will continue to receive this treatment every 14 days for 4 cycles.

All Treatment Groups

Filgrastim or sargramostim, medications that are stimulators of white blood cell growth, is recommended to be given as an injection under the skin, for about a week after each treatment, beginning 2 days after the treatment and continuing until about 10 days after the treatment. You, a family or a friend may be taught to give these injections at home. Pegfilgrastim is another medication that stimulates white blood cell growth. It is given as an injection under the skin, as a single dose, 24-36 hours following each cycle of treatment.

After you have completed the study treatment, if your breast cancer is the type that is responsive to hormones your doctor may recommend hormonal therapy. Tamoxifen may be recommended for women who have not yet experienced menopause. Tamoxifen, anastrozole, exemestane or letrozole may be recommended for women who have experienced menopause.

If your breast cancer is the type that is responsive to trastuzumab (also known as Herceptin®) your doctor may recommend treatment with this drug.

If the type of surgery you had to remove your breast cancer was a "lumpectomy" you will receive radiation therapy to the breast. If the type of surgery you had was a "mastectomy" your doctor may also recommend that you receive radiation therapy to the breast. If you are going to receive radiation therapy it may be given before the study chemotherapy has been started or after it has been completed.

Testing and Follow-up

If you take part in this study, you will have the following routine tests and procedures before the study treatment begins: you will be asked to give your medical history and have a physical examination, blood tests, an electrocardiogram (EKG) and a chest x-ray.

During the time that you are receiving the study treatment, a physical examination and blood tests will be done on day 1 of each treatment cycle. EKGs will be done as your study doctor feels necessary.

After the study treatment has been completed, the physical examinations will be done every 6 months for 2 years, then annually thereafter. The blood tests and chest x-rays will be done as your doctor feels necessary.

HOW LONG WILL I BE IN THE STUDY?

We think you will receive study treatment for about 2 months. After the treatment has been completed your doctor will follow your medical condition for up to 15 years to learn about the long-term effects of the study treatment.

Your study doctor will discontinue the study treatment if: the breast cancer returns in the breast or an area outside the breast; you experience intolerable side effects; you and your study doctor decide that it is in your best interest to stop; or if new information becomes available which suggests that the study treatment is unsafe or not effective for you.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to your study doctor first.

WHAT ARE THE RISKS OF THE STUDY?

While on the study, you are at risk for these side effects. You should discuss these with your study doctor. There also may be other side effects that we cannot predict. Other drugs will be given to make side effects less serious and uncomfortable. Many side effects go away shortly after the study drugs are stopped, but in some cases side effects can be serious or long-lasting or permanent.

CYCLOPHOSPHAMIDE + DOXORUBICIN (CA)

Likely

- Temporary lowering of the number of white blood cells (cells that help your body fight infection)
- Temporary lowering of the number of red blood cells (may cause a feeling of tiredness, and shortness of breath)
- Temporary lowering of the number of blood platelet cells (cells that help your blood clot)
- Nausea, vomiting or diarrhea
- Loss of appetite
- Temporary loss of scalp and body hair
- Skin and nail discoloration
- Sores in the mouth and/or throat
- Urine may turn red for 1-2 days (due to the color of the doxorubicin)
- Irritation of the bladder (where urine is stored)
- Sensitivity to sunlight
- Premenopausal women may experience irregular periods or stop menstruating altogether for a time. The ability to have children may be permanently impaired.

Less Likely, But Serious

- Blood in the urine
- Heart damage
- Irregular heart beat (may occur right after the drugs are given)
- Congestive heart failure (a decrease in the heart's ability to pump effectively, which may lead to shortness of breath)
- Skin tissue damage if some of the drug leaks from the vein while it is being given
- Acute leukemia
- Serious, potentially life threatening allergic reaction

PACLITAXEL

Likely

- Temporary lowering of the number of white blood cells (cells that help your body fight infection)
- Temporary lowering of the number of red blood cells (may cause a feeling of tiredness, and shortness of breath)
- Temporary lowering of the number of blood platelet cells (cells that help your blood clot)
- Diarrhea
- Loss of scalp and body hair
- Temporary or mild numbness or tingling in the fingers or the toes
- Temporary pain in the muscles and joints

Less Likely

- Nausea, vomiting
- Mouth sores (like canker sores)
- Problems with liver function (as seen on a blood test)

Less Likely, But Serious

- Allergic reactions, which may include rash and shortness of breath (may happen while the drug is being given)
- Slow or irregular heart beat
- Low blood pressure
- High blood pressure
- Numbness or tingling in the fingers or toes that may cause difficulty in walking or buttoning clothes on a long term basis

Reproductive risks: Because the drugs in this study can affect an unborn baby, you should not become pregnant while on this study. If you are a woman of childbearing potential you must practice an effective, non-hormonal method of birth control while receiving study treatment and for at least 2 months after completing or discontinuing study treatment. Ask about birth control counseling and more information about preventing pregnancy. You should not nurse your baby while on this study.

