AD_____

Award Number: DAMD17-97-1-7088

TITLE: Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer: Pilot Study to Evaluate Incidence

PRINCIPAL INVESTIGATOR: Charles A. Coltman, Jr., M.D.

CONTRACTING ORGANIZATION: CTRC Research Foundation
San Antonio, Texas 78229-3264

REPORT DATE: September 2001

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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13. ABSTRACT *(Maximum 200 Words)*

A serious late complication associated with breast cancer treatment is the increased risk for development of therapy-related hematologic malignancies. The goal of this biologic study is to determine whether dose-intensive adjuvant regimens for breast cancer induce genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis. Clonal hematopoiesis has been proposed as an early marker of hematopoietic stem cell damage, preceding the acquisition of critical, recurring genetic alterations associated with the development of therapy-related myelodysplastic syndromes and acute leukemia. Clonal hematopoiesis is being evaluated by two different methods, the X-linked HUMARA clonality and microsatellite instability assays. Positive clones will be further analyzed for *MLL* and *RAS* alterations. Study accomplishments to date: a) ancillary biological protocol (S9719) written and approved for study; b) clonality assays developed and standardized; c) specimen collection and data analysis of 188 samples from 29 patients completed; d) preliminary approval granted to incorporate S9719 into a new clinical treatment protocol, S0012; and e) submitted protocol revisions to DOD and CTEP on 8/23/01, awaiting final approval prior to study implementation.

14. SUBJECT TERMS
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Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer: Pilot Study to Evaluate Incidence

INTRODUCTION

Subject: Clonal Hematopoiesis As A Marker Of Genetic Damage Following Adjuvant Chemotherapy For Breast Cancer: Pilot Study To Evaluate Incidence. A Southwest Oncology Group (SWOG) Study.

Purpose and Scope of the Research: The goal of this study is to determine whether dose-intensive adjuvant regimens for breast cancer induce genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis. Originally, the study was designed to study sequential blood/bone marrow samples from 200 women enrolled in a single, randomized dose-intensive Southwest Oncology Group (SWOG) adjuvant breast cancer study for women with four or more positive nodes (**S9623**, "A Comparison of Intensive Sequential Chemotherapy using Doxorubicin plus Paclitaxel plus Cyclophosphamide with High Dose Chemotherapy and Autologous Hematopoietic Progenitor Cell Support for Primary Breast Cancer in Women with 4 or More Involved Axillary Lymph Nodes, Phase III, Intergroup"). Two different general assays are used to detect clonality: the HUMARA (human androgen receptor) assay to estimate the incidence of early genetic damage defined by the presence of clonal hematopoiesis, and microsatellite instability testing to screen for loss of heterozygosity or the presence of defective DNA mismatch repair mechanisms. In cases where the HUMARA and microsatellite repeat assays are positive for clonality, the incidence of *MLL* fusion gene transcripts and *RAS* gene mutations (*H-, K-,* and *N-RAS)* will be examined. Unfortunately, the Southwest Oncology Group clinical treatment protocol to which the clonal hematopoiesis investigation was attached was closed on February 15, 2001 because of poor accrual. After extensive correspondence with the Department of Defense, we received preliminary approval on August 21, 2001 to proceed with this DOD-funded correlative study using a new Phase III breast cancer treatment protocol, S0012, a randomized comparison of standard doxorubicin and cyclophosphamide versus weekly doxorubicin and daily oral cyclophosphamide plus G-CSF as neoadjuvant therapy for inflammatory and estrogen-receptor negative locally advanced breast cancer.

BODY

Statement Of Work Objectives/Problems

In year 1, the clonal hematopoiesis protocol (S9719) was activated as a companion protocol to S9623 on schedule (October 15, 1997). Despite the advertisements and protocol presentations, patient accrual was slower than anticipated. A 1998 survey indicated two barriers: (1) short staffing of research nurses/clinical research associates at member institutions required the necessity to delete non-treatment directed trials, and (2) the requirement of two separate consent forms was perceived as cumbersome for medical professionals and their patients. These concerns were ameliorated by drafting and submitting protocol amendments to the Department of Defense (DOD) and Cancer Therapy and Evaluation Program (CTEP) to incorporate S9719 directly into the primary treatment protocol (S9623). Draft amendments for S9623/S9719 were submitted to the Department of Defense, Human Use Review Specialist, on June 15, 1999 for DOD review and approval. The formal DOD and CTEP review process took ~13 months (official written approval received July 11, 2000). The amendments were incorporated into S9623/S9719 and distributed to SWOG investigators in the Group mailings dated 7/15/00 and 9/15/00. At the time this progress report was submitted last year, amendments were being re-evaluated by the Institutional Review Boards (IRBs) for each Southwest Oncology Group member institution. At the Fall 2000 meeting, the Southwest Oncology Group Data and Safety Monitoring Committee made the unfortunate announcement that S9623, the companion clinical treatment, was targeted

for closure due to poor accrual. One factor appeared to be that the highly emotional and controversial use of high dose chemotherapy and autologous stem cell transplantation (HDC-ABMT) for the treatment of breast cancer resulted in a significant decrease in patient accrual (see Controversy surrounding High Dose Chemotherapy submitted as part of the 1999-2000 progress report). Pleas to continue the study were submitted to Dr. Charles A. Coltman, Jr. However, after considerable review, S9623 and S9719 (the companion DOD clonal hematopoiesis protocol) were closed on February 15, 2001.

Immediately, plans were submitted to the DOD to revive the study using a different Southwest Oncology Group breast cancer protocol (see appendix, exhibit A). On August, 20, 2001, we received preliminary approval from the Acting Chair, HSRRB via Louise M. Pascal, RN, MS to proceed with our proposal to integrate the clonal hematopoiesis study into S0012, a randomized comparison of standard doxorubicin and cyclophosphamide versus weekly doxorubicin and daily oral cyclophosphamide plus G-CSF as neoadjuvant therapy for inflammatory and estrogen-receptor negative locally advanced breast cancer, Phase III (see appendix, exhibit B). The required revisions to the protocol and informed consent were incorporated and submitted to the Acting Chair, HSRRB on August 23, 2001 (see appendix, exhibit C). We are currently awaiting official notice of approval for implementation of the study. We have also informed Dr. Jeff Abrams at CTEP of these revisions to obtain approval by the NCI.

Thus, the progress update for year 4 below describes the results since the 1999-2000 progress report for patients accrued to S9719 and the strategy in place to study breast cancer patients accrued to S0012. **Consequently, the Southwest Oncology Group has not dispersed any DOD funds to support the research objectives outlined in this grant for Years 2 through 4.** Funding will be distributed to the testing centers when patient accrual resumes.

Experimental Design (S9719) Purpose: To test the hypothesis that genetic damage defined by the presence of clonal hematopoiesis can be detected in a subset of patients following dose-intensive adjuvant therapy.

Rationale for Clonal Hematopoiesis Project (S9719): Adjuvant therapy with anthracycline-based combination chemotherapy for patients with breast cancer improves disease-free and overall survival. Unfortunately, therapy-related myelodysplasia (t-MDS) or therapy-related acute myeloid leukemia (t-AML) has emerged as uncommon, but well-established, complications of adjuvant therapy using dose-intensive regimens for breast cancer. According to the Jacobs' model of leukemogenesis (1), t-MDS/t-AML evolves as a result of expansion of an abnormal clone of hematopoietic stem cells, which have acquired somatic mutations conferring a growth advantage. This damage may result in clonal proliferation which, according to the Jacobs model of neoplasia, is an essential early (? initial) step in leukemogenesis, occurring prior to the development of clinical abnormalities.

Year 4 Progress Update

During the past year, poor accrual to S9623 resulted in early closure of the randomized Phase III trial comparing cyclical dose intensive chemotherapy with induction therapy, followed by dose intensification and autologous stem cell rescue for women with 4-9 node positive breast cancer. Consequently, our companion study S9719 was closed. At the time of closure only 29 patients had entered S9719 from 17 Southwest Oncology Group centers (see enclosed report of study sheet dated October 26-28, 2001, Appendix, exhibit D). The objective of the companion study was to examine the incidence of clonal hematopoiesis following dose-intensive chemotherapy as a marker of chemotherapy-induced genetic damage. HUMARA and microsatellite instability (MSI) assays were used to assess the emergence of clonal hematopoiesis. Assay methodology was reported to the DOD in previous progress reports. To date, no evidence of clonality using either the HUMARA or the MSI assay was observed in pre-treatment or in the limited numbers of follow-up samples that have been obtained during and following completion of the chemotherapy. During the past year, eight pretreatment and 29 follow-up samples were analyzed for clonal hematopoiesis. These results are summarized in Table 1 (page 7). We are now beginning to correlate these data with the clinical course of

these patients. Importantly, we will continue to follow these patients for outcome, noting reports of the development of therapy-related myelodysplasia (t-MDS) or acute myeloid leukemia (t-AML). Lacking positive findings, there is no reason to perform *MLL* RT-PCR testing and *RAS* mutation studies in this subset of patients; however, these assays will be performed in the new study for all positive patients.

The poor accrual and premature closure of both studies precludes the ability to draw any significant conclusions, yet the concern about the potential for damage to hematopoietic stem cells as a result of dose intensive regimens that contain both alkylating agents and topoisomerase II inhibitors remains very real. Therefore, with the support of the Southwest Oncology Group Breast Cancer Committee and the Department of Defense, we will continue to pursue the objectives of our original study (S9719) by including them as additional objectives (1.5 and 1.6 – see attached protocol) of a new study that will compare two dose-intensive regimens for women with high risk locally advanced breast cancer (S0012). Importantly, the biologic studies are included as an optional component of the treatment study. We anticipate that "embedding" our biologic objectives into the clinical study, rather than asking patients to participate in a separate ancillary study, will simplify registration and provide us with a more certain accrual that will allow us to fulfill our original objectives.

The clinical question that is being asked in S0012 is whether more dose intensive delivery of doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan) may improve the disease-free and overall survival without increasing the risk of developing t-MDS/AML in women with high-risk breast cancer. We plan to determine whether these agents, given in different doses and schedules, with or without hematopoietic growth factor support (G-CSF), induces genetic damage to hematopoietic stem cells. The biologic question and the methodology remain the same as proposed in our original study; the chemotherapeutic approaches and the patient population differ. Again, a dose-intensity question is being raised in the clinical trial and patients will be randomized to one of two chemotherapy schedules. The treatment will be administered as neo-adjuvant chemotherapy to women with locally advanced breast cancer. We have chosen to study sequential blood samples from 200 women enrolled on this study (100 per arm). In this study, our biologic objective will be to determine whether the more dose-intensive regimen given for 15 weeks (daily oral cyclophosphamide, weekly Adriamycin with G-CSF support) induces genetic damage to hematopoietic stem cells with a higher frequency than in the other group who will receive a "standard" dose of Adriamycin and cyclophosphamide (AC) that is given every 21 days without growth factor support. A pretreatment blood sample will be used to control for variables such as age or damage that may have occurred as a result of other exposures or other pre-existing risk factors. Sequential blood samples will be obtained following completion of the neo-adjuvant chemotherapy (prior to surgical resection) and six and twelve months following surgical resection.

Reasons for lack of follow-up in three S9719 cases:

Three patients came off study for: toxicity (n=1), extreme difficulty in collecting blood (n=1), or patient became uncomfortable with proposed stem cell collection procedure (n=1). For past due follow-up samples, reminder letters were sent to the attending Southwest Oncology Group physicians on 9/5/2001.

Table 1. S9719: 29 patient samples at pretreatment and follow-up

Patient ID	Pretreatment HUMARA/MSI*	Apheresis HUMARA/MSI	3 Month HUMARA/MSI	~1 Year HUMARA/MSI	Addition Follow-up HUMARA/MSI
173752	1.95		1.35	Due3/02	
174043	1.45		1.55	Due5/02	
172865	1.4			Due9/01	
164513	1.33/ - MSI			1.20/ - MSI	1.20/ - MSI
169826	1.25/ - MSI			1.10/ - MSI	
172932	1.48			Due10/01	
163674	1.23/ - MSI			1.25/ - MSI	1.10/ - MSI
169380	1.20/ - MSI	1.45/ - MSI	1.30/ - MSI	1.6/ No gran	
166131	1.55	1.15	No gran	Due7/00	
166282	1.65/ - MSI	1.10/ - MSI	No gran	1.25/ - MSI	
169229	1.18/ - MSI		1.25/ - MSI	1.05/ - MSI	
163093**	No gran/- MSI				
164598***	1.15/ - MSI				
169452	1.45/ - MSI		1.10/ - MSI	1.70/ - MSI	
167501	1.18/ - MSI		1.45/ - MSI	1.15/ - MSI	
169820	1.13/ - MSI			1.30/ - MSI	2.4/ NA
167233***	2.00/ - MSI				
174311	2.05/ NA	1.27		Due 5/02	
173211	1.25/ - MSI		1.10/ - MSI	Due 11/01	
172127	1.10/ NA	1.40 / NA		1.55 / NA	
164192	1.05/ - MSI		1.15/ - MSI	1.20/ - MSI	
165292	1.18/ - MSI	1.70	No gran	1.15/ - MSI	
168599***	1.0/ - MSI				
168656	Monoallelic/- MSI		- MSI	- MSI	
168659	1.45/ - MSI	1.05/ - MSI	1.10/ - MSI	1.3/ - MSI	
172716	1.38/ - MSI	1.10/ - MSI		Due1/02	
166407	1.08/ - MSI		1.20/ - MSI	1.20/ - MSI	
167145	1.13/ - MSI	1.25/ - MSI	1.1/ - MSI	1.20/ - MSI	
166218	1.15/ - MSI	1.10/ - MSI		Due5/00	

*HUMARA results are expressed as a ratio. Random inactivation will show both maternal and paternal allele, signifying a polyclonal state; the presence of only one allele or shift of greater than 3-fold (to control for skewed X-inactivation over the other allele), will identify a clonal population (see reference 2 and previous progress reports). MSI is reported as positive (+) or negative (-).

**No follow up samples available for this patient

***Patient went off study

No gran: the granulocyte layer was not of sufficient quantity to yield results.

Monoallelic: HUMARA assay is non-informative because the alleles cannot be distinguished.

NA: not analyzed

Statistical Considerations

The length of accrual is anticipated to be three years. Compliance with the blood drawn at the time of surgery should be nearly complete; at 12 months following completion of treatment, approximately 15% of the patients might be anticipated to have relapsed or refused to participate and not have samples available. The probability of clonal hematopoiesis at a particular time point can be established to within $\pm$ 0.1 with a sample size of 100 per arm, and to within $\pm$ 0.11 with a sample size of 85. Change in status between pretreatment, at the time of surgery, and at six and twelve months post-surgery samples will be explored, as will concordance of the HUMARA and MSI assays. Association of treatment, pre-study patient characteristics, and tumor-related variables with presence or absence of clonality by HUMARA or MSI assays will also be explored.

The study will be monitored by the Southwest Oncology Group Data and Safety Monitoring Committee according to NCI guidelines and Southwest Oncology Group policy.

KEY RESEARCH ACCOMPLISHMENTS

- Southwest Oncology Group biologic protocol (S9719) activated. Clonality assays developed. 188 samples from 29 patients analyzed to date. S9719 closed on February 15, 2001.
- No evidence of clonal hematopoiesis by HUMARA assay detected. One patient was monoallelic and therefore non-informative.
- No evidence of microsatellite instability prior to the initiation of therapy or at time of follow-up detected.
- The HUMARA and microsatellite instability assays give reproducible and complementary results using sequentially obtained blood samples of women treated on this study.
- Peripheral blood T-lymphocytes are a useful internal, tissue-specific control for the HUMARA assay precluding the need for age-matched controls for skewed X-inactivation.
- Preliminary approval to incorporate this DOD biological investigation (S9719) into breast cancer treatment protocol S0012 has been received. The requested revisions to the protocol and IRB consent have been made and submitted to the DOD. We are awaiting final approval by the DOD and CTEP prior to study implementation.

REPORTABLE OUTCOMES

Analysis of additional patients with longer follow-up is essential to confirm these preliminary results; however, at this point, neither regimen used in this setting (dose-intensive therapy with growth factor support vs. high-dose therapy with stem cell reinfusion for stage II/III breast cancer as described in S9623) appears to **initiate** genetic damage that could result in development of hematologic malignancies. Due to short follow-up, no publications are possible at this time.

CONCLUSIONS AND FUTURE DIRECTIONS

This pilot study was designed to test the hypothesis that genetic damage, defined by the presence of clonal hematopoiesis, can be detected in a subset of patients following dose-intensive adjuvant therapy on a current Southwest Oncology Group trial for breast cancer. The salient points outlined in the grant application's "Statement of Work" for years 1-3 have been addressed, however, low accrual has resulted in a major set back and the inability to meet the original timelines proposed. During the past three years, the highly emotional controversy surrounding high dose chemotherapy with stem cell rescue for breast cancer has made a substantial deleterious impact on patient accrual to both the clonal hematopoiesis biological study and the clinical treatment protocol (S9623). Accordingly, to answer the important question of this study, we have incorporate this biological investigation into a less controversial but pertinent breast cancer treatment protocol. We are cautiously optimistic that the approved amendment changes will boost accrual and allow us to complete the study. The protocol is simple and consists of four blood drawings at four defined time points for those patients agreeing to participate in the study. The biological questions of why this late effect complication only occurs in a subset of breast cancer patients, what the synergistic effects of other drugs or

radiotherapy are, whether the period/sequence of drug administration matters, are patients predisposed, and are faulty DNA repair mechanism at play, remain problematic in the treatment of breast cancer. Therefore, this DOD funded project remains unanswered and very relevant for breast cancer patients.

Confirmation that adjuvant chemotherapy induces clonal hematopoiesis in a significant number of patients from this pilot study will provide a unique model to prospectively study the evolution of therapy-related leukemogenesis in patients being treated for breast cancer, and would be the focus of a subsequent grant proposal. The goals of a larger study would include the following: 1) to determine whether a relationship exists between detection of clonal hematopoiesis and subsequent evolution to t-MDS/AML; 2) to identify general mechanisms (e.g., faulty DNA repair and mutations in components of cell cycle checkpoints), which may predispose patients to genetic instability and leukemogenesis, following adjuvant therapy; 3) to determine the sequence of events (genomic instability, loss of heterozygosity, specific mutations/translocations, etc.) which participate in leukemogenesis; and 4) to determine whether specific adjuvant regimens place patients at an unacceptably high risk for the development of therapy-related hematologic malignancies. If the study is negative, two conclusions are possible: (1) high risk breast cancer patients can rest assured that they can receive high dose chemotherapy, without an increased risk of development of clonal hematopoiesis or subsequent evolution to a therapy-related hematopoietic disorder over the general population or (2) clonal hematopoiesis is not the route to leukemogenesis.

REFERENCES

Jacobs A. Genetic lesions in preleukemia. Leukemia 5:277, 1991

Allen RC, Zoghbi HY, Mosely AB, Rosenblatt HM, Belmont JW. Methylation of *Hpall* and *Hhal* sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229, 1992.

APPENDICES

The following exhibits are attached:

Exhibit A: Email Correspondence between the Southwest Oncology Group and the Department of Defense regarding the request to "embed" clonal hematopoiesis into a new Southwest Oncology Group breast cancer protocol, S0012 (dated March 18-19, 2001)

Exhibit B: Preliminary approval from the DOD, protocol changes submitted (email dated August 20-23, 2001)

Exhibit C: S0012 with amendment #1 embedding clonal hematopoiesis into treatment protocol

Exhibit D: Report of Studies listing S9719 registration by Southwest Oncology Group Institution

Exhibit A

Slovak, Marilyn

To: Slovak, Marilyn
Subject: FW: Request DAMD 17-97-1-7088

-----Original Message-----
From: "Oner, Tamra" <toner@swog.org>
Sent: Monday, March 19, 2001 7:04 AM
To: "Stotler, Karen" <karen.stotler@amedd.army.mil>
Cc: Marilyn Slovak; "Albain, Kathy" <kalbain@lumc.edu>; "Wendy Stock" <wstock@medicine.bsd.uchicago.edu>; "Livingston, Robert" <living@u.washington.edu>; "Green, Stephanie" <stephani@swog.fhcrc.org>
Subject: FW: Request DAMD 17-97-1-7088

Hi Karen,
Below is a message from Dr. Kathy Albain of the Southwest Oncology Group regarding our grant and protocol. Please feel free to contact me with any questions or concerns. Thank you for your patience.
Best regards, Tamra

Tamra N. Oner
Protocol Coordinator
Southwest Oncology Group
14980 Omicron Drive
San Antonio, TX 78245-3217
Phone: 210/677-8808
Fax: 210/677-0006
Email: toner@swog.org

> ----------
> From: Kathy Albain
> Sent: Sunday, March 18, 2001 6:51 PM
> To: toner@swog.org
> Cc: dsparks@swog.org
> Subject: request
>
>
> Karen Stotler
> Contract Specialist
> U.S. Army Medical Research Acquisition Activity
> Fort Detrick, MD
>
> RE: DAMD 17-97-1-7088
>
> Dear Ms. Stotler:
>
> As a result of a recent series of phone calls between myself and Dr. Earl Grant, as well as with Col. Julie Zadinsky of RCQ, we agree to the following plan. Dr. Grant indicated that he consulted with you and that we should email you with our decision. A "no cost" extension was approved by Dr. Grant.

We will "embed" the entire work of our DAMD grant into our new protocol S0012 in order to ensure a patient population for the blood samples that we need. This change was necessitated by the premature closure of S9623, the previous "parent" trial to our project. As Dr. Grant requested, we agree to provide you with a detailed cover letter plus the new protocol and informed consent, highlighting the places of embedment in each. We could not meet the March deadline, so will choose instead the April 18 date Dr. Grant also offered us in order to allow a full board review by Col. Zadinsky's group at their May meeting. Please let Tamra Oner (toner@swog.org) know the date that your office needs to receive our materials in order for you and Dr. Grant to forward to RCQ by April 18.

> Thank you very much for your help.
>
> Sincerely,

>
> Kathy S. Albain, M.D.
> Chair, Committee on Women and Special Populations
> Southwest Oncology Group

Exhibit B

Slovak, Marilyn

From: Oner, Tamra [toner@swog.org]
Sent: Thursday, August 23, 2001 1:55 PM
To: 'Pascal, Louise M Ms AMDEX'
Cc: Stotler, Karen S Ms USAMRAA; Bennett, Jodi Ms USAMRMC; Slovak, Marilyn; Stock, Wendy; Albain, Kathy; Ellis, Georgiana; Livingston, Robert; Gralow, Julie; Green, Stephanie
Subject: RE: HSRRB A-7569.1: S0012/S9719 protocol

0012/9719 combo.pdf
8/23/01

Dear Louise,

Thank you very much for your review of our response to your proposed protocol changes. Enclosed, please find a pdf file of the finalized protocol including the consent form changes that were agreed upon in our most recent correspondence along with the revised cover memo. I look forward to receiving final approval as you have described in your note below. Upon our receipt of formal approval from the USAMRAA, we will proceed with implementation.

Sincerely, Tamra

<<0012/9719 combo.pdf 8/23/01>>

Tamra N. Oner
Protocol Coordinator/IND Specialist
Southwest Oncology Group
14980 Omicron Drive
San Antonio, TX 78245-3217
Phone: 210/677-8808
Fax: 210/677-0006
Email: toner@swog.org

> ----------
> From: Pascal, Louise M Ms AMDEX
> Sent: Monday, August 20, 2001 1:01 PM
> To: 'toner@swog.org'
> Cc: Stotler, Karen S Ms USAMRAA; Bennett, Jodi Ms USAMRMC
> Subject: HSRRB A-7569.1: Review of 7 August 2001 correspondence
>
> RE: Protocol Entitled, "Correlative Studies to Determine Frequency of
> Clonal Hematopoiesis," Submitted by Charles A. Coltman, Jr., MD, Cancer
> Therapy Research Center Research Foundation, San Antonio, Texas, Southwest
> Oncology Group(SWOG), Integrated into SWOG Protocol #S0012, Award No.
> DAMD17-97-1-7088, Proposal Log No. BC960131, HSRRB Log No. A-7569.1
>
> To: Ms. Oner,
>
> Thank you for your follow-up and response regarding the requested
> revisions to the protocol documents. Your response of 7 August 2001 was
> very detailed and helpful. Upon review of same by the Acting Chair,
> HSRRB, the actions and explanations presented in your plan are acceptable;
> therefore, at this time, please proceed with completing the necessary
> revisions to the consent form document. When the items have been
> addressed, please submit the protocol and revised consent form to my
> attention at RCQ.
>
> Upon receipt of the protocol and revised consent form, the Acting Chair,
> HSRRB, will review again and if everything is in order, the notice of
> approval for implementation of the study will be forwarded to Karen
> Stotler, Contracting Specialist, USAMRAA, here at Ft. Detrick. As you are
> aware, the official notice of approval for implementation of the study
> will be issued by personnel at USAMRAA.
>
> Again, thank you for your assistance and support with this process.
> Please feel free to contact me at any time.
> Regards,
> Louise Pascal
>
> Louise M. Pascal, RN,MS

> Human Subjects Protection Specialist (AMDEX Corporation)
> U.S. Army Medical Research and Materiel Command
> Office of Regulatory Compliance and Quality
> Telephone #: 301-619-2607 or DSN 343-2607
> FAX #: 301-619-7803 or DSN 343-7803
> Electronic Mail: louise.pascal@det.amedd.army.mil
>
> Mailing Address:
> Commanding General
> U.S. Army Medical Research and Materiel Command
> ATTN: MCMR-RCQ-HR/Louise M. Pascal,RN
> 504 Scott Street
> Fort Detrick, Maryland 21702-5012
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Southwest
Oncology Group
A National Clinical Research Group

Draft July 12, 2001

TO: ALL SOUTHWEST ONCOLOGY GROUP, CCOP AND AFFILIATE MEDICAL ONCOLOGISTS, SURGEONS AND PATHOLOGISTS; EPP INSTITUTIONS; CTSU

FROM: Tamra N. Oner, Protocol Coordinator

RE: **S0012**," A Randomized Comparison of Standard Doxorubicin And Cyclophosphamide Vs. Weekly Doxorubicin And Daily Oral Cyclophosphamide Plus G-CSF As Neoadjuvant Therapy For Inflammatory And Estrogen-Receptor Negative Locally Advanced Breast Cancer, Phase III". Study Coordinators: Drs. G. Ellis and R. Livingston

AMENDMENT #1

Study Coordinator: Georgiana K. Ellis, M.D. Phone: 206/288-2048
Email: gellis@u.washington.edu

IRB Review Requirements (If you choose to participate in this study)

(√) Full board review required

( ) Expedited review allowed

( ) No review required

AMENDMENT #1

The above-noted study has been revised to include clonal hematopoiesis correlative studies. Specific changes are listed below:

1. Study coordinators for the clonal hematopoiesis correlative studies, Marilyn Slovak, Ph.D., Wendy Stock, M.D. and Kathy S. Albain, M.D., have been added to page 2.

2. New objectives 1.5 and 1.6 have been inserted into Section 1.0.

3. Additional background information, titled "Correlative Studies to Determine Frequency of Clonal Hematopoiesis", has been inserted into Section 2.0 prior to the "Inclusion of Minorities" paragraph.

4. A new Section 5.12 has been inserted to specify that for patients who consent to the clonal hematopoiesis sample submission, prestudy specimens must be submitted as described in Section 15.0. The rest of the section has been renumbered accordingly.

5. A parenthetical statement, "(See Appendix 19.6)", has been inserted at the end of Section 7.5 and a separate note has been added specifying that pre-surgical blood samples are to be submitted per Sections 14.9 and 15.0 for patients who consent to the clonal hematopoiesis sample submission.

6. A new item titled "Blood samples for clonal hematopoiesis" has been inserted in the "Laboratory" section of the Study Calendars, Sections 9.1 and 9.2, and a "¶" footnote inserted which states "For patients who consent to clonal hematopoiesis sample submission, see Section 15.0." The "§" footnote has been revised to state "Adjuvant treatment after post-chemotherapy surgery is at the physician's discretion. Post-surgery therapy must be documented on the Follow-Up Form (Form #61519). (See Appendix 19.6)."

7. A new paragraph has been inserted into Section 11.0 to provide statistical considerations for the correlative studies.

8. New sections 14.5, 14.9 and 14.12 have been inserted to provide submission instructions for the clonal hematopoiesis blood specimens. The rest of the section has been renumbered accordingly. A statement has been added to Section 14.11 which states, "Post-surgery therapy must be documented on the Follow-Up Form (Form #61519). (See Appendix 19.6)".

9. Section 15.0 has been inserted to provide specific specimen handling and shipping instructions for the clonal hematopoiesis samples.

10. Additional references (#28 - #65) have been inserted at the end of Section 17.0.

11. The Southwest Oncology Group Specimen Submission Form (Form #1951) has been inserted as a new Section 18.2i.

12. A new second paragraph has been inserted into the model informed consent under "Why Is This Study Being Done?" to provide information about the clonal hematopoiesis testing.

13. A new paragraph has been inserted into the model informed consent under "What Is Involved In This Study?" to provide specific information about the timing of specimen submission and the laboratories for the correlative studies. Also, a sentence has been added to the eighth paragraph in this section regarding follow-up in the event of secondary malignancy.

14. A paragraph has been added to the model informed consent regarding the possible risks of venipuncture in the "What Are The Risks Of The Study?" section.

15. A paragraph regarding the clonal hematopoiesis testing has been inserted into the model informed consent in the "What Other Options Are There?" section.

16. A new third paragraph has been inserted into the model informed consent under "Are There Benefits To Taking Part In the Study?" regarding the clonal hematopoiesis testing.

17. A new second paragraph has been added to the model informed consent in the "What About Confidentiality?" section providing information regarding data storage. Also, a sentence which states "Additionally, if you agree to submit samples for clonal hematopoiesis testing, the U.S. Army Medical Research and Material Command may inspect your research records." has been inserted into the third paragraph of this section. The first sentence of the wording for CTSU investigators has been updated in this section.

18. A new paragraph has been added to the model informed consent in the "What Are The Costs?" section regarding care for venipuncture.

19. A new paragraph and second signature line has been inserted in the "Signature" section of the model informed consent for providing consent for submission of the clonal hematopoiesis samples.

20. A space has been inserted into both participant signature blocks of the model informed consent for the participant to print their name. Also, a signature block has been added underneath both patient signature blocks for a witness to print their name, sign and date the form.

21. A new Appendix 19.5 has been added to the protocol that includes specific information regarding the clonal hematopoiesis assays. A new Appendix 19.6 has been inserted providing information for collecting post-surgical data.

Replacement pages are enclosed for the title page and pages 2, 4 - 6, 8 - 8b, 18, 20 - 21a, 26 - 27, 29 - 32c, 37 - 42a, 44, 56a, 66 and 67. Pages 8a - b, 21a, 32a - c, 37a - c, 42a and 56a have been inserted to prevent extensive repagination. The title page reflects the date of this revision. Please attach this memo to the front of your copy of the protocol and insert the replacement pages.

This memorandum serves to inform the NCI, EPP institutions, CTSU and the Southwest Oncology Group Statistical Center.

cc: PROTOCOL & INFORMATION OFFICE
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PRIVILEGED COMMUNICATION
FOR INVESTIGATIONAL USE ONLY

S0012
Activated May 1, 2001
Draft (Clonal Hematopoiesis Revision) July 12, 2001

SOUTHWEST ONCOLOGY GROUP

A RANDOMIZED COMPARISON OF STANDARD DOXORUBICIN AND CYCLOPHOSPHAMIDE VS. WEEKLY DOXORUBICIN AND DAILY ORAL CYCLOPHOSPHAMIDE PLUS G-CSF AS NEOADJUVANT THERAPY FOR INFLAMMATORY AND ESTROGEN-RECEPTOR NEGATIVE LOCALLY ADVANCED BREAST CANCER

PHASE III

PARTICIPANTS: ALL SOUTHWEST ONCOLOGY GROUP, CCOP AND AFFILIATE MEDICAL ONCOLOGISTS, SURGEONS AND PATHOLOGISTS; EPP INSTITUTIONS; CTSU

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(Protocol Last Changed ____)

AGENTS:
Doxorubicin (Adriamycin®)(NSC123127)
Cyclophosphamide (Cytoxan®) (NSC26271)
Filgrastim (r-metHuG-CSF) (NSC-614629)
Trimethoprim Sulfa (Bactrim®)

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

For patient enrollment or to report adverse events:

Phone: 1/888-462-3009
Fax: 1/888-691-8039

To mail forms or data

CTSU Data Processing Manager
CTSU Data Center
WB 408
1441 West Montgomery Avenue
Rockville, MD 20850-2062

All other questions (including forms - specific questions) should be communicated by phone or e-mail to:

CTSU General Information line: 1/888-823-5923
or ctsucontact@westat.com

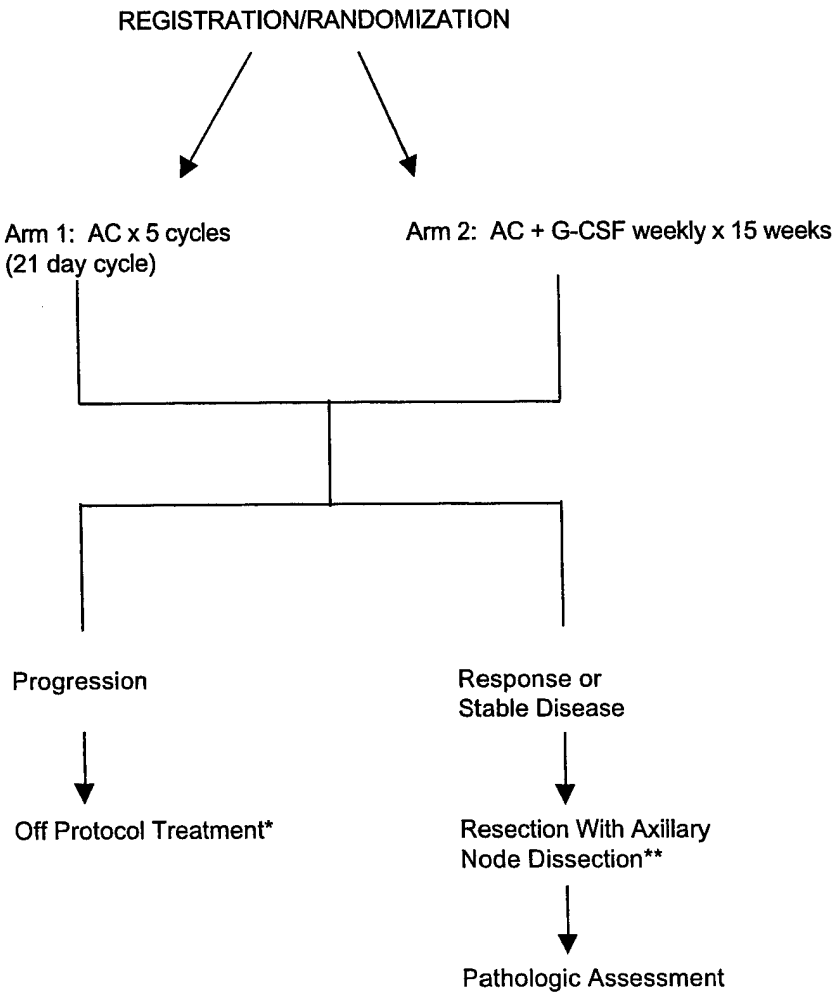
All calls and correspondence will be triaged to the appropriate CTSU representative.

The CTSU public website is located at: www.ctsu.org

The CTSU member website is located at: http://members.ctsu.org

Please refer all questions regarding chemotherapy treatment or dose modifications to Dr. Ellis.

SCHEMA



*After progression and removal from protocol treatment, further therapy is per treating physician's discretion.
**After resection with node dissection, further therapy per treating physician's discretion.

1.0 OBJECTIVES

1.1 To compare the microscopic pathologic response rates in patients with inflammatory and estrogen-receptor negative locally advanced breast cancer treated with weekly doxorubicin and daily oral cyclophosphamide given with G-CSF support to that in patients treated with the "standard" doxorubicin and cyclophosphamide regimen given every three weeks.

1.2 To compare the toxicities of these two regimens.

1.3 To compare the delivered dose intensity of these two regimens.

1.4 To assess the association between microscopic pathologic complete response and clinical complete response at the primary tumor site in these patients.

1.5 To estimate the incidence of early genetic damage during the course of treatment using two general clonal assays: a) the HUMARA (human androgen receptor assay) to screen for the presence of clonal hematopoiesis, and b) microsatellite instability (MSI) assays to screen for the presence of defective DNA mismatch repair mechanisms and loss of heterozygosity, in pretreatment blood and three sequential post-treatment specimens in breast cancer patients enrolled in this study.

1.6 To estimate the incidence of MLL (myeloid lymphoid leukemia) gene fusion transcripts and the frequency of RAS gene mutations (H-, K-, and N-RAS) in cases where either the HUMARA or microsatellite repeat assays are positive for clonal hematopoiesis.

2.0 BACKGROUND

Seminal work by Hryniuk, et al. has suggested that the dose intensity of chemotherapy (the amount of drug per square meter of body surface area per week) correlates with response rate and survival in advanced breast cancer and with relapse-free survival in the adjuvant setting, both for CMF (cyclophosphamide, methotrexate, 5-fluorouracil) and CAF (cyclophosphamide, doxorubicin, 5-fluorouracil) type regimens. *(1, 2)* Further, in those limited studies where necessary data are available, the use of actual doses delivered, rather than the intended doses specified in the various regimens, results in stronger correlation with outcome for metastatic disease. *(3)* There are too few adjuvant studies reporting dose delivery to allow such analysis in the adjuvant setting. Although controversial, such analyses have spurred ongoing interest in and evaluation of dose intensity. *(4)*

The Southwest Oncology Group has reported extensively on a continuous, or "Cooper-" type, CMF regimen in the setting of adjuvant chemotherapy for node-positive breast cancer, in which cyclophosphamide is administered orally on a daily basis and the 5-FU and methotrexate are given by weekly intravenous injection. The Southwest Oncology Group regimen also contains two additional drugs, vincristine and prednisone (CMFVP), and favorable 10 year and 20 year results have been reported with this combination. *(5, 27)* A Cancer and Leukemia Group B study, however, failed to identify a benefit to the additional two drugs (VP) in the adjuvant setting. *(6)*

Since the inception of regimens of this type, doxorubicin was developed and found to be one of the most active drugs in metastatic breast cancer, especially hormone-receptor negative disease. Combination chemotherapy regimens that included this drug repeatedly resulted in higher response rates than regimens that did not. Several adjuvant trials have also suggested greater efficacy. *(7, 8)* A common regimen of this type employs cyclophosphamide, doxorubicin, and 5-fluorouracil administered intravenously every three weeks. As there is potentially greater toxicity with the use of doxorubicin, many practitioners reserve the adjuvant use of this drug for patients with breast cancer at relatively high risk of relapse. The NSABP has reported a comparison between "classical" CMF administered for six months duration and short-course (12 week)

doxorubicin/cyclophosphamide, with comparable outcomes. *(9)* This would suggest increased efficacy for the use of doxorubicin in combination regimens, given the shorter duration of therapy on this arm. A subsequent study in our group along with ECOG of one year of CMFVP versus 20 weeks of FAC-M (5-fluorouracil, doxorubicin, cyclophosphamide, methotrexate) for receptor negative, node positive primary breast cancer showed no difference in overall survival between the two arms, though disease-free survival was marginally superior (p=0.06) on the CMFVP arm. *(10)*

Investigators at the University of Washington have obtained pilot toxicity data on a "weekly continuous FAC" regimen, modeled after Southwest Oncology Group-type CMF, but with the substitution of doxorubicin for methotrexate, in high risk Stage II and III breast cancer patients, administered as adjuvant and neoadjuvant therapy. *(11)* The intent of the regimen was to maximize dose intensity. In the first 29 patients treated, delivered dose intensity was 1.21 to 1.24 times higher than seen with two standard "FAC" regimens. Neutropenia was dose-limiting. A subsequent study added continuous daily G-CSF overlapping oral cyclophosphamide to overcome this limitation (see Table 1). *(15)* Pneumocystis pneumonia was seen in 2 of the first 7 patients treated. Prophylactic trimethoprim sulfa (Bactrim®) was added, and an additional two cases were seen in the next 65 treated patients, both of whom had discontinued their Bactrim® without notifying investigators.