Medications which must not be taken while receiving study treatment: Hormone therapy including oral contraceptives "the pill", postmenopausal hormone replacement therapy and raloxifene. If you are taking any of these medications you must discontinue their use before study entry. Your study doctor will discuss this with you.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope the information learned from this study will benefit other patients with breast cancer in the future.

WHAT OTHER OPTIONS ARE THERE?

Other treatment options include: adjuvant chemotherapy with CA, or different drugs, and possibly radiation therapy or hormonal therapy.

Your doctor will discuss the treatment options with you.

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

The results of this study may be published, but individual patients will not be identified in these publications.

Your medical record, including identifying information may be inspected and/or photocopied by: The Cancer and Leukemia Group B (CALGB), the National Cancer Institute (NCI), the Food and Drug Administration of the United States, or other Federal or state government agencies in the ordinary course of carrying out their governmental functions. If you are participating in this study through the Cancer Trials Support Unit (CTSU is a clinical trials mechanism sponsored by the NCI to provide greater access to phase III trials), a record of your progress will also be kept by the CTSU. If your record is used or disseminated for such purposes, it will be done under conditions that will protect your privacy to the fullest extent possible consistent with laws relating to public disclosure of information and the law-enforcement responsibilities of the agency.

WHAT ARE THE COSTS?

Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury.

You or your insurance company will be charged for continuing medical care and/or hospitalization.

You will receive no payment for taking part in this study.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or willingness to continue in this study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

A Data and Safety Monitoring Board, an independent group of experts, will be reviewing the data from this research throughout the study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact the researcher *NAME(S)* at *TELEPHONE NUMBER*.

For questions about your rights as a research participant, contact the *NAME OF CENTER* Institutional Review Board (which is a group of people who review the research to protect your rights) at *TELEPHONE NUMBER*. *[And, if available, list patient representative (or other individual who is not on the research team or IRB).]*

It may be necessary to contact you at a future date regarding new information about the treatment you have received. For this reason, we ask that you notify the institution where you received treatment on this study of any changes in address.

If you move, please provide your new address to the following person:
(name)_____ (title)_____
(address)_____
(phone number) _____

WHERE CAN I GET MORE INFORMATION?

You may call the NCI's **Cancer Information Service** at **1–800–4–CANCER (1–800–422–6237)** or **TTY: 1–800–332–8615**

Visit the NCI's Web sites…
cancerTrials: comprehensive clinical trials information **http://www.cancer.gov.clinical_trials/**

CancerNet™: accurate cancer information including PDQ **http://www.cancer.gov/cancer_information/**

You will get a copy of this form. You may also request a copy of the protocol (full study plan).
[Attach information materials and checklist of attachments. Signature page should be at the end of package.]

SIGNATURE

I agree to take part in this study.

Participant _____ Date _____

RELATED TISSUE STUDIES

In addition to the treatment study, the researchers are also interested in studying some of the tissue that was taken from you in the normal course of treatment and care. If you agree, a sample of your breast cancer tissue, which was taken at the time of diagnosis for routine testing, will be sent to a CALGB research laboratory for future research studies.

Where does tissue come from?
After a person has had a biopsy (or surgery) and all tests have been done, there may be some left over tissue. Sometimes, this tissue is thrown away because it is not needed for the patient's care. Instead, a patient can choose to have the tissue kept for future research. People who are trained to handle tissue and protect donors' rights make sure that the highest standards of quality control are followed by the CALGB. If you agree, only left over tissue will be saved for research. Your doctor will not take more tissue during surgery than needed for your care.

Why do people do research with tissue?
Research with tissue can help to find out more about what causes cancer, how to prevent it, how to treat it, and how to cure it. Research using tissue can also answer other health questions. Some of these include finding the causes of diabetes and heart disease, or finding genetic links to Alzheimer's.

The research that may be done with your breast cancer tissue samples probably will not help you. However, it might help people in the future who have breast cancer or other diseases.