Thrombocytopenia (platelets below 100,000) occurred in 5% without versus 26% with concurrent G-CSF who received higher chemotherapy doses, and platelets below 50,000 occurred in 0% versus 5% (also received higher chemotherapy doses). No patient required platelet transfusion. Hand-foot syndrome occurred in 9% of patients without G-CSF versus 74% in those who received higher chemotherapy doses with its administration. This was the most common indication for dose delay in the concurrent growth factor study. This pilot trial was supported by Amgen, Inc. (Thousand Oaks, California) under an investigator-initiated IND from the Food and Drug Administration (BB 4482).

With the high incidence of hand-foot syndrome, the widespread use of the NSABP "AC" combination in the treatment of breast cancer, and the desire to dose intensify Adriamycin in the regimen, University of Washington investigators next examined weekly Adriamycin with daily oral cyclophosphamide and G-CSF. Phase I dose escalation of Adriamycin proceeded to 24 mg/m^2/week. In the first 37 patients there was one admission for neutropenic fever at this dosing level, and hand-foot syndrome was much decreased, to approximately 24%. Delivered dose intensity of Adriamycin appears to be in the 20 - 22 mg/m^2/week range, as compared to 18.1 mg/m^2/week for FAC + G. Preliminary experience suggests a "gross complete response" rate nearly twice as high as that seen for FAC + G. This study was also supported by Amgen under the same IND (BB 4482).

Expanded Phase II data on this regimen as neoadjuvant treatment for locally advanced breast cancer were obtained in the Southwest Oncology Group. **S9625** accrued 122 patients over a two year period, of which 96 were eligible at the time of evaluation. Median delivered dose of Adriamycin was 21.8 mg/m^2/week. No treatment related deaths occurred. Dose limiting toxicity was hematologic: Grade 4 neutropenia in 13 patients, Grade 3 in 46. No febrile neutropenia was seen. Other Grade 4 toxicites included herpetic encephalopathy (1), diarrhea (1) and hematuria (1). In locally advanced breast cancer, combined rates of pCR (pathologic or "microscopic" complete response) and mCR (macroscopic CR, or no gross evidence of residual tumor at pathologic evaluation), pCR alone, and nodal status after neoadjuvant therapy have all been reported as prognostic for disease-free and overall survival. In **S9625**, pathologic endpoints were evaluable in 88 patients, of whom 37 (42%) met pathologic response criteria with microscopic complete response (pCR) in 23/88 (26%). Among 84 patients who underwent node dissection, 25 were N0. The proportion with pCR+N0 was 21%. This compares to the pathologic CR and macroscopic CR combined of 39%, and pCR alone of 12%, from combined experience of others, primarily in non-inflammatory disease, all in single institution studies. The largest comparable experience is from MD Anderson, reported by Kuerer, of 372 patients with locally advanced, non-inflammatory disease, treated on two Adriamycin-based neoadjuvant regimens, with a combined pCR and mCR rate of 16%, 12% of the total also node negative. *(25)*

In **S9625**, results appear especially encouraging for patients with inflammatory breast cancer, with 12/49 (24%) pCR, and 22/49 (45%) with pCR+mCR. Similarly encouraging are the respective results in ER-negative disease, with pCR in 16/45, or 36%. This confirms the report from MD Anderson that pathologic complete response was more likely to be seen in patients with ER-negative disease, a relationship first speculated upon by Livingston. *(25, 26)* We believe these results justify comparison, in this present study, of AC+G to "standard" AC (60/600 q3w) in patients with locally advanced breast cancer that is inflammatory or estrogen-receptor negative.

Using the criteria that were specified in **S9625** under Statistical Considerations, the combined incidence of macroscopic CR and pathologic CR meets the required level of interest for disease that was defined as inflammatory. Among 45 patients currently considered as fully eligible, there were 21 mCR + pCR (47% of all entered, 51% of those who underwent resection), and a response probability of 0.4 had been required to be of interest. For patients defined as non-inflammatory, the observed response rate does not meet the level required for interest (26% of all entered, 31% of those who underwent resection). Further evaluation of the pilot data by hormone receptor status resulted in very similar results in ER-negative patients, where 12/28, or 43%, achieved a pathologic complete response with AC+G in **S9625**. This high complete response rate was not seen in ER-positive patients.

For the Phase III trial (present study), we elected to evaluate only that patient subgroup-Stage III disease which was ER-negative-which showed pathologic response superior to that expected from previous studies, and which met the statistical framework of our previous study. Further, because pCR seems to be a much more readily defined criterion than mCR, we proposed Statistical Considerations to consider only pCR as the main endpoint. Adopting the most conservative definition of denominator (all patients entered = 45) in the inflammatory group, there were, in **S9625**, 11 pCR (24%). If we assume that the pCR rate for inflammatory patients receiving "standard" AC is the same as that reported by NSABP for resectable patients in B-18, it would be 13%. We reiterate that NSABP B-18 was a trial largely (90%) of Stage II patients. It seems very unlikely that AC would be as effective in inflammatory disease as in operable disease, but no historical data exist for the inflammatory group. We believe it is realistic to expect a pCR rate of 10% for AC and of 25% for AC+G in the Phase III trial, which would give us a need for about 110 patients in each group. Our statistician recommended rounding upward to 150 patients per arm.

The NSABP has used four cycles of their AC regimen as adjuvant therapy, and four cycles as neoadjuvant in NSABP B-18. But eligibility for B-18 included having "operable" breast cancer, nearly all of which was clinical Stage I or II disease. MD Anderson has reported using 4 - 7 cycles of FAC chemotherapy, with treatment to maximum response. Since it is difficult to specify maximum response in a cooperative group setting, we elected in **S9625** to treat for a specified period of 16 weeks. In fact, average delivered duration of therapy was 15 weeks. We therefore feel, in parallel with our previous Southwest Oncology Group Phase II experience with AC+G, that the comparison of 5 cycles of standard NSABP AC regimen with 15 weeks of the dose-intense AC+G regimen is most appropriate.

Table 1.

Delivered Dose Intensity of Adjuvant Breast Cancer Chemotherapy Regimens Containing 5-Fluorouracil, Doxorubicin and Cyclophosphamide

	MDAH FAC *(13,14)*	ECOG CAF *(12)*	Continuous FAC *(11)* (RDI*)	Continuous FAC + G-CSF*(15)* (RDI*)
5-fluorouracil	267	175	242 (0.91/1.38)	270 (1.01/1.54)
doxorubicin	13.3	10.5	13.2 (0.99/1.26)	19.8 (1.49/1.89)
cyclophosphamide	133	245	250 (1.88/1.02)	414 (3.11/1.69)
adjusted cyclophosphamide**	133	221	225 (1.69/1.02)	373 (2.80/1.69)
RDI, *** regimen	1.00	1.09	1.26	1.87
RDI, *** regimen, adjusted	1.00	1.04	1.20	1.77

* RDI = Relative dose intensity, continuous weekly FAC or continuous weekly FAC + G-CSF vs. other. This is the delivered dose intensity of each agent of our regimen (without and with G-CSF) divided by that from the reported reference regimens (MDAH FAC, ECOG CAF).

** Bioavailability based on 90% absorption of oral cyclophosphamide.

*** RDI, average of each of the three drugs divided by MDAH FAC as reference regimen, as per the method of Hryniuk, who did not adjust for the oral bioavailability of cyclophosphamide. Presented, American Society of Clinical Oncology, May 1994, Dallas, Texas.

Table 2 reviews the published results from eight trials of neoadjuvant chemotherapy in locally advanced breast cancer. It is of note that the reported range of observed clinical complete responses is very broad, from 4 to 49%. The likelihood of observing a clinical complete response appears related to initial tumor size, and was as high as 71% for FAC with T2 tumors as reported in Hortobagyi's series. *(16)* Of perhaps greater interest is the poor correlation of clinical assessment of response compared to pathologic assessment for the few studies in which this was reported and the fact that the correlation can be poor in either direction. For example, the McCready study, also at M.D. Anderson, reported a higher pathologic than clinical complete response rate, though in most reports of primary chemotherapy for operable breast cancers the discordance has been in the opposite direction. *(17)* Swain's series (the Georgetown experience) is the most impressive, with a pathologic complete response rate estimated at 30% (62% of the 49% who had clinical complete responses). *(18)* This study employed CAMF with "hormone synchronization." Our expectation would be that higher delivered dose intensity would improve upon these results.

Table 2.

Neoadjuvant Chemotherapy in Locally Advanced Breast Cancer

Reference	Number of Patients	Induction Regimen	Duration (cycles)	Clinical RR CR	Clinical RR Overall	Path CR
De Lena *(19)*	74	A + VLB		14	73	
De Lena *(20)*	132	A + VLB		4	52	
Lesnick *(21)*	99	CMFpV		18	72	
Jacquillat *(22)*	98	FAVThMp		23	91	
McCready *(17)*	136	FAC	3 - 6	13	87	23*
Swain *(18)*	76	CAMF**	3 - 5	49	93	30 (est)
O'Reilly *(23)*	21		4	33	86	
Hortobagyi *(16)*	100	VCR + ACP	3	19 (13 - 71)	89	

*No gross tumor, with or without microscopic complete response.
**Plus "hormonal synchronization" with tamoxifen and Premarin.

In those series which have reported on pathologic response and its correlation with clinical outcome, the most important variable has been achievement of a macroscopic complete response (CR) (no visible or palpable remaining tumor, with or without microscopic residual). For example, in McCready's series from MDAH, patients who achieved a macroscopic CR (23% of the total) had a 65% five-year survival, compared to a five-year survival of 49% in the remainder who had gross residual ($p = 0.03$). *(17)* No difference in outcome was cited between gross and microscopic CRs. Similarly, Armstrong, et al., from Johns Hopkins reported that the only factor predicting outcome in their neoadjuvant series was pathologic response: at a median follow-up of 45 months, relapse had occurred in 1/11 (9%) patients with macroscopic CR versus 9/13 (69%) with macroscopic residual disease. *(24)*

Correlative Studies to Determine Frequency of Clonal Hematopoiesis.

As disease-free and overall survival for patients with breast cancer following treatment for breast cancer with anthracycline-based combination chemotherapy continues to improve, concerns relating to late effect complications of therapy must be investigated. Therapy-related myelodysplastic disorders and leukemia (t-MDS/AML) associated with chemotherapy, particularly alkylating agents and topoisomerase II inhibitors, are being reported with increasing frequency in the literature, in particular after breast cancer treatment. *(28 - 30)* t-MDS/AML evolve as a result of expansion of an abnormal clone of hematopoietic stem cells that have acquired somatic mutations conferring a growth advantage.

According to the Jacobs model for leukemogenesis, the mutations resulting in clonal hematopoiesis may occur without any obvious hematological change (no dysplasia or cytopenias noted). *(31)* Subsequently, the acquisition of a variety of additional genetic lesions may be essential for the development of MDS (preleukemia) or overt leukemia. Clonal chromosomal abnormalities have been reported in the majority of cases of t-MDS/AML. The most frequently reported abnormalities involve complete loss or interstitial deletions of the long arm of chromosomes 5 and/or 7. Typically, these leukemias develop following alkylating agent-induced damage at a median of 3 - 5 years following therapy. *(32)* The second group of t-MDS/AML is associated with rearrangements of the *MLL* gene localized to chromosome band 11q23. *(33 - 36)*

The 11q23-associated t-AMLs often develop following treatment with drugs that target DNA-topoisomerase II (e.g., epipodophyllotoxins, anthracyclines) with a very short (12 to 18 months) latency following treatment. *(35)*

Over the last ten years, anthracyclines have become a major component of combination chemotherapy regimens for breast cancer. Two adjuvant breast cancer trials, NSABP-B25 and NCIC, employing dose-intensive anthracycline-based chemotherapy, reported rates of t-MDS/AML that are two to four-fold higher than in previous adjuvant studies. *(29 - 30)* Notably, the most dose intensive arms of these studies employed the use of hematopoietic growth factors to facilitate blood count recovery following the high dose chemotherapy. The leukemias that developed in these patients had a monocytic morphology and occurred following a short latency period (within two years of adjuvant therapy), a characteristic finding of the hematologic disorders linked to the topoisomerase II inhibitors. Cytogenetic analysis revealed rearrangements of 11q23 in five of eight of these cases. *(32 - 37)* Further concern about the development of t-MDS/AML following high-dose chemotherapy for breast cancer may be warranted based on the alarming data emerging on high rates of development of t-MDS/AML following autologous transplantation for lymphoma, where the incidence of therapy-related leukemias has been estimated to be as high as 18% at six years following transplantation. *(38 - 41)*

These studies raise three major concerns: 1) Does genetic damage leading to the development of clonal hematopoietic stem-cell disorders occur with unacceptable frequency in patients receiving these intensive anthracycline-based adjuvant regimens for the treatment of breast cancer? (and, accordingly, what frequency is unacceptable?) 2) Will careful monitoring of this patient population reveal additional t-MDS/AML with long-term follow-up? and 3) Does the administration of recombinant hematopoietic growth factors (i.e., G-CSF) used to minimize morbidity and facilitate scheduled drug dosing play a potentiating role in the development of these secondary malignancies? This study will focus on the first concern; in addition, insights into the potential contributing role of hematopoietic growth factors in the induction of genetic damage to hematopoietic stem cells may be gained by comparison of the two treatment arms since only one group will receive G-CSF support.

Chemotherapeutic agents used in the treatment of breast cancer may induce genetic damage. This damage may result in clonal proliferation, which, according to the Jacobs model of neoplasia is an essential early, possibly initial, step in leukemogenesis, occurring prior to the development of clinical abnormalities. *(31)* Data confirming the presence of clonal proliferation following chemotherapy exist. Carter and others described clonal hematopoiesis in more than 30% of 70 clinically asymptomatic patients who had received prior cytotoxic chemotherapy for lymphoma. *(31, 42 - 43)* Busque, et al. found that clonal hematopoiesis existed in 8 of 12 (67%) patients with Hodgkin's or Non-Hodgkin's lymphomas studied prior to autologous transplantation (all had received prior chemotherapy), and that this value was significant ($p < 0.0033$) when compared to normal control donors. *(44)* Gale et al. have shown that sequential X-linked clonality assays are predictive of subsequent evolution to frank MDS/AML. *(45)* These provocative studies suggest that the presence of clonal hematopoiesis following chemotherapy may be a relatively common event. Pilot studies are warranted to determine the clinical relevance of these interesting findings.

The development of clonal hematopoiesis may be one of the earliest events that occur in an evolving neoplastic process. *(31, 46)* Thus, assays to detect clonality, such as the polymerase chain reaction (PCR)-based HUMARA (human androgen receptor assay), may define the primary steps in the evolution to t-MDS/AML. *(46, 47)* The HUMARA is informative in more than 90% of females and is; therefore, probably the optimal clonality assay for testing female blood or marrow samples for clonal hematopoiesis at regular intervals. *(47, 48)* Genomic instability at simple repeated DNA sequences, or microsatellites, is a sensitive marker of a genetic damage. *(49, 50)* It

appears that instability in these repeated sequences is a result of defective DNA replication/repair mechanisms. In two recent publications, genomic instability in microsatellite variants arising from genomic instability can be used as clonal markers in hematologic malignancies. *(51, 52)* Therefore, the microsatellite instability and the HUMARA assays are complementary PCR-based methods of detecting genetic damage, and can be done using a very small amount of DNA obtained from blood. To determine the incidence of specific genetic lesions following anthracycline-based regimens for breast cancer, *MLL* gene rearrangements and *RAS* mutations, genetic alterations frequently observed in therapy-related hematopoietic disorders will be evaluated in those cases where either or both of the general clonality assays show evidence of early genetic damage.

The question that is being asked in this clinical trial is whether more "dose intensive" delivery of Adriamycin and cyclophosphamide may improve upon disease free and overall survival without increasing the risk of developing t-MDS/AML in women with high-risk breast cancer. As part of this study, we would like to determine whether these agents, given in different doses and schedules, induces genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis utilizing two different assays (the HUMARA and microsatellite instability). To answer this question and to determine whether this more dose intensive regimen (daily oral cyclophosphamide, weekly Adriamycin and hematopoietic growth factor support) induces genetic damage with a higher frequency than "standard" AC (administered every 21 days without hematopoietic growth factor support), we have chosen to study sequential blood samples from 200 women enrolled on this study (100 per arm). We will evaluate/compare the frequency of clonal hematopoiesis in both standard and dose intensive arms of this study and control for variables such as age or damage that may have occurred due to other risk factors/exposures induced damage by using pretreatment (baseline) samples.

Inclusion of Minorities:

Anticipated accrual by race for this study follows:

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Female	0	12	20	25	243	0	300

Race-treatment interactions are not anticipated, so the trial has not been powered to address specific race questions. However, we will do exploratory analysis of treatment by race at the end of the study.

3.0 DRUG INFORMATION

3.1 Cyclophosphamide (Cytoxan®) (NSC-26271)

a. DESCRIPTION

2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxidemonohydrate. Cyclophosphamide is biotransformed principally in the liver to active alkylating metabolites which cross-link to tumor cell DNA.

b. TOXICOLOGY

Human Toxicology: Toxicity from cyclophosphamide includes bone marrow suppression which usually occurs 10 to 12 days after administration, nausea, vomiting, anorexia, abdominal discomfort, diarrhea, stomatitis, hemorrhagic colitis, jaundice, reversible alopecia, hemorrhagic cystitis which can frequently be prevented with increased hydration, hematuria, ureteritis, tubular necrosis, fibrosis of the bladder, cardiac toxicity which may potentiate doxorubicin-induced cardiotoxicity, rare anaphylactic reaction, skin rash, hyperpigmentation of the skin and nails, interstitial pulmonary fibrosis, and cross sensitivity with other aklylating agents. Treatment with cyclophosphamide may cause significant suppression of the immune system.

Second malignancies, most frequently of the urinary bladder and hematologic systems, have been reported when cyclophosphamide is used alone or with other anti-neoplastic drugs. It may occur several years after treatment has been discontinued. It interferes with oogenesis and spermatogenesis and may cause sterility in both sexes which is dose and duration related. It has been found to be teratogenic, and women of childbearing potential should be advised to avoid becoming pregnant. Increased myelosuppression may be seen with chronic administration of high doses of phenobarbital. Cyclophosphamide inhibits cholinesterase activity and potentiates effect of succinylcholine chloride. If patient requires general anesthesia within 10 days after cyclophosphamide administration, the anesthesiologist should be alerted. Adrenal insufficiency may be worsened with cyclophosphamide. Cyclophosphamide is excreted in breast milk, and it is advised that mothers discontinue nursing during cyclophosphamide administration. The occurrence of acute leukemia has been reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.

c. PHARMACOLOGY

Kinetics: Cyclophosphamide is activated principally in the liver by a mixed function microsomal oxidase system. PO administration is well absorbed, with bioavailability greater than 75%. Five to twenty-five percent of unchanged drug is excreted in the urine. Several active and inactive metabolites have been identified with variable plasma protein binding. There appears to be no evidence of clinical toxicity in patients with renal failure, although elevated levels of metabolites have been observed.

Formulation: Cyclophosphamide is supplied in 100 mg, 200 mg, 500 mg, 1 gram and 2 gram vials as a white powder. The drug should be reconstituted with Sterile Water for Injection, USP, and may be diluted in either normal saline or D5W. The PO form is supplied as 50 mg and 25 mg tablets.

Storage and Stability: Although the reconstituted cyclophosphamide is stable for six days under refrigeration, it contains no preservatives and therefore should be used within 6 hours. Tablets are stable at room temperature.

Administration: Cyclophosphamide should be diluted in about 150 cc of normal saline or D5W and infused IV. An added dose of IV fluids may help prevent bladder toxicity. The tablet form of the drug may also be administered PO.

Supplier: Cyclophosphamide is commercially available and should be purchased by a third party. This drug will not be supplied by the NCI.

3.2 Doxorubicin (Adriamycin®)(NSC-123127)

a. DESCRIPTION

Mechanism of Action: Doxorubicin is a cytotoxic anthracycline antibiotic different from daunorubicin by the presence of a hydroxyl group in the C-14 position. Doxorubicin is produced by fermentation from S. *Peucetius* var. *caesius*. Its mechanism of action is thought to be the binding of nucleic acids, preventing DNA and possibly RNA synthesis.

b. TOXICOLOGY

Human Toxicology: Studies with doxorubicin have shown that the major toxic effects of this drug are alopecia, which is often total but always reversible; nausea and vomiting, which develops shortly after drug administration, occasionally persisting for 2 - 3 days; fever on the day of administration; and phlebitis at the site of the drug's injection. Extravasation of the drug will lead to soft tissue necrosis. Phlebosclerosis, cellulitis, vesication and erythematous streaking have also been seen. Mucositis may be seen 5 - 10 days after administration. Ulceration and necrosis of the colon, particularly the cecum, with bleeding and severe infection have been reported with concomitant administration of cytarabine. Anorexia and diarrhea have also been observed. Hyperpigmentation of nailbeds and dermal creases, onycholysis and recall of skin reaction from prior radiotherapy may occur. Cardiac toxicity manifested as acute left ventricular failure, congestive heart failure, arrhythmia or severe cardiomyopathy has been reported, but appears to occur predominantly in patients who receive total doses in excess of 550 mg/M^2. Myelosuppression, predominantly neutropenia, is common with nadir occurring approximately two weeks after a single injection; lesser degrees of anemia and thrombocytopenia have been reported. Rapid recovery of the blood counts approximately two and a half weeks after a single injection generally permits an every three week schedule. Patients with obstructive liver disease have more severe myelosuppression due to impaired drug excretion. Thus, patients with hepatic dysfunction may need to have reduced dosage or to be excluded from therapy. Renal excretion of doxorubicin is minimal, but enough to color the urine red; thus impaired renal function does not appear to increase the toxicity of doxorubicin. Other side effects include fever, chills, facial flushing, itching, anaphylaxis, conjunctivitis and lacrimation. The occurrence of acute leukemia has been reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.

c. PHARMACOLOGY

Kinetics: Intravenous administration is followed by a rapid plasma clearance with significant tissue binding. Urinary excretion is negligible; biliary excretion accounts for 40 to 50% of the administered dose being recovered in the bile or the feces in 7 days. The drug does not cross the blood-brain barrier.

Formulation: Doxorubicin is supplied in 10, 20 and 50 mg single-use vials, and 150 mg multidose vials as a red-orange, lyophilized powder which has a storage stability of at least two years - see expiration date on vial. Doxorubicin should be reconstituted with 5, 10, 25 and 75 ml respectively, of Sodium Chloride Injection, USP (0.9%) to give a final concentration of 2 mg/ml.

Storage and Stability: The reconstituted doxorubicin is stable for 24 hours at room temperature and 48 hours under refrigeration (2° - 8°C). It should be protected from exposure to sunlight. Discard any unused solution from the vials. Bacteriostatic diluents with preservatives are NOT recommended as they might possibly worsen the reaction to extravasated drug.

Administration: Doxorubicin may be further diluted in 5% dextrose or sodium chloride injection and should be administered slowly into tubing of a freely flowing intravenous infusion with great care taken to avoid extravasation.

Supplier: This drug is commercially available for purchase by the third party. This drug will not be supplied by the NCI.

3.3 Filgrastim (r-metHuG-CSF) (NSC-614629) (BB-IND-2704)

a. DESCRIPTION

Filgrastim, Neupogen®, recombinant-methionyl human granulocyte-colony stimulating factor, granulocyte-colony stimulating factor, r-methHuG-CSF, is a protein produced by E. coli into which has been inserted the human granulocyte colony-stimulating factor gene. Filgrastim differs from the natural protein in that the N-terminal amino acid is a methionine and it is not o-glycosylated. G-CSF functions as a hematopoietic growth hormone; it increases the proliferation, differentiation, maturation and release of precursor cells into mature blood cells of the neutrophil lineage. G-CSF has demonstrated in vitro effects on mature neutrophils, including an increased expression of chemotactic receptors, enhanced phagocytosis and intracellular killing of certain organisms, as well as enhanced killing of target cells that are bound by antibodies.

Approximately 6,400 patients in U.S. and international based trials have participated in clinical trials of filgrastim to date, and the worldwide commercial populations receiving filgrastim totaled approximately 190,000. The drug has been found to be well tolerated at dosages up to 69 µg/kg/day given IV or SC, with no toxic effects attributable to filgrastim. A maximum tolerated dose has not yet been determined.

Classification: Colony stimulating factor; cytokine.

b. TOXICOLOGY

The most frequently reported adverse effect was medullary bone pain, occurring in 20 - 25% of patients in Phase II and III trials. When bone pain was reported it often preceded a rise in the circulating neutrophil count; it occurred more frequently in patients treated with 20 - 100 µg/kg/day of intravenously administered filgrastim and less often in lower subcutaneous doses. The pain was generally mild to moderate in severity, and usually controlled with non-narcotic analgesics such as acetaminophen. Other side effects include transient but reversible increases of alkaline phosphatase, lactate dehydrogenase and uric acid levels. These occurred in 27 - 58% of patients, without clinical sequelae

observed. Elevations of leukocyte alkaline phosphatase levels have also been noted but the significance is not yet known. Less frequently reported adverse events related to filgrastim administration include subclinical splenomegaly, exacerbation of pre-existing skin rashes, alopecia, and thrombocytopenia, and cutaneous vasculitis. Ischemic or infarcted colon, sometimes with involvement of other parts of the gastrointestinal tract, has been seen in patients receiving paclitaxel and G-CSF therapy. Patients reporting abdominal discomfort should be monitored closely. The specific etiologic role of paclitaxel, other chemotherapeutic agents or G-CSF is not entirely defined. It is conceivable that the high doses of chemotherapy used in these studies induced sufficiently severe neutropenia that these patients were at risk for this complication based on the myelotoxicity alone. If this is the case, then the use of G-CSF may actually assist in preventing this occurrence in other patients receiving high-dose paclitaxel chemotherapy. A review of the Amgen database of over 10,000 patients treated on company-sponsored trials reveal that the occurrence of only one case of typhlitis, two instances of intestinal ischemia, and six occurrences of intestinal perforation. However, it is remotely possible that the G-CSF may have contributed in some unforeseen way to these events.

Rarely, allergic-type reactions have occurred. Since the commercial introduction of filgrastim there have been reports (< 1 in 4,000 patients) of symptoms suggestive of an allergic-type reaction, but in which an immune component has not been demonstrated. These have generally been characterized by systemic symptoms involving at least two body systems, most often skin (rash, urticaria, edema), respiratory (wheezing, dypsnea), and cardiovascular (hypotension, tachycardia). Some reactions occurred on initial exposure. Reactions tended to occur within the first thirty minutes after administration and appeared to occur more frequently in those patients who received filgrastim intravenously. Rapid resolution of symptoms occurred in most cases after administration of standard supportive care, and symptoms recurred in more than half the patients when rechallenged.

Precautions: filgrastim should be used with caution in patients with pre-existing cardiac conditions such as hypertension, angina pectoris and cardiac dysrhythmias. Until further data become available, precaution should be exercised if filgrastim is administered to those patients with myeloid malignancies.

Pregnancy and Lactation: No clinical trials have been performed in pregnant or lactating women. Therefore, administration of filgrastim, (r-metHuG-CSF) during pregnancy or lactation is not recommended until further data are available.

Contraindications: filgrastim is contraindicated in those patients with known hypersensitivity to E. coli-derived proteins.

c. PHARMACOLOGY

Formulation: Recombinant G-CSF, filgrastim, NEUPOGEN®, is supplied as a clear, colorless preservative-free liquid for parenteral administration. Single use vials contain filgrastim 300 µg/ml in a preservative-free solution with 0.59 mg/ml acetate, 50 mg/ml sorbitol, 0.004% Tween® 80, 0.035 mg/ml sodium, and water for injection, USP, pH 4.0 to make 1 ml filgrastim Neupogen® is commercially available in 2 vial sizes: 300 µcg/1 ml and 480 µg/1.6 ml.

Dilution: If required, filgrastim may be diluted in 5% dextrose. Filgrastim diluted to concentrations between 5 and 15 µg/ml should be protected from adsorption to plastic materials by addition of albumin (Human) to a final concentration of 2 mg/ml. When diluted in 5% dextrose or 5% dextrose plus albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes. Dilution of filgrastim to a final concentration of less than 5 µg/ml is not recommended at any time. Do not dilute with saline at any time; product may precipitate.

Mode of Action: Hematopoietic regulator with effects on both immature bone marrow progenitors and mature myeloid cells; it acts by supporting growth of human bone-marrow-derived, colony-forming units and enhancing neutrophil-mediated, antibody-dependent cellular toxicity.

Storage and Stability: Filgrastim should be refrigerated and not allowed to freeze. It is stable for 24 hours at room temperature if the solution remains clear. At a concentration of 5 mcg/ml or greater in D5W, filgrastim is stable for 7 days at room or refrigerator temperatures. At dilutions from 5 to 14 mcg/ml, albumin in a final concentration if 2 mcg/ml should be added to protect against adsorption. Addition of albumin is unnecessary when the drug is diluted to a concentration greater than or equal to 15 mcg/ml in D5W. Concentrations of less than 5 mcg/ml should not be used. Dilutions in D5W are stable in glass bottles, polyvinyl chloride, polyolefin or polypropylene bags and IV sets, and Travenol Infusors.

Preparation: Draw appropriate dose into syringe for subcutaneous injection.

Incompatibilities: Normal saline.

Side Effects

Musculoskeletal: In clinical trials medullary bone pain was the only consistently observed adverse event attributed to Filgrastim and was reported in approximately 24% of patients across all indications. The bone pain was generally mild to moderate in severity and controllable in most patients with non-narcotic analgesia; infrequently, bone pain was severe enough to require narcotic analgesia.

Cardiovascular: Rarely fluid retention; transient hypotension; pericardial effusion.

Dermatologic: Local inflammation at the injection site; rarely cutaneous vasculitis.

Other: Transient, mild to moderate elevations of uric acid, LDH, alkaline phosphatase and leukocyte alkaline phosphatase when given with cytotoxic drugs.

Nursing Guidelines

Filgrastim should be kept in the refrigerator until needed and the vials should not be shaken.

The drug should be administered at the same time each day. Vials of filgrastim are single-dose and the remaining drug should be discarded.

Refer to protocol for information regarding requirements for documentation of doses administered, temperatures, side effects, etc.

Acetaminophen is the recommended analgesic for mild bone pain.

Duration of therapy will be determined by the return of blood counts (WBC/ANC) to specific values.

Administration: Filgrastim is administered as a single daily injection by SC bolus injection, by short IV infusion (15 - 30 minutes), or by continuous SC or continuous IV infusion.

Supplier: G-CSF (Filgrastim) is commercially available. However, for this study it is being supplied free-of-charge by Amgen, Inc. and is available from Oncology Therapeutics Network (OTN). To obtain a supply of G-CSF, complete the G-CSF (Filgrastim) Drug Request Form supplied in Appendix 19.2, and fax or send the form to OTN at the following address:

Oncology Therapeutics Network (OTN)
395 Oyster Point Boulevard, Suite 405
South San Francisco, CA 94080
General Phone (800) 370-2508
Fax: 650/952-1588

OTN's office hours are 8:00 a.m. to 5:00 p.m. PST, phone message may be left at other times.

Orders received by 3:00 p.m. PST Monday through Thursday will be shipped for next day delivery. The initial shipment to each study site will be delivered by 3:30p.m. Orders received by 3:00 p.m. PST on Friday will be shipped for receipt the following Monday, unless the institution specifically requests Saturday delivery by checking the appropriate box ("Saturday Delivery") on the form, and can guarantee their institution will accept delivery. **G-CSF orders from USA sites only will be accepted.** Patients must be registered to the study before study drug can be obtained.

For this study, G-CSF is supplied in 480 mcg/1.6 ml vials; initial order quantities will be 100 vials; reorder quantities will be in 30 vial increments. Unused drug at the site upon termination of the study will need to be returned to OTN, with a completed Return Medication Packing Slip (see Appendix 19.3) paperwork included identifying for which study the drug was originally shipped.

3.4 Trimethoprim Sulfa (Bactrim®)

a. DESCRIPTION

Chemistry: Trimethoprim-sulfa is an anti-bacterial compound which is a combination of a pyrimidine (trimethoprim) together with a sulfanilamide (sulfamethoxazole).

b. TOXICOLOGY

Human Toxicity: Human toxicity includes myelosuppression, allergic reactions including erythema multiforme, Stevens-Johnson syndrome, and other dermatitis, mucositis, nausea, vomiting, abdominal pain, hepatitis, headache, mental depression, convulsions, drug fever, chills and toxic nephrosis.

c. PHARMACOLOGY

Microbiology: Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid. Trimethoprim blocks the production of tetrahydrofolic acid and dihydrofolic acid by binding and reversibly inhibiting dihydrofolate reductase. Thus two consecutive steps in the biosynthesis of nucleic acids essential to many bacteria are inhibited.

Human Pharmacology: This drug is rapidly absorbed following oral administration. Blood levels of each component are similar to those achieved when each is given alone. Peak blood levels occur one to four hours after oral administration. Both drugs are present in the blood as free, conjugated, and protein bound forms. Free forms are considered to be therapeutically active drug. Excretion of the compound is chiefly by the kidneys through glomerular filtration and tubular secretion.

Formulation: Tablets containing 80 mg trimethoprim and 400 mg sulfamethoxazole and suspension containing 40 mg of trimethoprim and 200 mg sulfamethoxazole per teaspoon are available. DS (double strength) tablets containing 160 mg trimethoprim and 800 mg sulfamethoxazole are white notched tablets.

Administration: PO.

Supplier: Trimethoprim sulfamethoxazole is commercially available and should be purchased through a third party. This drug will NOT be supplied by the NCI.

4.0 STAGING CRITERIA

DEFINITION OF TNM

Primary Tumor (T)

Definitions for classifying the primary tumor (T) are the same for clinical and for pathologic classification. The *telescoping* method of classification can be applied. If the measurement is made by physical examination, the examiner will use the major headings (T3, T4). If other measurements, such as mammographic or pathologic, are used, the telescoped subsets of T1 can be used.*

T1 Tumor 2 cm or less in greatest dimension

T2 Tumor more than 2 cm but not more than 5 cm in greatest dimension

T3 Tumor more than 5 cm in greatest dimension

T4† Tumor of any size with direct extension to chest wall or skin.

- T4a Extension to chest wall
- T4b Edema (including peau d'orange) or ulceration of the skin of the breast or satellite skin nodules confined to the same breast
- T4c Both (T4a and T4b)
- T4d Inflammatory carcinoma

*Note: Paget's disease associated with a tumor is classified according to the size of the tumor.
†Note: Chest wall includes ribs, intercoastal muscles, and serratus anterior muscle but not pectoral muscle.

Regional Lymph Nodes (N)

N0 No regional lymph node metastasis

N1 Metastasis to movable ipsilateral axillary lymph node(s)

N2 Metastasis to ipsilateral axillary lymph node(s) fixed to one another or to other structures

N3 Metastasis to ipsilateral internal mammary lymph node(s)

Distant Metastasis (M)

M0 No distant metastasis

STAGE GROUPING

Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1, N2	M0
Stage IIIB	T4	Any N	M0
	Any T	N3	M0

5.0 ELIGIBILITY CRITERIA

Each of the criteria in the following section must be met in order for a patient to be considered eligible for registration. Use the spaces provided to confirm a patient's eligibility. For each patient, this section must be photocopied, completed and submitted to the Statistical Center (see Section 14.4).

SWOG Patient No. _____

Patient's Initials (L, F, M) _____

_____ 5.1 Patients must be women with a histologically confirmed diagnosis of locally advanced or inflammatory (see Section 10.1a) breast carcinoma. Histologic confirmation shall be by either core needle biopsy or incisional biopsy. Patients with locally advanced, non-inflammatory breast cancer must be estrogen receptor negative.

Inflammatory? (circle one) YES NO If no, estrogen receptor status _____

_____ 5.2 Patients must meet one of the criteria defined below (indicate one):

_____ a. Selected Stage IIB (T3, N0, M0) or IIIA (T3, N1-2, M0 or T0-2, N2, M0) disease judged primarily unresectable by an experienced breast surgeon; or otherwise deemed appropriate candidates for neoadjuvant treatment.

_____ b. Stage IIIB (T4, Any N, M0) or (Any T, N3, M0) disease.

_____ 5.3 Patients must not have any distant metastases.

_____ 5.4 Patients must not have received any prior chemotherapy or hormonal therapy for breast cancer.

_____ 5.5 Patients must not have received prior radiation therapy and must not have undergone prior definitive surgery for breast cancer.

_____ 5.6 Physical examination, chest x-ray and any x-rays or scans needed for tumor assessment must be performed within 42 days prior to registration.

Date of physical examination _____

Date of chest x-ray _____

Date of x-rays/scans for tumor assessment _____

_____ 5.7 Patients with the clinical diagnosis of congestive heart failure or angina pectoris are NOT eligible. Patients with hypertension or age > 60 years must have a MUGA or echocardiogram scan performed within 42 days prior to registration (indicate NA if no MUGA required) and LVEF % must be greater than the institutional lower limit of normal.

Hypertension or age > 60 years? (circle one) YES NO

Date of baseline MUGA/echocardiogram _____ LVEF % _____

ILLN _____

SWOG Patient No. _____

Patient's Initials (L, F, M) _____

_____ 5.8 Patients must have a serum creatinine and bilirubin ≤ the institutional upper limit of normal, and an SGOT or SGPT ≤ 2 x the institutional upper limit of normal. These tests must have been performed within 28 days prior to registration.

Serum creatinine _____ IULN_____ Date obtained_____

Bilirubin _____ IULN _____ Date obtained _____

SGOT/SGPT (circle one) _____ IULN _____ Date _____

_____ 5.9 Patients must have an ANC of ≥ 1,500/µl and a platelet count of ≥ 100,000/µl. These tests must have been performed within 28 days prior to registration.

ANC _____ Platelets _____ Date obtained _____

_____ 5.10 No prior malignancy is allowed except for adequately treated basal cell (or squamous cell) skin cancer, in situ cervical cancer or other cancer for which the patient has been disease-free for five years.

_____ 5.11 Patients must have a performance status of 0 - 2 by Zubrod criteria (see Section 10.2).

_____ 5.12 For patients who consent to the clonal hematopoiesis sample submission, a pretreatment sample of forty (40) ml of peripheral blood (four 10 ml EDTA tubes supplemented with or without 2 ml of tissue culture medium) must be submitted per Section 15.0.

_____ 5.13 Patients known to be HIV positive are not eligible due to the fact that the compromised immune system of these patients and the possibility of early death may compromise study objectives.

_____ 5.14 Pregnant or nursing women may not participate due to the possibility of fetal harm or of harm to nursing infants from this treatment regimen. Women of reproductive potential may not participate unless they have agreed to use an effective contraceptive method.

5.15 **In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28. This allows for efficient patient scheduling without exceeding the guidelines. If Day 28 or 42 falls on a weekend or holiday, the limit may be extended to the next working day.**

_____ 5.16 All patients must be informed of the investigational nature of this study and must sign and give written informed consent in accordance with institutional and federal guidelines.

_____ 5.17 At the time of patient registration, the treating institution's name and ID number must be provided to the Statistical Center in order to ensure that the current (within 365 days) date of institutional review board approval for this study has been entered into the data base.

6.0 STRATIFICATION FACTORS

Patients will be randomly assigned to Arm 1 or Arm 2 according to a dynamic allocation scheme. Treatment arms will be balanced with respect to the following stratification factor.

Disease status: inflammatory (see Section 10.1a) vs. other.

7.0 TREATMENT PLAN

For treatment or dose modification related questions, please contact Dr. Ellis at 206/288-2048 or Dr. Livingston at 206/288-1085.