What type of research will be done with my tissue?
Many different kinds of studies use tissue. Some researchers may develop new tests to find diseases. Others may develop new ways to treat or even cure diseases. In addition, some of this tissue may be used to establish products that could be patented and licensed. There are no plans to provide financial compensation to you should this occur.

Will I find out the results of the research using my tissue?
You will receive the results of your biopsy, but you will not receive the results of research done with your tissue. This is because research can take a long time and must use tissue samples from many people before results are known. Results from research using your tissue may not be ready for many years and will not affect your care right now, but they may be helpful to people like you in the future.

How am I protected?
Tissue samples will be stored at a CALGB Pathology Coordinating Office. The CALGB Statistical Center will perform all analyses of data and store all study results. Your tissue sample will not be stored with your name on it. Instead, it will be labeled with a special CALGB identification number. The only location where your name and special identification number will be stored together is at the CALGB Statistical Center. The greatest effort will be made to see that all personal information that can identify you is kept under conditions that protect your privacy.

Information about your participation in future studies and results of any tests performed on your sample will be kept only at the CALGB Statistical Center. This information will not be made available to your doctors or to individual researchers at CALGB. Test results from this study will not be put in your medical records. All study information, including test results, is stored under conditions that limit access in order to protect the privacy of the women participating in this study.

If you decide now to allow your tissue sample to be used in these and future studies and then change your mind at any time about participating in the studies, just contact your institution and let them know that you do not want the researchers to use your sample. Then it will no longer be used for research.

The CALGB will protect your records so that your name, address and phone number will be kept private. Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

The results from these studies may be published, but individual patients will not be identified in these publications.
There will be no charge to you for participating in these research studies.

Your participation in these research studies is entirely voluntary. You do not have to participate in this portion of the study to receive the study treatment.

My tissue may be kept for use in research to learn about, prevent, treat or cure cancer.

____Yes ____No _____Initials _____ Date_____

My tissue may be kept for research about other health problems (for example: causes of diabetes, Alzheimer's disease and heart disease).

_____Yes _____No _____Initials _____ Date_____

Someone from the CALGB may contact me in the future to ask me to take part in more research.

_____Yes _____No _____Initials _____ Date_____

RELATED BLOOD STUDIES

Many factors contribute to the success of chemotherapy, including the way the human body processes the chemotherapy drugs. The researchers conducting the treatment study would also like to collect an additional sample of your blood for the purpose of learning about how certain genes influence the effectiveness and side effects of adjuvant breast cancer treatment with the chemotherapy drug combination of cyclophosphamide plus doxorubicin or paclitaxel given alone. In order to study the genes the DNA must be removed from your blood sample. DNA is the substance that makes up your genes. Genes are the units of inheritance that are passed down from generation to generation. They are responsible for eye color, hair color, blood type and hundreds of other traits.

These tests will not involve the study of cancer genes that can be inherited (passed from parents to children).

About 1 to 2 teaspoons of blood will be drawn before you begin the study treatment.

The results of these research studies will not be given to your doctor or to you, nor will the results have any effect on your treatment.

There will be no charge to you for participating in these studies.

Safeguards of Confidentiality in Studies Involving Genes

- Blood samples will be stored at a CALGB laboratory. Your blood sample will not be stored with your name on it. Instead, it will be labeled with a special CALGB identification number. The CALGB Statistical Center will perform all analyses of data and store all study results. The only location where your initials, special identification number and the results of the laboratory tests will be stored together is at the CALGB Statistical Center. The results from these studies may be published, but individual patients will not be identified in the publications.
- Your blood will be used only for the study of genes involved in cancer.
- If you decide now to give a sample of blood and then later change your mind at any time about participating in the study, you should contact your institution and let them know that you do not want the researchers to use your sample. Then it will no longer be used for research.
- Because it is not possible for the CALGB to know what studies of breast cancer may be appropriate in the future we would like to store your DNA for future studies. Your DNA samples for future studies would be protected as described above. Future investigators must apply to the CALGB and have their research project reviewed and approved by the CALGB. There will be no charge to you for participating in the future research studies.

Your participation in the studies described above and/or in this DNA specimen bank for future studies is entirely voluntary. You do not have to participate in this portion of the study to receive the study treatment.

In addition, your blood may be used to develop products that could be patented and licensed. There are no plans to provide financial compensation to you should this occur.

My blood may be used for the purpose of learning about how certain genes influence the effectiveness and side effect of adjuvant breast cancer, as described above.