7.1 Good Medical Practice

The following pre-study tests should be obtained within 42 days prior to registration in accordance with good medical practice. Results of these tests do not determine eligibility and minor deviations would be acceptable if they do not impact on patient safety in the clinical judgement of the treating physician. The Study Coordinator must be contacted if there are significant deviations in the values of these tests.

It is recommended that the following tests be done to rule out metastatic disease:

a. CT scan of abdomen and chest.

b. Bone scan.

7.2 ARM 1: DOXORUBICIN AND CYCLOPHOSPHAMIDE (AC)

The "standard" AC regimen consists of intravenous administration of doxorubicin (Adriamycin) followed by cyclophosphamide (Cytoxan) every 21 days. The regimen is administered at the doses below for a total of five cycles, unless clinical progression is documented. Patients with progressive disease at any time will be removed from protocol treatment.

Therapy will be administered on Day 1 for five 21-day cycles.

AGENT	DOSE	ROUTE	DAYS	INTERVAL
Doxorubicin*	60 mg/m^2	IV, bolus	1	every 21 days x 5 cycles
Cyclophosphamide**	600 mg/m^2	IV	1	every 21 days x 5 cycles

* Doxorubicin should be administered into a vein with secure IV access.
** Rounded to the nearest 25 mg dose. All patients should be instructed on the importance of vigorous hydration during cyclophosphamide therapy.

7.3 ARM 2: WEEKLY DOXORUBICIN WITH DAILY ORAL CYCLOPHOSPHAMIDE AND G-CSF (AC+G)

The weekly AC + G regimen consists of weekly intravenous administration of doxorubicin (Adriamycin) and daily oral administration of cyclophosphamide (Cytoxan). Subcutaneous filgrastim (G-CSF) is administered every day, **except the day of intravenous chemotherapy administration**.

To order filgrastim (G-CSF) for patients on Arm 2 of this study, please refer to the ordering instructions in Section 3.3c and the drug order form in Section 19.2.

Therapy will be administered for 15 weekly courses.

AGENT	DOSE	ROUTE	DAYS	INTERVAL
Doxorubicin	24 mg/m^2	IV, bolus	1	weekly x 15 weeks
Cyclophosphamide*	60 mg/m^2	PO	daily weeks	continuous for 15
Filgrastim**	5 µg/kg	SQ	2 - 7	weekly x 15 weeks
Prophylactic Trimethoprim Sulfa***	1 double-strength tablet bid	PO	4 and 5	weekly x 15 weeks

* Rounded to the nearest 25 mg dose. All patients should be instructed on the importance of vigorous hydration (drinking 8 - 10 glasses of water daily) during cyclophosphamide therapy.

** Begin 24 hours after the administration of doxorubicin.

*** For patients who are allergic to trimethoprim sulfa, oral trimethoprim/sulfamethoxazole desensitization may be administered at the discretion of the treating physician (see Appendix 19.4).

7.4 ARMS 1 and 2: RESPONSE ASSESSMENT

Patients will have the primary disease site evaluated at least every 3 weeks with at minimum a physical examination documentation and any clinically indicated x-rays and scans for tumor measurement (see Sections 9.1 and 9.2).

7.5 ARMS 1 and 2: SURGERY

Post-chemotherapy surgery for patients with a response or stable disease must take place no sooner than 21 days following the completion of IV chemotherapy to allow for normalization of blood counts. Unless there are exceptional circumstances, surgery should be modified radical mastectomy (mastectomy with axillary node dissection). For patients with excellent clinical response who decline modified radical mastectomy, minimal surgical resection should include lumpectomy with clear surgical margins and axillary dissection. Surgery should take place within 6 weeks after completion of chemotherapy unless complications require a delay.

Patients who progress at any time will be removed from protocol treatment. Further treatment will be per the treating physician's discretion. Any additional treatment must be documented on the Follow-Up Form (Form #61519). (See Appendix 19.6.)

NOTE: For patients who consent to the clonal hematopoiesis sample submission, pre-surgical blood samples are to be submitted per Sections 14.9 and 15.0.

7.6 Criteria for Removal from Protocol Treatment:

a. Progression of disease (as defined in Section 10.3).

b. Delay of treatment for more than 3 weeks for hematologic toxicity or more than 2 weeks for other toxicity.

c. Unacceptable toxicity.

d. Completion of planned treatment.

e. The patient may withdraw from the study at any time for any reason.

7.7 All reasons for discontinuation of treatment must be documented clearly on the Off-Treatment Notice (Form #61571).

7.8 All patients will be followed for one year or until death, whichever occurs first.

8.0 TOXICITIES TO BE MONITORED AND DOSAGE MODIFICATIONS

8.1 This study will utilize the CTC (NCI Common Toxicity Criteria) Version 2.0 for toxicity and Adverse Event reporting. A copy of the CTC version 2.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). **All appropriate treatment areas should have access to a copy of the CTC version 2.0.**

8.2 **Toxicities and Dose Modifications for ARM 1: doxorubicin and cyclophosphamide (AC)**

Chemotherapy delays and dose modifications for hematologic toxicity are based on counts from Day 1 of the cycle.

Dose reduction of either doxorubicin or cyclophosphamide is **not** allowed, except as noted below.

If a patient develops multiple toxicities included in the list below, delay treatment or modify dose based on the greatest toxicity. Dose re-escalations are not allowed after dose reductions unless otherwise specified.

NOTE: If G-CSF administration is deemed necessary for patients on Arm 1, the drug should be obtained from commercial sources. G-CSF is not being provided for patients treated on Arm 1 of this study.

a. HEMATOLOGIC TOXICITIES - ARM 1 (AC)

Toxicity	Treatment Modification
ANC < 1,500/mm^3	Hold both doxorubicin and cyclophosphamide until ANC ≥ 1,500. Repeat counts at least weekly. Resume **at full dose** with G-CSF support when counts have recovered. G-CSF should begin on Day 2 at 5 µg/kg/day and continue for 10 days. G-CSF should be discontinued at least 24 hours prior to the next cycle of chemotherapy. All remaining cycles of AC will be given with G-CSF support. If, despite G-CSF support, ANC < 1,500 on Day 1 of subsequent cycles, do the following: IF ANC recovers to ≥ 1,500 in ≤ **1 week**, give AC at full dose. IF ANC recovers to ≥ 1,200 in **2 - 3 weeks**, give AC as follows: Doxorubicin 50 mg/m^2 Cyclophosphamide 500 mg/m^2 IF ANC < 1,200 **after the 3-week delay**, remove patient from protocol treatment.
Platelets < 100,000	Hold both doxorubicin and cyclophosphamide until platelets are ≥ 100,000. Resume **at full dose**. If platelet count fails to recover to ≥ 100,000 **within 3 weeks**, remove the patient from protocol treatment.
Febrile Neutropenia* Grade 3	Give all remaining cycles with G-CSF support. If a second episode occurs, all remaining cycles will be given with G-CSF support and ciprofloxacin (500 mg po, bid) or antibiotic of choice. If a third episode occurs, the remaining cycles of AC will be reduced by 25% (based on the current dose) when chemotherapy is resumed. If a fourth episode occurs, remove the patient from protocol treatment.

*Febrile neutropenia is defined as a fever ≥ 38.5°C in the presence of neutropenia (ANC < 1,000).

b. OTHER TOXICITIES - ARM 1 (AC)

TOXICITY	TREATMENT MODIFICATION
Infection ≥ Grade 3	Give all remaining cycles with G-CSF support and ciprofloxacin (500 mg po, bid) or antibiotic of choice. If a second episode of Grade 3 or 4 infection occurs, the remaining cycles of AC will be reduced by 25% (based on current dose) when chemotherapy is resumed.
Gastrointestinal Grade ≥ 3	If full doses cannot be administered, hold both doxorubicin and cyclophosphamide. Resume **at full dose** when can be tolerated. No more than a two week delay will be allowed for this recovery. If, after a two week delay, the toxicity is not resolved, remove the patient from protocol treatment.
Mucositis Grade ≥ 3	Hold doxorubicin. Resume at full dose next cycle if toxicity recovers to ≤ Grade 2. If patient continues to have mucositis ≥ Grade 3 when next cycle is due, contact the Study Coordinator.
Liver Function Abnormalities Grade ≥ 2 Bilirubin > 1.5 x IULN or SGOT/SGPT > 2.5 x IULN	Hold chemotherapy while cause is determined. If rise is not due to metastatic disease and levels return to < Grade 2 within two weeks, resume at full dose. If delay is longer than 2 weeks, contact the Study Coordinator.
Cardiac changes** Grade ≥ 3	AC therapy must be discontinued and the patient removed from protocol treatment if the patient has symptoms of CHF (e.g., dyspnea, tachycardia, cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, etc.) and a diagnosis of CHF is confirmed or if the patient has a myocardial infarction.
Hematuria (Hemorrhagic cystitis) Grade 3 or 4	Discontinue cyclophosphamide and contact the Study Coordinator.
Grade ≥ 3*** Hand-foot syndrome with desquamation, vesicle formation, or pain which interferes with walking	Hold doxorubicin, cyclophosphamide and G-CSF for one week. Resume at full previous dose in one week if improved. Otherwise, reduce current dose of doxorubicin by 25%.

** The presence of PACs or PVCs without cardiac dysfunction is **not** an indication to stop doxorubicin. Acute dysrhythmias, which may occur during and shortly after doxorubicin infusion, are not an indication to stop doxorubicin.

*** Hand-foot syndrome often begins as tenderness or mild erythema at the lateral margins of the nails (usually of the hands) or as tenderness and edema over the calluses of the feet. Patients who show these early symptoms or have persistent significant involvement should do the following:

1. Take vitamin B6 (pyridoxine) 100 mg three times daily;

2. Regularly use Bag Balm or Australian tea tree lotion or oil on the hands and feet.

8.3 Toxicities and Dose Modifications for ARM 2: Weekly doxorubicin and daily cyclophosphamide + G-CSF (AC+G)

Chemotherapy dose modifications and delays for toxicities on the day IV therapy is due shall be based on the guidelines below.

If a patient develops multiple toxicities included in the list below, delay treatment or modify dose based on the greatest toxicity. Dose re-escalations are not allowed after dose reductions unless otherwise specified.

a. HEMATOLOGIC TOXICITIES - ARM 2 (AC+G)

TOXICITY	TREATMENT MODIFICATION
ANC ≤ 1,200/mm^3	Hold both doxorubicin and cyclophosphamide for **one week**; continue G-CSF. Then proceed as follows: IF ANC recovers to > 1,200 in ≤ **1 week**, resume both doxorubicin and cyclophosphamide **at full doses.** IF ANC < 1,200 but > 1,000 hold doxorubicin and resume cyclophosphamide **at full dose.** IF ANC remains ≤ 1,000 for **another week**, reduce current dose of AC as follows when therapy resumes: Doxorubicin 18 mg/m^2 IV Day 1 Cyclophosphamide 45 mg/m^2 PO qd. IF ANC fails to recover to > 1,000 by **week 3**, remove the patient from protocol treatment.
Platelets < 100,000/mm^3	Hold both doxorubicin and cyclophosphamide, but continue G-CSF. Hold until platelets are ≥ 100,000. Resume **at full dose**. If platelet count fails to recover to ≥ 100,000 **within 3 weeks**, remove the patient from protocol treatment.
Febrile Neutropenia* Grade 3	Give all remaining cycles with ciprofloxacin (500 mg po, bid) or antibiotic of choice. If a second episode occurs, the remaining cycles of AC will be reduced by 25% (based on current dose) when chemotherapy is resumed. If a third episode occurs, remove patient from protocol treatment.

*Febrile neutropenia is defined as a fever ≥ 38.5° C in the presence of neutropenia (ANC < 1,000).

b. OTHER TOXICITIES - ARM 2 (AC+G)

TOXICITY	TREATMENT MODIFICATION
Infection ≥ Grade 3	Give all remaining cycles with ciprofloxacin (500 mg po, bid) or antibiotic of choice. If a second episode of Grade 3 or 4 infection occurs, the remaining cycles of AC will be reduced by 25% (based on current dose) when chemotherapy is resumed.
Gastrointestinal Grade ≥ 3	If full doses cannot be administered, hold both doxorubicin and cyclophosphamide. Resume **at full dose** when can be tolerated. No more than a **two week** delay will be allowed for this recovery. If, after a **two week** delay, the toxicity is not resolved, remove the patient from protocol treatment.
Mucositis Grade ≥ 3	Hold doxorubicin; hold cyclophosphamide **only** if patient is unable to take oral medication. Resume at full dose the next week if mucositis ≤ Grade 2. If patient continues to have mucositis ≥ Grade 3 the next week, call the Study Coordinator.
Liver Function Abnormalities Grade ≥ 2 Bilirubin > 1.5 x IULN or SGOT/SGPT > 2.5 x IULN	Hold chemotherapy while cause is determined. If rise is not due to metastatic disease and levels return to < Grade 2within **two weeks**, resume at full dose. If delay is longer than 2 weeks, contact the Study Coordinator.
Cardiac changes** Grade ≥ 3	AC therapy must be discontinued and the patient removed from protocol treatment if the patient has symptoms of CHF (e.g. dyspnea, tachycardia, cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, etc.) and a diagnosis of CHF is confirmed or if the patient has a myocardial infarction.
Hematuria (Hemorrhagic cystitis) Grade ≥ 3	Discontinue cyclophosphamide and contact the Study Coordinator.
Grade ≥ 3 Hand-foot syndrome with desquamation, vesicle formation or pain which interferes with walking***	Hold doxorubicin, cyclophosphamide and G- CSF for one week. Resume at full dose in one week if improved. Otherwise, reduce dose of doxorubicin by 25% (based on current dose).

** The presence of PACs or PVCs without cardiac dysfunction is **not** an indication to stop doxorubicin. Acute dysrhythmias, which may occur during and shortly after doxorubicin infusion, are not an indication to stop doxorubicin.

*** Hand-foot syndrome often begins as tenderness or mild erythema at the lateral margins of the nails (usually of the hands) or as tenderness and edema over the calluses of the feet. Patients who show these early symptoms or have persistent significant involvement should:

1. Take vitamin B6 (pyridoxine) 100 mg three times daily;

2. Regularly use Bag Balm or Australian tea tree lotion or oil on the hands and feet.

8.4 For treatment or dose modification related questions, please contact Dr. Ellis at 206/288-2048 or Dr. Livingston at 206/288-1085.

8.5 Unexpected or fatal toxicities (including suspected reactions) must be reported to the Operations Office, to the Study Coordinator, to the IRB and the NCI. The procedure for reporting adverse reactions is outlined in Section 16.0.

9.0 STUDY CALENDAR S0012, "A Randomized Comparison of Standard Doxorubicin and Cyclophosphamide vs. Weekly Doxorubicin and Daily Oral Cyclophosphamide Plus G-CSF as Neoadjuvant Therapy for Inflammatory and Estrogen-Receptor Negative Locally Advanced Breast Cancer, Phase III"

9.1 S0012, ARM 1: Doxorubicin and Cyclophosphamide (AC)

		Cycle 1			Cycle 2			Cycle 3			Cycle 4			Cycle 5			Ω	§ √
REQUIRED STUDIES	PRE	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Wk	Post-Chemo-	Follow-
	STUDY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	therapy surgery	Up
PHYSICAL																		
History & Physical Exam ¥	X				X			X			X			X		X		X
Weight & Performance Status	X				X			X			X			X		X		X
Disease Assessment π	X				X			X			X			X		X		X
Toxicity Notation		X			X			X			X			X		X		X
LABORATORY																		
CBC, Platelets & Differential	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Serum Creatinine	X				X			X			X			X				X
Bilirubin	X				X			X			X			X				X
SGOT or SGPT	X				X			X			X			X				X
Estrogen-receptor Status	Xμ																X	X
Blood samples for clonal hematopoiesis ¶	X																	
X-RAYS AND SCANS																		
Chest x-ray	X																	
MUGA or Echocardiogram	X *																	
X-rays/scans for disease assessment £	X																	
CT of chest/abdomen	XØ																	
Bone scan	XØ																	
TREATMENT (See Section 7.0)																		
Doxorubicin ß		X ß			X			X			X			X				
Cyclophosphamide ß		X ß			X			X			X			X				
Post Chemotherapy Surgery																	X	

NOTE: Data submission forms are found in Section 18.0. Forms submission guidelines may be found in Section 14.0.

π A detailed description of the primary tumor, including results of physical exam or results of imaging studies, must be provided.
Ø These tests are recommended prestudy for good medical practice.
* MUGA or echocardiogram will be obtained prestudy for patients with hypertension or age > 60 years.
μ Estrogen-receptor status must be determined for all non-inflammatory breast cancer patients, and for those patients it must be negative (see Section 5.1).
ß Doxorubicin and cyclophosphamide are administered on Day 1 of each cycle.
Ω Definitive surgery for patients with response or stable disease must take place no sooner than 21 days following the end of the chemotherapy to allow for normalization of blood counts and within 6 weeks after the completion of chemotherapy unless complications require a delay.
§ Adjuvant treatment after post-chemotherapy surgery is at the physician's discretion. Post-surgery therapy must be documented on the Follow-Up Form (Form #61519). (See Appendix 19.6).
√ After off treatment, follow-up assessments will be done every 6 months for one year.
¥ History and physical exam will be performed every 3 weeks while on protocol treatment.
£ X-rays/scans for disease assessment should be done every 3 weeks or as clinically indicated and the same methods used at baseline should be used throughout.
¶ For patients who consent to the clonal hematopoiesis sample submission: See Section 15.0.

9.0 STUDY CALENDAR S0012, "A Randomized Comparison of Standard Doxorubicin and Cyclophosphamide vs. Weekly Doxorubicin and Daily Oral Cyclophosphamide Plus G-CSF as Neoadjuvant Therapy for Inflammatory and Estrogen-Receptor Negative Locally Advanced Breast Cancer, Phase III"

9.2 S0012, ARM 2: Doxorubicin and Cyclophosphamide + G-CSF(AC+G)

																£							Ω	§ √
		WEEK 1							WEEK 2							WEEK 3							Post-Chemo-	Follow-
REQUIRED STUDIES	PRE	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	therapy Surgery	Up
	STUDY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
PHYSICAL																								
History & Physical Exam ¥	X																							X
Weight & Performance Status	X								X							X								X
Disease Assessment ƒ	X																							X
Toxicity Notation		X							X							X								
LABORATORY																								
CBC, Platelets & Differential	X								X							X								X
Serum Creatinine	X								X							X								X
Bilirubin	X								X							X								X
SGOT or SGPT	X								X							X								X
Estrogen-receptor Status	Xμ																							
Blood samples for clonal hematopoiesis ¶	X																						X	X
X-RAYS AND SCANS																								
Chest x-ray	X																							
MUGA or Echocardiogram	X *																							
X-rays/scans for disease assessment ≠	X																							
CT of chest/abdomen	XØ																							
Bone scan	XØ																							
TREATMENT (See Section 7.0)																								
Doxorubicin		X							X							X								
Cyclophosphamide		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Trimethoprim sulfa ß					X	X						X	X						X	X				
Filgrastim (G-CSF)			X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X		
Post-Chemotherapy Surgery																							X	

NOTE: Data submission forms are found in Section 18.0. Forms submission guidelines may be found in Section 14.0.

Ø These tests are recommended prestudy for good medical practice.

ƒ Disease should be assessed at least every 3 weeks during treatment. A detailed description of the primary tumor, including results of physical exam or results of imaging studies, must be provided.

* MUGA or echocardiogram will be obtained prestudy for patients with hypertension or age > 60 years.

μ Estrogen-receptor status must be determined for all non-inflammatory breast cancer patients, and for those patients it must be negative (see Section 5.1).

ß Omit if the patient is allergic to sulfa drugs.

Ω Definitive surgery for patients with response or stable disease must take place no sooner than 21 days following the end of the chemotherapy to allow for normalization of blood counts and within 6 weeks after the completion of chemotherapy unless complications require a delay.

£ Weekly cycles of AC+G therapy will repeat as specified for 15 weeks.

§ Adjuvant treatment after post-chemotherapy surgery is at the physician's discretion. Post-surgery therapy must be documented on the Follow-Up Form (Form #61519). (See Appendix 19.6)

√ After off treatment, follow-up assessments will be done every 6 months for one year.

¥ History and physical exam performed every 3 weeks while on treatment.

≠ X-rays/scans for disease assessment should be done every 3 weeks or as clinically indicated and the same methods used at baseline should be used throughout.

¶ For patients who consent to the clonal hematopoiesis sample submission: See Section 15.(

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

10.1 Definitions

a. **Inflammatory Disease**: Erythema AND peau d'orange involving half or more of the breast with a histologic diagnosis of breast cancer. The findings of focal dermal lymphatic involvement on histology does not constitute inflammatory breast cancer.

b. **Microscopic path CR (pCR)**: No evidence of microscopic invasive tumor at the primary tumor site in the surgical specimen.

c. **Clinical CR**: Normal breast on physical exam. No mass, no thickening, no erythema, no peau d'orange.

10.2 **Performance Status**: Patients will be graded according to the Zubrod performance status scale.

POINT	DESCRIPTION
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

10.3 **Time to Progression**: From date of registration to date of first documentation of progressive disease defined as: clear increase in disease sites present at registration, or development of new sites of disease.

11.0 STATISTICAL CONSIDERATIONS

One hundred fifty patients per arm will be required for a two-sided .05 level test of equality of probabilities to have power .9 to detect a difference of .15 between the arms (assuming the probability of pCR is approximately .1 on the standard arm).

Patients who fail treatment before surgery will be assumed not to have a pCR. Treatment failure before surgery includes death, progression or start of non-protocol treatment after early discontinuation of protocol treatment.

One interim analysis will be performed after pCR is determined on the first 150 patients. If the arms are significantly different at the .01 level, or the alternative of a .15 improvement due to Arm 2 is ruled out at the .01 level, consideration will be given to stopping the trial. The final analysis will be done at the .045 level to adjust for the early test.

Samples for clonal hematopoiesis determination will be made for one hundred patients on each arm of the **S0012** study. The length of accrual is anticipated to be three years. Compliance with the blood draw at the time of surgery should be nearly complete; at 12 months following completion of treatment, approximately 15% might be anticipated to have relapsed or refused and not have samples available. The probability of clonal hematopoiesis at a particular time point can be established to within ± 0.1 with a sample size of 100 per arm, and to within ± 0.11 with a sample size of 85. Change in status between pretreatment, at the time of surgery and six and twelve months post-surgery samples will be explored, as will concordance of the HUMARA and microsatellite assays. Association of treatment, pre-study, patient characteristics, and tumor-related variables with presence or absence of clonality by HUMARA or microsatellite assays will also be explored. For example, a two-sided .05 level test of the association of the treatment group with presence or absence of clonality will have adequate power to detect differences of .25 or greater (power at least .93 for the pretreatment and three month time point and .88 for the 12 month post-treatment time point, if sample size decreased to 85 per arm).

The study will be monitored by the Southwest Oncology Group Data and Safety Monitoring Committee according to NCI guidelines and Southwest Oncology Group policy.

Based on accrual to **S9625**, accrual to this protocol should be completed in approximately 3 years.

12.0 DISCIPLINE REVIEW

There will be no discipline review for this study.

13.0 REGISTRATION GUIDELINES

13.1 Patients must be registered prior to initiation of treatment (no more than one working day prior to planned start of treatment).

13.2 For either method of registration, the individual registering the patient must have completed the appropriate Southwest Oncology Group Registration Form. The completed form must be referred to during the registration but should not be submitted as part of the patient data.

The individual registering the patient must also be prepared to provide the treating institution's name and ID number in order to ensure that the current (within 365 days) date of institutional review board approval for this study has been entered into the data base. Patients will not be registered if the IRB approval date has not been provided or is > 365 days prior to the date of registration.

13.3 Registration procedures

a. Patients from member, CCOP or approved affiliate institutions may be registered via the Web Reg program (http://www.swog.org/members/wrstart.html) at any time except maintenance down times (the logon page shows down times). Institutions with internet access are encouraged to register this way. For first time users, a "Starter Kit" can be accessed at:

https://www.swogstat.org/webapps/secured/starterkit.htm

The person registering the patient must be in the Southwest Oncology Group Roster. Call the Operations Office (210/677-8808) if an addition to the roster is necessary. A valid password allowing Web registration is also necessary. Each institution has a Web Registration Administrator (listed on the "Starter Kit" site) who may add Web Reg users at their institution and assign passwords to new users. Any Web Reg user can change their own password using the Administrator program (as explained in the Starter Kit). For other password problems or problems with the Web Reg program, email webreghelp@swog.fhcrc.org.

b. If the Web Reg program is not used, the registration must be done by phone.

Member and Affiliate Institutions

Registration by phone of patients from member and affiliate institutions must be done through the Southwest Oncology Group Statistical Center by telephoning 206/667-4623, 6:30 a.m. to 5:00 p.m. Pacific time, Monday through Friday, excluding holidays.

CCOP Institutions

Registration by phone of patients from CCOP Institutions must be done through the Southwest Oncology Group CCOP Office by telephoning 206/652-CCOP (206/652-2267), 7:00 a.m. to 4:00 p.m., Pacific Time, Monday through Friday, excluding holidays.

13.4 For either method of registration, exceptions to Southwest Oncology Group registration policies will not be permitted.

a. Patients must meet all eligibility requirements.

b. Institutions must be identified as approved for registration.

c. Registrations may not be cancelled.

d. Late registrations (after initiation of treatment) will not be accepted.

13.5 CTSU Investigators:

Prior to the recruitment of a patient for this study, investigators and their institutions must be registered with the CTSU. Each CTSU investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit all IRB/regulatory documents to the CTSU before they can enroll patients. All forms and documents associated with this study can be downloaded from the **S0012** webpage on the CTSU member website (http://members.ctsu.org). Patients can be registered only after pre-treatment evaluation is complete, all eligibility criteria have been met, and all pertinent forms and documents are approved and on file with the CTSU.

CTSU Procedures for Patient Enrollment: Contact the CTSU Patient Registration Office by calling 1-888-462-3009 to alert the CTSU Patient Registrar that an enrollment is forthcoming. To enroll the patient, the investigator (or designee) should complete the following forms:

- CTSU Enrollment Coversheet
- **S0012** Eligibility Check (Section 5.0)
- **S0012** Registration Form (Complete all sections of form except for 'Tx Assignment', and any Southwest Oncology Group-specific data fields.)

Fax these forms to the CTSU Patient Registrar at 1-888-691-8039 between the hours of 8:00 a.m. and 4:30 p.m. Eastern time, Monday-Friday. The CTSU registrar will verify that the investigator is CTSU-credentialed, that enrollment forms are complete, and that all regulatory and patient eligibility requirements have been met. The CTSU registrar will follow-up with the CTSU investigative site to resolve any discrepancies. Once investigator and patient eligibility are confirmed, the CTSU registrar will contact SWOG to obtain a treatment assignment and assignment of a unique patient ID. The CTSU registrar will then contact the enrolling site and convey the patient ID number (to be used on all future forms and correspondence) and the patient's treatment assignment. This will be confirmed by an e-mail or fax to the enrolling site.

14.0 DATA SUBMISSION SCHEDULE

14.1 Data must be submitted according to the protocol requirements for **ALL** patients registered, whether or not assigned treatment is administered, including patients deemed to be ineligible. Patients with inadequate documentation to determine eligibility will be deemed ineligible.

14.2 Copies of all forms used for this study are included in the Master Forms Set in Section 18.0. With the exception of the model consent form and the **S0012** Registration Form, these forms should be photocopied, completed, and submitted for all patients.

14.3 Group Members and Affiliates

Group members must submit one copy of all data forms directly to the Statistical Center in Seattle. Affiliates must submit (number of copies to be determined by the Group member) copies of all forms to their Group member institution for forwarding to the Statistical Center.

CCOP Institutions

CCOP Institutions must submit one copy of all data forms to the Southwest Oncology Group CCOP Office in Seattle at the following address:

Cancer Research and Biostatistics (CRAB)
ATTN: SWOG CCOP Office
1100 Olive Way, Suite 1150
Seattle, Washington 98101-1892

OR CCOP members may submit data via facsimile to 206/652-4612. Faxed data must be accompanied by the Data Submission Facsimile Cover Sheet.

14.4 CTSU INVESTIGATORS

All data forms for this study are available for download from the CTSU member website at http://members.ctsu.org. CTSU investigators should use the protocol-specific SWOG forms, adhere to the SWOG schedule for data submission, and forward all forms and reports to the CTSU Data Center in the following manner:

- Patient registration and adverse event forms should be *faxed* to the CTSU Data Center according to the CTSU-specific instructions in the patient registration and adverse event reporting sections of the protocol.

- All other original forms and reports must be *mailed* directly to the CTSU Data Center accompanied by a completed CTSU Data Transmittal Form; the CTSU will then forward all information to the Southwest Oncology Group.

CTSU REPORT FORMS AND DATA MUST BE SENT TO:

CTSU Data Processing Manager
CTSU Data Center
WB 408
1441 W. Montgomery Avenue
Rockville, MD 20850-2062

Phone: 1-888-823-5923
Fax: 1-888-691-8039

14.5 FOR PATIENTS WHO CONSENT TO THE CLONAL HEMATOPOIESIS SAMPLE SUBMISSION: AT THE TIME OF REGISTRATION:

Submit pre-study blood sample to the City of Hope National Medical Center per Section 15.0 (also see Section 5.12).

A copy of the Specimen Submission Form (Form #1951) should be submitted along with each sample and a copy should also be submitted to the Southwest Oncology Group Statistical Center.

14.6 WITHIN 14 DAYS OF REGISTRATION:

Submit copies of the following:

a. **S0012** Breast Cancer Prestudy Form (Form #64004)

b. **S0012** Assessment Form (Form #4072)

c. A completed copy of Section 5.0 of the protocol documenting history and physical and prestudy tests/exam results

d. Initial Pathology Report

14.7 AT 6 WEEKS AND 12 WEEKS DURING TREATMENT AND WITHIN 14 DAYS OF THE COMPLETION OF CHEMOTHERAPY, AND AFTER FINAL TOXICITY ASSESSMENT:

Submit copies of the **S0012** Assessment Form (Form #4072) documenting required parameters as specified in the Study Calendar.

14.8 WITHIN 14 DAYS OF DISCONTINUATION OF CHEMOTHERAPY:

Submit copies of the Off Treatment Notice (Form #61571) and the **S0012** Dose Form for Arm 1 (Form #23840) or Arm 2 (Form # 53865).

14.9 FOR PATIENTS WHO CONSENT TO THE CLONAL HEMATOPOIESIS SAMPLE SUBMISSION: AT THE TIME OF POST-CHEMOTHERAPY SURGERY (BEFORE SURGERY):

Submit pre-surgical blood sample to the City of Hope National Medical Center per Section 15.0.

A copy of the Specimen Submission Form (Form #1951) should be submitted along with each sample and a copy should also be submitted to the Southwest Oncology Group Statistical Center.

14.10 WITHIN 14 DAYS OF POST-CHEMOTHERAPY SURGERY:

Submit copies of the Operative Report and Pathology Report.

14.11 AFTER OFF-TREATMENT: EVERY SIX MONTHS FOR ONE YEAR: Post-surgery therapy must be documented on the Follow-Up Form (Form #61519). (See Appendix 19.6.)

Submit the Southwest Oncology Group Follow-Up Form (Form #61519).

14.12 FOR PATIENTS WHO CONSENT TO THE CLONAL HEMATOPOIESIS SAMPLE SUBMISSION: AT SIX AND TWELVE MONTHS AFTER POST-CHEMOTHERAPY SURGERY:

Submit six and twelve month post-surgical blood samples to the City of Hope National Medical Center per Section 15.0.

A copy of the Specimen Submission Form (Form #1951) should be submitted along with each sample and a copy should also be submitted to the Southwest Oncology Group Statistical Center.

14.13 WITHIN 14 DAYS OF PROGRESSION/RELAPSE:

Submit copies of the Southwest Oncology Group Follow-Up Form (Form #61519) documenting date, site, and method for determining progression/relapse.

14.14 WITHIN 4 WEEKS OF KNOWLEDGE OF SECOND MALIGNANCY:

Submit copies of the Southwest Oncology Group Follow-Up Form (Form #61519) documenting date, site and method for determining malignancy. (See Appendix 19.6.)

If the second malignancy is a hematologic malignancy, submit blood sample to the the City of Hope National Medical Center per Section 15.0 for clonal hematopoiesis studies. A copy of the Specimen Submission Form (Form #1951) should be submitted along with each sample and a copy should also be submitted to the Southwest Oncology Group Statistical Center.

14.15 WITHIN 4 WEEKS OF KNOWLEDGE OF DEATH:

Submit a copy of the Notice of Death (Form #61554). Also submit either a final **S0012** Assessment Form (Form #4072) if death occurs while on treatment, or a Southwest Oncology Group Follow-Up Form (Form #61519) if death occurs after off treatment.

15.0 SPECIAL INSTRUCTIONS

15.1 **For patients who consent to the clonal hematopoiesis sample submission: Samples will be submitted as described below:**

Forty ml of peripheral blood (four 10 ml EDTA tubes supplemented with or without tissue culture medium) must be collected from each patient at each time point.

Samples will be submitted at the following time points:

- Prior to the initiation of treatment
- Just prior to surgery after the completion of neoadjuvant therapy
- At six months following surgery
- At twelve months following surgery
- In the case of diagnosis of a secondary hematologic malignancy

15.2 Mailing Tubes for Sample Submission

Institutions may use their own EDTA (purple top) tubes.

If a delay in shipment is expected, please prepare the tubes using any standard tissue culture formation such as RPMI 1640, Alpha MEM, or McCoy's 5A, containing 10% fetal calf serum with EDTA (20 mg/ml) added as an anticoagulant.

15.3 Handling of Required Study Samples

All samples should be sent to the attention of Ms. Margaret Lo in Dr. Marilyn Slovak's Laboratory at room temperature by overnight courier to arrive within 24 hours at the City of Hope National Medical Center.

Blood samples will each be placed in EDTA tubes supplemented with tissue culture medium and sent **the day it is obtained** by Federal Express to:

City of Hope National Medical Center
ATT: Ms. Margaret Lo
1500 East Duarte Road
Northwest Building, Room 2265
Duarte, CA 91010
Phone: 626/359-8111 ext. 62025

Tubes should be labeled with the patient's name, Southwest Oncology Group patient number, study number (**S0012**), and the date and time of collection. Each tube should be tightly capped and wrapped with paraffin film to prevent leakage. The tube should then be placed in a standard biohazard specimen resealable bag.

15.4 Shipping

A Specimen Submission Form (Form #1951) must be completed at the institution of origin and sent with EACH specimen, even if two samples are sent on the same day to the laboratory. The Specimen Submission Form should be placed in the pocket of the specimen bag if it has one, or in a separate resealable bag.

A copy of the Specimen Submission Form (Form #1951) with the top part filled out must also be sent to the Southwest Oncology Group Statistical Center for each sample.

The bags should be shipped in a standard styrofoam shipping container which the sites can supply. NOTE: Federal Express shipping cost reimbursement is available through the Southwest Oncology Group Operations Office. Upon receipt, specimens will be logged in the City of Hope Laboratories for the HUMARA assay. MLL gene rearrangements and the RAS gene mutation assay will be on all positive genetic instability samples.

Weekday Shipping (Arrival Monday-Friday)

PLEASE PACK TUBES CAREFULLY. Cardboard express mail envelopes alone are NOT ADEQUATE--please additionally pack the tubes in styrofoam or with extra padding. If freezing conditions or extreme heat conditions are anticipated, insulated containers are recommended. Please include the laboratory telephone number listed above on the label.

Weekend Shipping (arrival on Saturday)

Samples will be accepted on Saturdays and holidays; however, the laboratory MUST be contacted (626/359-8111 ext 62025) one or two days before the sample is shipped so that special mailing instructions for weekend specimens can be obtained. Make sure to indicate Saturday delivery on the overnight mailing label.

15.5 **The Federal guidelines for shipment are as follows:**

a. The specimen must be wrapped in an absorbable material;

b. The specimen must then be placed in an AIRTIGHT container (like a resealable bag);

c. Pack the resealable bag and specimen in a styrofoam shipping container;

d. Pack the styrofoam shipping container in a cardboard box.

e. The cardboard box must be marked as "BIOHAZARD".

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

Informed Consent

The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

Drug Accountability

For each drug supplied for a study, an accountability ledger containing current and accurate inventory records covering receipt, dispensing, and the return of study drug supplies must be maintained. Drug supplies must be kept in a secure, limited access storage area under the recommended storage conditions. During the course of the study, the following information must be noted on the accountability ledger; the identification code of the subject to whom drug is dispensed, the date(s) and quantity of drug dispensed to the subject, and the date(s) and quantity of drug returned by the subject; subjects should return empty containers to the investigator, with the return noted on the ledger. These Accountability Forms must be readily available for inspection and are open to FDA inspection at any time.

Adverse Experiences

Any adverse experience, if deemed drug related, must be reported to the Operations Office Adverse Drug Reaction (ADR) representative (210/677-8808), who will obtain information on the ADR. Depending on the nature of the reaction and whether it was caused by an investigational or commercial agent, the ADR representative will advise whether the report to the NCI should be phoned in, written in, or both. See guidelines below. On Phase II and III studies, all deaths considered drug-related must be reported immediately to the ADR representative. On double-blinded studies, if the investigator must know what treatment the subject received to make therapeutic decisions, the code for that particular subject can be broken by telephoning the Statistical Center.

All adverse experiences must also be reported to the Institutional Review Board within 10 days and documentation of this report sent to the Operations Office.

All adverse experiences must also be recorded in the appropriate section of the case report form. The report should include, whenever possible, the investigator's written medical judgment as to relationship of the adverse experience to study medication(s) (i.e., "probable", "possible" or "unrelated").

Monitoring

This study will be monitored by the Clinical Data Update System (CDUS) version 2.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31.

GUIDELINES FOR REPORTING OF
ADVERSE EVENTS (AE) / ADVERSE DRUG REACTIONS (ADR)
OCCURRING WITH **COMMERCIAL** AGENTS

1. **WITHIN 24 HOURS OF THE EVENT CALL THE OPERATIONS OFFICE AT 210-677-8808.**
2. WITHIN 10 DAYS, SEND TO THE OPERATIONS OFFICE
   a) A COPY OF THE FDA FORM 3500, including Investigator's attribution[1] of the event in item 5 (or the NCI/CTEP Secondary AML/MDS Report Form for cases of secondary AML or MDS[5]).
   b) COPIES OF PRESTUDY FORM(S), AND FLOW SHEETS FROM PRESTUDY THROUGH THE EVENT
   c) IRB NOTIFICATION DOCUMENTATION
   d) OTHER DATA AS REQUESTED DURING TELEPHONIC REPORT.
3. IN ADDITION, FOLLOW THE GUIDELINES BELOW

These guidelines apply to patients accrued to NCI research protocols which use commercial anticancer agents. The following events, when attributed as possibly, probably, or definitely related to the commercial agent(s), must be reported:

(a) Any AE/ADR which is life threatening (Grade 4) or fatal (Grade 5) and unexpected (is not listed as a known toxicity, or is of greater severity or specificity than listed toxicity).[2,3,4] Any occurrence of secondary AML or MDS must also be reported.[5]

(b) Any AE/ADR which is fatal (Grade 5), even if an expected toxicity.[4]

The AE report, documented on FDA Form 3500 (or NCI/CTEP Secondary AML/MDS Report Form) should be sent within 10 days to FDA, with a copy to NCI, as indicated below:

To FDA:		**AND**	**NCI:**	
Via Internet at	www.fda.gov/medwatch		Mail one	
or mail to	MedWatch		copy to	Investigational Drug Branch
	5600 Fishers Lane			P.O. Box 30012
	Rockville, MD 20852-9787			Bethesda, MD 20824-0012
or fax to	800-332-0178		or fax to	301-402-1584

Send a copy of the FDA Form 3500 or NCI/CTEP Secondary AML/MDS Report Form, plus prestudy form, flowsheets, and a copy of IRB notification to the **Operations Office** within 10 days:

Southwest Oncology Group Operations Office
ATTN: ADR Program
14980 Omicron Drive
San Antonio, TX 78245-3217

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[1] Attribution: Whether the event was *definitely not, unlikely, possibly, probably, or definitely* related to protocol treatment.