____Yes ____No Initials _____ Date_____

I agree that my DNA obtained from my whole blood may be kept for use in future DNA research studies to learn more about cancer.

_____Yes _____ No Initials _____ Date _____

APPENDIX I

CANCER TRIALS SUPPORT UNIT (CTSU) PARTICIPATION PROCEDURES

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION:

To submit site registration documents:
CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103
Phone: 1-888-823-5923
Fax: 215-569-0206

For patient enrollments:
CTSU Data Operations Center
Phone: 1-888-462-3009
Fax: 1-888-691-8039
Hours: 9:00 AM – 5:30 PM Eastern Time, Monday – Friday (excluding holidays)
[Registrations received after 5:00 PM ET will be handled the next business day. For CTSU patient enrollments that must be completed within approximately one hour, or other extenuating circumstances, call 301-704-2376 between 9:00 AM and 5:30 PM.]

Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:

CALGB Statistical Center
Hock Plaza
2424 Erwin Road, Suite 802
Durham, NC 27705
Tel: 919-668-9350
Data Operations Fax: 919-668-9348
Teleform Fax: 919-416-4990

Sites should submit Teleforms via Fax or Mail. See section 6.2 Data Submission Section for details on forms submission.

Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.

For patient eligibility or treatment related questions: Contact the CALGB 40101 Study Chair.

For questions unrelated to patient eligibility, treatment, or data submission contact the CTSU Help Desk by phone or e-mail:

CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.

The CTSU Public Web site is located at: www.ctsu.org

The CTSU Registered Member Web site is located at http://www.ctsu.org

REGISTRATION/RANDOMIZATION

Prior to the recruitment of a patient for this study, investigators must be registered members of the CTSU. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU registered member Web site or by calling the PMB at 301-496-5725 Monday through Friday between 8:30 a.m. and 4:30 p.m. Eastern time.

Each CTSU investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site at http://www.ctsu.org

All forms and documents associated with this study can be downloaded from the CALGB-40101 Web page on the CTSU registered member Web site (https://www.ctsu.org). Patients can be registered only after pre-treatment evaluation is complete, all eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS.

Requirements for CALGB-40101 site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

Pre-study requirements for patient enrollment on CALGB-40101:

- Patient must meet all inclusion criteria, and no exclusion criteria should apply
- Patient has signed and dated all applicable consents and authorization forms, and the patient decision whether to permit use of tissue for related studies and future studies has been documented.
- All baseline laboratory tests and prestudy evaluations performed within the timeframes specified in the protocol.

CTSU Procedures for Patient Enrollment

1. Contact the CTSU Patient Registration Office by calling 1-888-462-3009 between 9:00 AM and 5:30 PM Eastern Time, Monday-Friday. Leave a voicemail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs, e.g. within one hour, call the registrar cell phone at 1-301-704-2376.

2. Complete the following forms:

   • CTSU Patient Enrollment Transmittal Form
   • CALGB 40101 Eligibility Checklist
   • CALGB Registration Worksheet

3. Fax these forms to the CTSU Patient Registrar at 1-888-691-8039 between the hours of 8:00 a.m. and 8:00 p.m., Mon-Fri, Eastern Time (excluding holidays). This is limited to the operating hours of the CALGB Registration Office. The CTSU registrar will check the investigator and site information to ensure that all regulatory requirements have been met. The registrar will also check that forms are complete and follow-up with the site to resolve any discrepancies.

4. Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will contact the CALGB, within the confines of CALGB's registration hours. The CTSU registrar will access the CALGB's on-line registration system, to obtain assignment of a treatment arm and assignment of a unique patient ID (to be used on all future forms and correspondence). The CTSU registrar will confirm registration by fax.

<u>Procedures for late enrollment onto CALGB 60202 (pharmacogenomic companion):</u>

- Submit CTSU Patient Enrollment transmittal form (with note indicating delayed registration to the pharmacogenetic companion study).
- Submit revised CALGB 40101 Registration Worksheet (indicating patient consent for CALGB 60202).

Note: Although it is preferable that patients are registered to 60202 at the same time they are registered to 40101, registration to 60202 may occur up to 60 days following registration to the treatment trial.

DATA SUBMISSION AND RECONCILIATION

1. All case report forms (CRFs) associated with this study must be downloaded from the CALGB-40101 Web page located on the CTSU registered member Web site (https://www.ctsu.org). Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.

2. Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and transmittals directly to the CALGB Statistical Center, [see contact table and section 6.2 of protocol] unless an alternate location is specified in the protocol. Do not send study data to the CTSU. A completed CTSU-CALGB coversheet should accompany all data submissions.