[2] For grading reactions, see NCI Common Toxicity Criteria, Section 19.0.

[3] Known toxicities are listed in the Drug Information, Background or Informed Consent Form sections of the protocol.

[4] **A report shall be submitted if the adverse event is *definitely* related, *probably* related, or *possibly* related to the agent(s).** Reactions judged definitely not treatment related should not be reported, except that all deaths while on treatment or within 30 days after treatment must be reported. Any death more than 30 days after treatment which is felt to be treatment-related must also be reported.

[5] Secondary AML or MDS should be reported using the NCI/CTEP Secondary AML/MDS Report Form in lieu of FDA Form 3500. The Operations Office will forward this form to the Statistical Center within one working day of receipt.

ADVERSE EVENT (AE) REPORTING FOR CTSU INVESTIGATORS

This study will utilize the CTC version 2.0 for toxicity and Adverse Event (AE) reporting. A hyperlink to the CTC guidelines is available on the CTSU member website. Adverse Event reporting forms for this study can be downloaded from the **S0012** webpage located on the CTSU member website (http://members.ctsu.org). CTSU investigators are responsible for notifying their local IRB of adverse events as defined in the guidelines above.

All adverse events (both written and telephonic) should be reported according to Southwest Oncology Group guidelines. A copy of all written adverse event reports should also be faxed to the CTSU Data Center at1/888/462-3009.

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18.0 MASTER FORMS SET

18.1 Attached are copies of all data forms which must be completed for this study. The Model Informed Consent form is also included, and must be reviewed and approved by the Institutional Review Board prior to registration and treatment of patients on this study.

18.2 Forms to be used for patients treated on this study include:

a. **S0012** Registration Form (Form #63439)

b. **S0012** Breast Cancer Prestudy Form (Form #64004)

c. **S0012** Assessment Form (Form #4072)

d. **S0012** Dose Form - Arm 1 (Form #23840)

e. **S0012** Dose Form - Arm 2 (Form #53865)

f. Off Treatment Notice (Form #61571)

g. Notice of Death (Form #61554)

h. Southwest Oncology Group Follow-Up Form (Form #61519)

I. Southwest Oncology Group Specimen Submission Form (Form #1951)

For IRB use only, not to be included in patient information.

This model informed consent form has been reviewed by the DCT/NCI and is the official consent document for this study. Local IRB changes to this document are allowed. (Institutions should attempt to use sections of this document which are in bold type in their entirety.) Editorial changes to these sections may be made as long as they do not change information or intent. If the institutional IRB insists on making deletions or more substantive modifications to the risks or alternatives sections, they may be justified in writing by the investigator and approved by the IRB. Under these circumstances, the revised language, justification and a copy of the IRB minutes must be forwarded to the Southwest Oncology Group Operations Office for approval before a patient may be registered to this study.

Readability Statistics:	Flesch Reading Ease	57.8 (targeted above 55)
	Flesch-Kincaid Grade Level	9.1 (targeted below 8.5)

S0012, "A Randomized Comparison of Standard Doxorubicin and Cyclophosphamide vs. Weekly Doxorubicin and Daily Oral Cyclophosphamide plus G-CSF as Neoadjuvant Therapy for Inflammatory and Estrogen-Receptor Negative Locally Advanced Breast Cancer, Phase III"

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 family and friends.

You are being asked to take part in this study because you have breast cancer that cannot be cured by surgery alone or because your doctor believes that it is best to give you chemotherapy to shrink your tumor before surgery.

WHY IS THIS STUDY BEING DONE?

The main purpose of this study is to compare two different treatments (or "regimens") for breast cancer prior to surgery to see if one works better against breast cancer than the other. One treatment includes the drugs doxorubicin and cyclophosphamide given through a needle in a vein on Day 1 every 21 days, five times. The other treatment includes the same two drugs but the doxorubicin is given through a needle in the vein once a week for 15 weeks and the cyclophosphamide is given by pill every day for 15 weeks. The two drugs filgrastim and trimethoprim sulfa are also given with this second treatment regimen. The other purpose of this study is to compare the type and severity of the side effects of each of these two treatment regimens.

The researchers would also like to learn whether the treatment for your breast cancer causes gene damage to your hematopoietic cells (early blood cells) which may be linked with the development of

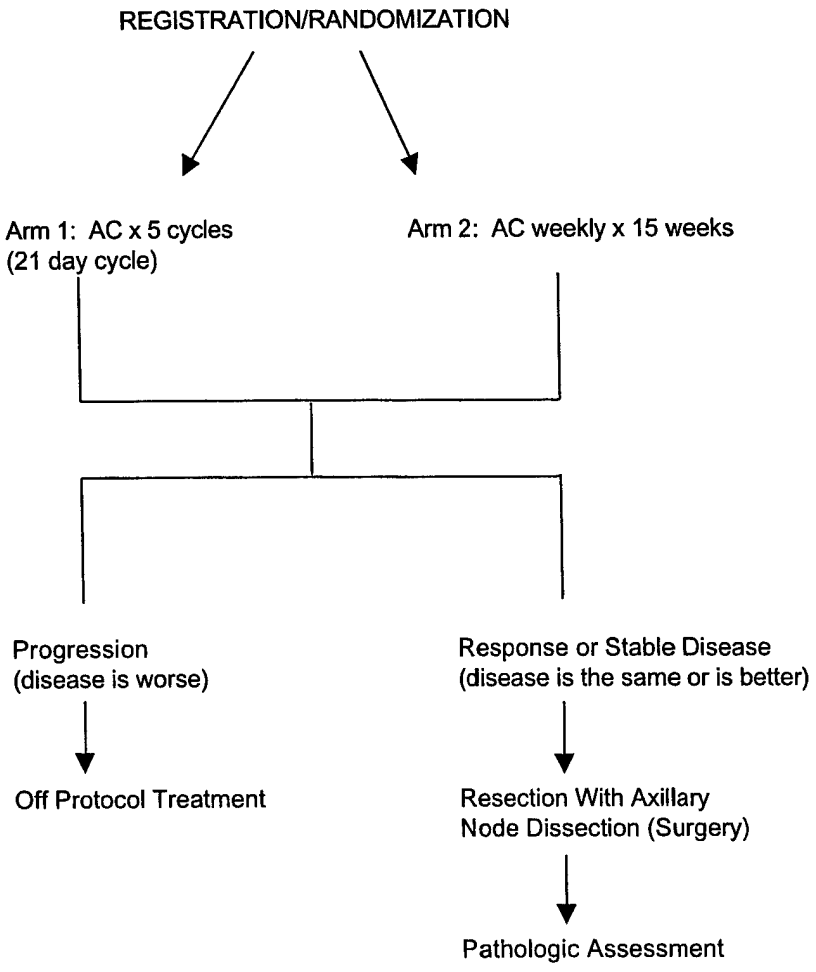
leukemia in a small subgroup of breast cancer patients. The Department of Defense (DOD) has provided funds to find out this information. If you agree to submit blood samples for this purpose, small amounts of your blood (four 10 ml tubes) will be sent to a central laboratory where it will be tested for genetic damage. The submission of these samples will help in finding out if genetic damage is related to a second cancer. The studies done on your blood cells may lead to discoveries which help future patients with breast cancer. *(paragraph added)*

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 300 people will take part in this study.

WHAT IS INVOLVED IN THE STUDY?

SCHEMA



You will be "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance. It is like flipping a coin. Which group you are put in is done by a computer. Neither you nor the researcher will choose what group you will be in. You will have an equal chance of being placed in either group.

Regardless of which group you are assigned to, you will receive two chemotherapy drugs that are commonly used to treat breast cancer: Doxorubicin (also called Adriamycin) and cyclophosphamide (also called Cytoxan). However, in each of these two treatment groups, Arm 1 or Arm 2 of the study, the doxorubicin and cyclophosphamide are given differently.

If you are assigned to Arm 1, you will receive the chemotherapy drugs doxorubicin and cyclophosphamide through a needle in your vein on Day 1 every 21 days for a total of five cycles (with each cycle being a 21-day timeframe). Three to six weeks after you finish your chemotherapy treatment, you will have surgery to remove the remaining breast cancer.

If you are assigned to Arm 2, you will receive the chemotherapy drug doxorubicin through a needle in your vein once a week for 15 weeks. You will receive the chemotherapy drug cyclophosphamide by mouth (by taking a pill) every day for 15 weeks. You will also receive two additional non-chemotherapy drugs: filgrastim (also called G-CSF) and trimethoprim sulfa (also called Bactrim). Filgrastim is a "growth factor" that helps your body produce high numbers of the white blood cells that fight infection. (Chemotherapy can lower your white blood cell count and increase your chance of infection.) You will receive an injection of filgrastim every day except for the day that you receive the doxorubicin in your vein. You will receive information on the administration of filgrastim. You will also receive the drug trimethoprim sulfa by mouth by taking two pills on Days 4 and 5 of each week. Trimethoprim sulfa is an antibiotic that protects you from developing a specific type of pneumonia that is associated with the high doses of cyclophosphamide that are used in this treatment. Three to six weeks after you complete your chemotherapy treatment, you will have surgery to remove the remaining breast cancer.

The following procedures are part of regular cancer care and may be done even if you do not join the study.

- Medical history and physical exam
- Chest x-ray
- Blood tests for liver and kidney function
- Blood counts
- Other blood tests or x-rays may be done if you physician feels that they are necessary to help determine your "baseline" condition.

- If you have high blood pressure or are over 60 years of age, you will need to have a test, called a MUGA scan or echocardiogram, to determine how well your heart is working as a pump.

The following are standard procedures being done because you are in this study.

- Your weight will be measured regularly during the study: This is to make sure that the doxorubicin, cyclophosphamide and filgrastim are given at the correct dose as they are all based on your weight and height.

- You will have a physical exam and possibly x-rays and scans at least once every 3 weeks while you are receiving treatment to check on your condition.

If you agree to submit samples for the clonal hematopoiesis testing funded by the Department of Defense (DOD) looking for genetic damage in blood cells, the following will be done. Blood samples (four 10 ml tubes) will be submitted before you begin treatment, just before your surgery and at 6 and 12 months after your surgery. Also, if a secondary hematologic cancer such as leukemia is diagnosed any time after you begin treatment, you are requested to submit a blood sample at that time. The blood samples can be taken from those needed for the diagnosis and treatment of your secondary cancer. If this is not possible, an additional venipuncture may be needed. The samples will be sent to the following laboratories for genetic testing. The "microsatellite instability assays" and "MLL gene rearrangement" testing will be performed at the University of Illinois at Chicago, Department of Hematology/Oncology, under the direction of Dr. Wendy Stock. Dr. Marilyn Slovak will direct the "HUMARA Clonality Assay" testing at the City of Hope National Medical Center in Duarte, California, Department of Cytogenetics. In the final year of the study (year three), the "RAS gene mutation analysis" will be done only for those samples that are positive for clonality. This testing (if there are any positive samples), will be done at the Fred Hutchinson Cancer Research Center in Seattle, Washington by Dr. Jerry Radich. All of these assays are described in Appendix 19.5 of the **S0012** protocol. *(paragraph added)*

We think you will be in the study for about 15 weeks to complete your chemotherapy before surgery. Your surgery is expected to take place 3 - 6 weeks after you complete your chemotherapy. After that, you will continue with regular doctor visits for checkups every 6 months for one year. If you agree to submit samples for the clonal hematopoiesis testing and if you are diagnosed with a secondary hematologic malignancy at any time after you complete treatment on this study, you will see your doctor again and a blood sample will be taken at that time.
( )

The researcher may decide to take you off this study if your disease gets worse despite the treatment; the side effects of the treatment are too dangerous for you; new information about the treatment becomes available and this information suggests the treatment will be ineffective or unsafe for you. It is unlikely, but the study may be stopped early due to lack of drug supply or lack of funding.

WHAT ARE THE RISKS OF THE STUDY?

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

Risks and side effects related to the doxorubicin, cyclophosphamide, filgrastim and trimethoprim sulfa treatment include:

Likely:

- **Nausea and vomiting**
- **Loss of appetite**
- **Heartburn**
- **Hair loss**
- **Lowered white blood cell count, red blood cell count and platelet count leading to increased risk of infection, fatigue, or bruising or bleeding more easily**

Less Likely:

- **Sores in the mouth**
- **Hand-foot syndrome (tingling pain and redness of the hands and feet)**
- **Change in color of fingernails and toenails**
- **Loosening of fingernails and toenails**
- **Inflammation or damage to the skin and around the IV tubing.**
- **Bladder inflammation (prevent this by drinking 8 - 10 glasses of water each day and emptying your bladder frequently)**
- **Pneumocystis pneumonia (for patients on Arm 2) may be caused by the lowered white blood cell counts and may be prevented by the use of an antibiotic, trimethoprim sulfa)**
- **Bone or joint pain (for patients on Arm 2)**
- **Cramps in the legs or back (for patients on Arm 2)**

Less Likely but Serious:

- **Heart damage**
- **Increased risk of leukemia**

Risks from venipuncture (needed for drawing blood samples for the clonal hematopoiesis testing): The risk from venipuncture is very small. There may be some bruising or bleeding at the site the blood is drawn from. *(paragraph added)*

Reproductive risks: Because the drugs in this study can affect an unborn baby, you should not become pregnant while on this study. You should not nurse your baby while on this study. Ask about counseling and more information about preventing pregnancy. Doxorubicin and cyclophosphamide may also damage your reproductive cells (eggs) and, if you are still menstruating, you may begin having irregular menstrual periods or stop menstruating altogether. This side effect may be permanent. You may not be able to have children after receiving this treatment. This is more likely if you are over the age of 40.

Very rarely, severe bleeding or infection may result from lowered blood counts, and could be fatal.

You may receive other drugs as part of your treatment (erythropoietin [also known as "epo"], antibiotics, anti-nausea drugs) that are not part of this study and may never be needed. If your doctor feels it is necessary to use any of these drugs to treat the side effects of your

chemotherapy, he/she will discuss the risks and benefits with you at that time. Other drugs not mentioned in this consent may be used to prevent or treat the side effects of chemotherapy. The risks, benefits and possible side effects of any drug prescribed will be explained to you. As with any drug, there may be unanticipated side effects.

For more information about risks and side effects, ask the researcher or contact _____.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

We cannot and do not guarantee that you will benefit if you take part in this study. The treatment you receive may even be harmful. Your doctors feel that your participation in this study will give you at least as good a chance as you might expect from other treatments. We hope the information learned from this study will benefit other patients with breast cancer in the future.

The possible benefits of taking part in the study are the same as receiving chemotherapy prior to surgery without being in the study.

There is no benefit for your taking part in the clonal hematopoiesis testing portion of this protocol. There may be some benefit to patients with breast cancer in the future. *(paragraph added)*

WHAT OTHER OPTIONS ARE THERE?

Instead of being in this study, you have these options:

You may choose to participate in other studies giving neoadjuvant chemotherapy (chemotherapy before surgery) or other chemotherapy. You may choose to receive other chemotherapy combinations commonly used for this type of breast cancer, most containing the drug doxorubicin, without being in a study. You may choose treatment with other new experimental drugs.

You may also choose to have no anti-cancer treatment at this time (with care to make you feel more comfortable).

You can get treatment for breast cancer without being on this study. All of the treatment on this study may be available at this center or at other locations.

There may be other ways of determining if there is genetic damage in your cells. The methods used in this study are comparable to others that may be available. You also have the option of not having this procedure done on your blood samples.

Please talk to your regular doctor about these and other options.

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.

Data is stored in a secured and confidential computer record at the Southwest Oncology Group Statistical Center. No submitted forms, reports or internal applications include name, SSN, zip code or country and only patient initials are used. *(paragraph added)*

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as: the National Cancer Institute, the Food and Drug Administration, Amgen Pharmaceutical Company and the Southwest Oncology Group. Additionally, if you agree to submit samples for the clonal hematopoiesis testing, the U.S. Army Medical Research and Material Command may inspect your research records. *( )*

If we publish the information we learn from this study in a medical journal, you will not be identified by name or in any other way.

It is suggested that CTSU institutions incorporate the following paragraph in their consent forms . ( ) While you are participating in this study a record of your progress on this study will be kept in a confidential form at [INSERT NAME OF INSTITUTION] and sent to the sponsor who will add this information to a computer file. The confidentiality of any central computer record will be carefully guarded and no information by which you can be identified will be released or published. You have been informed that authorized representatives of [INSERT GROUP NAME and the CANCER TRIALS SUPPORT UNIT], the National Cancer Institute, the Food and Drug Administration (FDA), and [INSERT NAME OF INSTITUTION AND INSTITUTIONAL REVIEW BOARD HERE] may inspect and copy the records. ([Optional, if applicable] *An authorized representative of the manufacturers of the drugs used in this study may also have access to your study records.)* Your identity will remain confidential and your records will be used by these authorized representatives only in connection with carrying out their obligations relating to the clinical trial and they shall not be used for any other purpose or disclosed to any third party except with your express permission.

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/funds have been set aside to compensate you in the event of injury. *(local institutions must choose the option that best fits the hospital's situation)*

In the case of injury from the venipuncture blood draws for the clonal hematopoiesis testing: Other than medical care that may be provided at the discretion of the treating institution, and any other payment specifically stated in this consent form, there is no other compensation available for your participation in this part of the study. *(paragraph added)*

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.

Administration of the drug will be (provided free of charge/charged in the usual way). The parts of the research consisting of keeping research records will be paid by those organizing and conducting the research. The research requires that you receive certain standard medical tests and examinations. These standard tests and examinations will be (charged in the usual way/provided at a reduced rate). *(local institutions must choose the option that best fits the hospital's situation)*

Doxorubicin, cyclophosphamide and trimethoprim sulfa are commercially available. Filgrastim is also commercially available but will be supplied for this study by Amgen Pharmaceutical.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

A Data Safety and Monitoring Board, an independent group of experts, will be reviewing the data from this research throughout the study. We will tell you about important new information from this or other studies that may affect your health, welfare, or willingness to stay in this 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).]*

WHERE CAN I GET MORE INFORMATION?

[To IRB/Investigators: Attach information materials and checklist of attachments. Signature page should be at the end of package. You may also wish to include the following informational resources]

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://cancertrials.nci.nih.gov.

CancerNet™: accurate cancer information including PDQ
http://cancernet.nci.nih.gov.

You will get a copy of this form. You may also request a copy of the protocol (full study plan).

SIGNATURE

You are deciding whether or not to take part in this study. If you sign, it means that you have decided to volunteer to take part in this study, and that you have read and understood all the information on this form.

(changes on signature and lines)

Participant signature _____ Date _____

Participant printed name _____

Consent for submission of samples for clonal hematopoiesis testing: You are agreeing to submit blood samples for the purposes of looking for genetic damage as explained in this form. *(paragraph and lines below added)*

Participant signature _____ Date _____

Participant printed name _____

Witness:

Witness signature _____ Date _____

Witness printed name _____

Southwest Oncology Group Statistical Center
1100 Fairview Avenue North, MP557
PO Box 19024
Seattle, WA 98109-1024
Patient Registration (206) 667-4623
CCOP Patient Registration (206) 652-2267

Southwest Oncology Group Operations Office
14980 Omicron Drive
San Antonio, TX 78245-3217
(210) 677-8808

Southwest Oncology Group Registration Form

SWOG Study No.	Registration Step	Assigned Treatment Arm	Activation Date: May 1, 2001
S0012	1		Last Amended Date:

A Randomized Comparison of Standard Doxorubicin and Cyclophosphamide Vs. Weekly Doxorubicin and Daily Oral Cyclophosphamide Plus G-CSF as Neoadjuvant Therapy for Inflammatory and Estrogen-Receptor Negative Locally Advanced Breast Cancer

Patient's Name _____

SWOG Patient ID ☐☐☐☐☐☐

INSTRUCTIONS: All of the information on this Registration Form and the Protocol Eligibility Section must be answered appropriately for a patient to be considered eligible for registration. This Registration Form must be entirely filled out and referred to during the registration. Do NOT submit this form as part of the patient data.