3. The CALGB Statistical Center will send (generally via email but may be sent via postal mail or fax) query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the CALGB Statistical Center (via postal mail) and do not copy the CTSU Data Operations. Each site should have a designated CTSU Administrator and Data Administrator and must keep their CTEP AMS account contact information current. This will ensure timely communication between the clinical site and the CALGB Statistical Center.

SPECIAL MATERIALS OR SUBSTUDIES

1. Pathology Submission (Protocol section 6.3)
    - Patient consent required.
    - Collect, prepare, and submit specimens as outlined in the protocol
    - Do not send specimens, supporting clinical reports, or transmittals to the CTSU
2. Whole Blood Submission (CALGB 60202) (Protocol section 6.4)
    - Patient consent required.
    - Collect, prepare, and submit specimens as outlined in the protocol
    - Do not send specimens, supporting clinical reports, or transmittals to the CTSU

SERIOUS ADVERSE EVENT (AE) REPORTING (SECTION 16.0)

1. CTSU sites must comply with the expectations of their local Institutional Review Board (IRB) regarding documentation and submission of adverse events. Local IRBs must be informed of all reportable serious adverse reactions.

2. CTSU sites will assess and report adverse events according to the guidelines and timelines specified in the protocol. You may navigate to the CTEP Adverse Event Expedited Report System (AdEERS) from either the Adverse Events tab of the CTSU member homepage (https://www.ctsu.org) or by selecting Adverse Event Reporting Forms from the document center drop down list on the protocol number Web page.

3. Do not send adverse event reports to the CTSU.

4. Secondary AML/MDS/ALL reporting: Report occurrence of secondary AML, MDS, or ALL via the NCI/CTEP AML-MDS Report Form in lieu of AdEERS. Submit the completed form and supporting documentation as outlined in the protocol.

DRUG PROCUREMENT (SECTION 11.0)

<u>Commercial agents:</u> Cyclophosphamide, Doxorubicin, and Paclitaxel

1. Information on drug formulation, procurement, storage and accountability, administration, and potential toxicities are outlined in section 11.0 of the protocol.

2. You may navigate to the drug forms by selecting Pharmacy Forms from the document center drop down list on the CALGB-40101 Web page.

REGULATORY AND MONITORING

Study Audit

To assure compliance with Federal regulatory requirements [CFR 21 parts 50, 54, 56, 312, 314 and HHS 45 CFR 46] and National Cancer Institute (NCI)/ Cancer Therapy Evaluation Program (CTEP) Clinical Trials Monitoring Branch (CTMB) guidelines for the conduct of clinical trials and study data validity, all protocols approved by NCI/CTEP that have patient enrollment through the CTSU are subject to audit.

Responsibility for assignment of the audit will be determined by the site's primary affiliation with a Cooperative Group or CTSU. For Group-aligned sites, the audit of a patient registered through CTSU will become the responsibility of the Group receiving credit for the enrollment. For CTSU Independent Clinical Research Sites (CICRS), the CTSU will coordinate the entire audit process.

For patients enrolled through the CTSU, you may request the accrual be credited to any Group for which you have an affiliation provided that Group has an active clinical trials program for the primary disease type being addressed by the protocol. Per capita reimbursement will be issued by the credited Group provided they have endorsed the trial, or by the CTSU if the Group has not endorsed the trial. Details on audit evaluation components, site selection, patient case selection, materials to be reviewed, site preparation, on-site procedures for review and assessment, and results reporting and follow-up are available for download from the CTSU Operations Manual located on the CTSU Member Web site.

Health Insurance Portability and Accountability Act of 1996 (HIPAA)

The HIPAA Privacy Rule establishes the conditions under which protected health information may be used or disclosed by covered entities for research purposes. Research is defined in the Privacy Rule referenced in HHS 45 CFR 164.501. Templated language addressing NCI-U.S. HIPAA guidelines are provided in the HIPAA Authorization Form located on the CTSU website.

The HIPAA Privacy Rule does not affect participants from outside the United States. Authorization to release Protected Health Information is NOT required from patients enrolled in clinical trials at non-US sites.

Clinical Data System (CDS) Monitoring

This study will be monitored by the Clinical Data System (CDS-web). Cumulative CDS data will be submitted quarterly to CTEP by electronic means. The sponsoring Group fulfills this reporting obligation by electronically transmitting to CTEP the CDS data collected from the study-specific case report forms.