Caller's SWOG Roster ID ☐☐☐☐☐

SWOG Investigator Number ☐☐☐☐☐

SWOG Treating Institution Number ☐☐☐☐☐

IRB Approval Date ☐☐/☐☐/☐☐☐☐

Date Informed Consent Signed ☐☐/☐☐/☐☐☐☐

Projected Start Date of Treatment ☐☐/☐☐/☐☐☐☐

Patient's Date of Birth: ☐☐/☐☐/☐☐☐☐

Patient's Race / Ethnicity: ☐☐/☐☐

Patient's Sex: ☐ Female ☐ Male

Method of Payment: ☐☐

If a U.S. resident:

Patient's Social Security Number: ☐☐☐-☐☐-☐☐☐☐

Patient's ZIP Code: ☐☐☐☐☐

Country of Residence, if not USA: _____

If a resident of Canada:

Social Insurance Number: ☐☐☐-☐☐☐-☐☐☐

Postal Code: ☐☐☐-☐☐☐

Stratification Factor:

Inflammatory disease: ☐ No ☐ Yes

Notes:

Southwest Oncology Group Registration Form Code Sheet

Patient's race:

0 - Unknown	1 - Caucasian	2 - African American	3 - Native American
4 - Eskimo	5 - Aleut	6 - Chinese	7 - Filipino
8 - Hawaiian	9 - Korean	10 - Vietnamese	11 - Japanese
12 - Asian Indian	13 - Samoan	14 - Guamanian	15 - Hmong
16 - Fijian	17 - Laotian	18 - Thai	19 - Tongan
20 - Pakistani	21 - Cambodian	22 - Other API	23 - Other race

Patient's Ethnicity (Spanish/Hispanic Origin):

0 - Unknown	1 - No (not Spanish)	2 - Yes, Mexican	3 - Yes, Puerto Rican
4 - Yes, Cuban	5 - Yes, Central American		6 - Yes, South American
7 - Yes, Other	8 - Yes, NOS		

Method of Payment:

1 - Private	2 - Medicare	3 - Medicare and Private	4 - Medicaid
5 - Medicaid and Medicare		7 - No insurance (self-pay)	
8 - No insurance (no means)		9 - Other - specify _____	
10 - Unknown		11 - Veterans Admin	12 - Military

Other Group Registration Code:

9977 - ACOSOG	9981 - NCIC	9982 - CALGB	9984 - GOG
9987 - MDACC	9995 - ECOG	9996 - NCCTG	9997 - RTOG

SOUTHWEST ONCOLOGY GROUP
S0012 BREAST CANCER PRESTUDY FORM

SWOG Patient ID ☐☐☐☐☐☐ **SWOG Study No.** S0012 **Registration Step** 1

Patient Initials _____ (L, F M)

Institution / Affiliate _____ Physician _____

Instructions: All dates are **MONTH, DAY, YEAR.** Explain any blank fields or blank dates in the **Notes** section at the bottom of the form. Place an [X] in appropriate boxes. Circle **AMENDED** items in red.

PATIENT CHARACTERISTICS

Menopausal status (select one):

☐ Pre (< 6 mo since LMP and no prior bilateral ovariectomy and not on estrogen replacement)

☐ Post (prior bilateral ovariectomy OR > 12 mo since LMP with no prior hysterectomy)

☐ Above not applicable AND age < 50 (pre) ☐ Above not applicable AND age ≥ 50 (post)

Height: ☐☐☐ cm **Weight:** ☐☐☐.☐ kg **Performance Status:** ☐ 0 ☐ 1 ☐ 2

DISEASE DESCRIPTION

Date of Initial Diagnosis of Primary Tumor: ☐☐ / ☐☐ / ☐☐☐☐

Stage: ☐ IIB ☐ IIIA ☐ IIIB

Is there a palpable mass or thickening? ☐ No ☐ Yes **If yes, size of mass:** ☐☐.☐ cm

Is there erythema or redness? ☐ No ☐ Yes **If yes, indicate extent:** _____

Is there peau d'orange? ☐ No ☐ Yes **If yes, indicate extent:** _____

Receptor Status: (≥ 10 is positive if measured in fmols/mg cytosol protein. Otherwise use institutional standards; borderline results should be reported as positive.)

ER: ☐ ER- ☐ ER+ ☐ Unknown ER

PgR: ☐ PgR- ☐ PgR+ ☐ Unknown PgR

Was HER-2/neu status determined? ☐ No ☐ Yes

If yes, methods used (select all that apply):

☐ DAKO Herceptest ☐ FISH ☐ Other IHC, specify: _____

HER-2 Status (select one):

☐ Positive. One or more of the following results were obtained:

a) 3+ by DAKO Herceptest OR
b) strongly positive by another immunohistochemical method OR
c) Her-2 positive as demonstrated by FISH

☐ Negative: otherwise.

PRIOR TREATMENT RELATED TO THIS CANCER

Prior Surgery: ☐ Core needle biopsy ☐ Incisional biopsy **Date:** ☐☐ / ☐☐ / ☐☐☐☐

Notes:

SOUTHWEST ONCOLOGY GROUP
S0012 ASSESSMENT FORM

SWOG Patient ID ☐☐☐☐☐☐ **SWOG Study No.** S 0 0 1 2 **Registration Step** 1

Patient Initials _____ (L, F M)

Institution / Affiliate _____ Physician _____

Instructions: Complete after every disease assessment during treatment, and submit all completed forms at 6 weeks, 12 weeks, and at time of discontinuation of treatment (after final toxicity assessment). All dates are **MONTH, DAY, YEAR**. Explain any blank fields or dates in the **Notes** section. Place an [X] in appropriate boxes. Circle **AMENDED** data in red.

DISEASE STATUS

Date of Last Disease Assessment: ☐☐ / ☐☐ / ☐☐☐☐

Date of Last Contact or Death: ☐☐ / ☐☐ / ☐☐☐☐

Vital Status:
☐ Alive
☐ Dead *(submit Notice of Death form)*

Is breast normal on physical exam? ☐ No ☐ Yes

Is there a palpable mass or thickening? ☐ No ☐ Yes

Is there erythema or redness? ☐ No ☐ Yes

Is there peau d'orange? ☐ No ☐ Yes

Is there a clear increase in disease since registration? ☐ No ☐ Yes

If Yes, describe increase: _____

New site of disease? ☐ No ☐ Yes, specify: _____

TREATMENT STATUS

Assigned treatment arm: ☐ AC every 3 weeks ☐ AC + G-CSF weekly

Is patient still on protocol treatment? ☐ No *(submit Off Treatment Notice)* ☐ Yes

Were there any dose modifications or additions/omissions to protocol treatment? ☐ No ☐ Yes

If yes, type and reason: _____

CTC Adverse Event (submit Grades 3 - 5 toxicities since last form only)

Date of Last Toxicity Assessment: ☐☐ / ☐☐ / ☐☐☐☐

Stat Ctr Use Only	GRADE / TERM
CA07	☐ Supraventricular arrhythmia
CA08	☐ Ventricular arrhythmia
CA10	☐ LVEF decrease/CHF
GI00	☐ Nausea
GI10	☐ Vomiting
GI20	☐ Diarrhea
GI60	☐ Stomatitis/pharyngitis
GI61	☐ Esophagitis/dysphagia

Stat Ctr Use Only	GRADE / TERM
SK13	☐ Hand-Foot Syndrome
FL40	☐ Fatigue/malaise/lethargy
HE20	☐ Anemia
HE00	☐ Leukopenia
HE10	☐ Thrombocytopenia
HE30	☐ Neutropenia/ Granulocytopenia
LU00	☐ Dyspnea

Stat Ctr Use Only	GRADE / TERM
IN00	☐ *Infection w/o 3-4 neutropenia
IN05	☐ *Infection with 3-4 neutropenia
IN99	☐ *Infection, unk ANC
IN30	☐ Febrile Neutropenia
PA99	☐ Pain - other
	☐ Other_____
	☐ Other_____

*Indicate site of infection: _____

Notes:

SOUTHWEST ONCOLOGY GROUP
S0012 DOSE FORM – ARM 1

SWOG Patient ID [ ][ ][ ][ ][ ][ ] **SWOG Study No.** [S][0][0][1][2] **Registration Step** [1]

Patient Initials _____ (L, F M)

Institution / Affiliate _____ Physician _____

Instructions: Complete this form if the patient was assigned to **Treatment Arm 1**. Submit this form **only once**, after the patient has **completed chemotherapy**. All dates are **MONTH, DAY, YEAR**. Explain any blank dates or fields in the **Notes** section. Place an [X] in appropriate boxes. Circle **AMENDED** items in red.

Did the patient start treatment on the assigned arm?

☐ No (Do not complete the rest of the form.)

☐ Yes (Do complete the rest of the form.)

DOSE RECEIVED

For each cycle the patient remained on the assigned treatment arm, specify dates of administration and total dose (in mg/m^2) received by the patient for each agent. Note: Total dose in mg/m^2 is calculated by dividing the total dose the patient received in mg by the patient's BSA. Also note: 1 gm = 1,000 mg.

Specify totals for the remaining agent even if the other agent was discontinued early.

BSA (m^2): [ ].[ ]
(measured at baseline)

Cycle 1
Start date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
CTX total mg/m^2: [ ][ ][ ].[ ]
DOX total mg/m^2: [ ][ ].[ ]
Was G-CSF given? ☐ No ☐ Yes
If Yes: Total µg/kg per day: [ ][ ].[ ]
Number of days: [ ][ ]

Cycle 2
Start date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
CTX total mg/m^2: [ ][ ][ ].[ ]
DOX total mg/m^2: [ ][ ].[ ]
Was G-CSF given? ☐ No ☐ Yes
If Yes: Total µg/kg per day: [ ][ ].[ ]
Number of days: [ ][ ]

Cycle 3
Start date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
CTX total mg/m^2: [ ][ ][ ].[ ]
DOX total mg/m^2: [ ][ ].[ ]
Was G-CSF given? ☐ No ☐ Yes
If Yes: Total µg/kg per day: [ ][ ].[ ]
Number of days: [ ][ ]

Cycle 4
Start date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
CTX total mg/m^2: [ ][ ][ ].[ ]
DOX total mg/m^2: [ ][ ].[ ]
Was G-CSF given? ☐ No ☐ Yes
If Yes: Total µg/kg per day: [ ][ ].[ ]
Number of days: [ ][ ]

Cycle 5
Start date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ]
CTX total mg/m^2: [ ][ ][ ].[ ]
DOX total mg/m^2: [ ][ ].[ ]
Was G-CSF given? ☐ No ☐ Yes
If Yes: Total µg/kg per day: [ ][ ].[ ]
Number of days: [ ][ ]

Notes:

SOUTHWEST ONCOLOGY GROUP
S0012 DOSE FORM -- ARM 2

SWOG Patient ID ☐☐☐☐☐☐ **SWOG Study No.** S 0 0 1 2 **Registration Step** 1

Patient Initials _____(L, F M)

Institution / Affiliate _____ Physician _____

Instructions: Complete this form if the patient was assigned to **Treatment Arm 2**. Submit this form **only once**, after the patient has **completed chemotherapy**. All dates are **MONTH, DAY, YEAR**. Explain any blank dates or fields in the **Notes** section. Place an ☒ in appropriate boxes. Circle **AMENDED** items in red.

Did the patient start treatment on the assigned arm?

☐ No (Do not complete the rest of the form.)

☐ Yes (Do complete the rest of the form.)

DOSE RECEIVED

For each cycle the patient remained on the assigned treatment arm, specify dates of administration and total dose (in mg/m^2) received by the patient for each agent. Note: Total dose in mg/m^2 is calculated by dividing the total dose the patient received in mg by the patient's BSA. Also note: 1 gm = 1,000 mg.

Specify totals for the remaining agent even if the other agent was discontinued early.

BSA (m^2): ☐.☐☐ (measured at baseline)

	Course Begin date	CTX total mg/m^2	DOX total mg/m^2	G-CSF given
Week 1	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 2	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 3	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 4	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 5	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 6	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 7	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 8	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 9	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 10	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 11	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 12	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 13	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 14	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No
Week 15	☐☐ / ☐☐ / ☐☐☐☐	☐☐☐.☐	☐☐.☐	☐ Yes ☐ No

Notes:

SOUTHWEST ONCOLOGY GROUP
OFF TREATMENT NOTICE

SWOG Patient ID ☐☐☐☐☐☐ SWOG Study No. S☐☐☐☐ Registration Step ☐

Patient Initials _____ (L, F M)

Institution / Affiliate _____ Physician _____

Groups other than SWOG: Group Name/Study No./Pt. ID _____ / _____ / _____

Instructions: For each registration step, submit this form within 2 weeks after completion (or discontinuation) of treatment.

Systemic Therapy: List regimens, start and end dates. For multidrug regimens, do not list individual drugs separately; end date would be the date all drugs in the regimen were discontinued.

Surgery: List type of surgery, and in the "end date" column, the date of surgery.

Radiation: List sites, start and end dates (inclusive of boosts and implants).

All dates are **MONTH, DAY, YEAR**. Explain any blank fields or blank dates in the **Notes** section. Place an ☒ in appropriate boxes. Circle AMENDED items in red.

Treatment Start Date	**Treatment End Date**	**Regimen or Procedure or Site(s)**
☐☐ / ☐☐ / ☐☐☐☐	☐☐ / ☐☐ / ☐☐☐☐	_____
☐☐ / ☐☐ / ☐☐☐☐	☐☐ / ☐☐ / ☐☐☐☐	_____
☐☐ / ☐☐ / ☐☐☐☐	☐☐ / ☐☐ / ☐☐☐☐	_____
☐☐ / ☐☐ / ☐☐☐☐	☐☐ / ☐☐ / ☐☐☐☐	_____
☐☐ / ☐☐ / ☐☐☐☐	☐☐ / ☐☐ / ☐☐☐☐	_____

(If more room is needed, please continue on a separate page)

Off Treatment Reason (select one):

- ☐ Treatment completed per protocol criteria
- ☐ Medically required, due to toxicity, specify: _____
- ☐ Patient refused, due to toxicity, specify: _____
- ☐ Patient refused, other than toxicity, specify: _____
- ☐ Progression or relapse. Sites: _____
- ☐ Death (attach Notice of Death form)
- ☐ Other, specify: _____

Off Treatment Date

Date of completion, progression, death or decision to discontinue therapy: ☐☐ / ☐☐ / ☐☐☐☐

Will patient receive further treatment?

☐ No ☐ Yes, specify: _____ ☐ Unknown

Date of Last Contact (or death): ☐☐ / ☐☐ / ☐☐☐☐

Vital Status: ☐ Alive ☐ Dead (attach Notice of Death form)

Notes:

SOUTHWEST ONCOLOGY GROUP
NOTICE OF DEATH

SWOG Patient ID ☐☐☐☐☐☐ Most Recent SWOG Study No. S☐☐☐☐

Patient Initials _____ (L, F M)

Institution / Affiliate _____ Physician _____

Groups other than SWOG: Group Name/Study No./Pt. ID _____ / _____ / _____

Instructions: Answer all questions and explain any blank fields or blank dates in the **Notes** section. Place an [X] in appropriate boxes. Circle AMENDED items in red.

Date of Death: ☐☐ / ☐☐ / ☐☐☐☐ (month / day / year)

CAUSES OF DEATH

Any cancer (select one):

☐ No ☐ Primary Cause ☐ Contributory ☐ Possible ☐ Unknown

If cancer was the primary cause or if cancer possibly or definitely contributed to death, and the patient had had multiple tumor types, specify those which were causes of death:

☐ Cancer of most recent SWOG study, specify cancer: _____

☐ Cancer of other SWOG study, specify cancer: _____

☐ Other cancer, specify: _____

Toxicity from disease related treatment (select one):

☐ No ☐ Primary Cause ☐ Contributory ☐ Possible ☐ Unknown

If Primary Cause, Contributory or Possible, specify treatment and toxicity:

Non-cancer and non-treatment related causes (select one):

☐ No ☐ Primary Cause ☐ Contributory ☐ Possible ☐ Unknown

If Primary Cause, Contributory or Possible, specify:

Autopsy done? ☐ No ☐ Yes ☐ Unknown

Death information obtained from (select all that apply):

☐ Autopsy report
☐ Medical record / Death certificate
☐ Physician
☐ Relative or friend
☐ Other, specify: _____

Notes:

SOUTHWEST ONCOLOGY GROUP
FOLLOW UP FORM

SWOG Patient ID ☐☐☐☐☐☐ SWOG Study No. S☐☐☐☐ Registration Step ☐

Patient Initials _____ (L, F M)

Institution / Affiliate _____ Physician _____

Groups other than SWOG: Group Name/Study No./Pt. ID _____ / _____ / _____

Instructions: Please submit at each follow up after completion of treatment until recurrence, at time of recurrence, and at protocol specified intervals after recurrence. All dates are **MONTH, DAY, YEAR**. Answer all questions and explain any blank fields or blank dates in the **Notes** section. Place an ☒ in appropriate boxes. Circle AMENDED items in red.

VITAL STATUS

Vital Status: ☐ Alive ☐ Dead Date of last contact or death: ☐☐ / ☐☐ / ☐☐☐☐

If vital status is Dead, complete and submit Notice of Death form.

DISEASE FOLLOW UP STATUS

Has the patient had a documented clinical assessment for this cancer since submission of the previous follow-up form?

☐ No ☐ Yes If Yes, Date of Last Clinical Assessment: ☐☐ / ☐☐ / ☐☐☐☐

NOTICE OF FIRST RELAPSE OR PROGRESSION

Has the patient developed a first relapse or progression that has not been previously reported?

☐ No ☐ Yes If Yes, Date of Relapse or Progression: ☐☐ / ☐☐ / ☐☐☐☐

Site(s) of Relapse or Progression: _____

NOTICE OF NEW PRIMARY

Has a new primary cancer or myelodysplastic syndrome (MDS) been diagnosed that has not been previously reported?

☐ No ☐ Yes If Yes, Date of Diagnosis: ☐☐ / ☐☐ / ☐☐☐☐

New Primary Site: _____

NON-PROTOCOL TREATMENT

Has the patient received any non-protocol cancer therapy (prior to progression/relapse) not previously reported?

☐ No ☐ Yes If Yes, Date of First Non-Protocol Therapy: ☐☐ / ☐☐ / ☐☐☐☐

Agents: _____

LONG TERM TOXICITY

Has the patient experienced (prior to treatment for progression or relapse or a second primary, and prior to non-protocol treatment) any severe (grade ≥ 3) long term toxicity that has not been previously reported?

☐ No ☐ Yes If Yes, Toxicities and Grades: _____

Notes:

19.0 APPENDIX

19.1 Expanded Participation Project (EPP) Instructions

19.2 G-CSF Drug Order Form

19.3 Returned Medication Packing Slip

19.4 Oral Trimethoprim/Sulfamethoxazole Desensitization Procedure

19.5 Clonal Hematopoiesis Assay Descriptions

19.6 Post-Surgical Treatment Data Collection Information

19.1 Expanded Participation Project (EPP) Instructions

PROTOCOL: **S0012**

A Randomized Comparison of Standard Doxorubicin and Cyclophosphamide Vs. Weekly Doxorubicin and Daily Oral Cyclophosphamide Plus G-CSF As Neoadjuvant Therapy For Inflammatory and Estrogen-Receptor Negative Locally Advanced Breast Cancer

1.0 EPP Randomization and Registration Procedures

I. EPP institutions will register a patient on-line through the Clinical Trials Management Unit (CTMU). Questions pertaining to eligibility criteria should be directed to the CTMU, medical questions should be directed to the Study Chair.

II. A signed HHS 310 form documenting the Institutional Review Board (IRB) approval for this study must be on file at the CTMU before the EPP institution can enter a patient. IRB approval date must be less than one year prior to the date of registration.

III. The CTMU will check the investigator and site information provided to ensure that all regulatory requirements have been met. The CTMU protocol monitor will also check the forms for completeness and follow-up with the site to resolve any discrepancies. Once investigator and patient eligibility have been confirmed, the CTMU will contact the SWOG to obtain a randomization assignment. The CTMU will then contact the enrolling site and convey the patient's treatment assignment. This will be followed by a confirmation of registration e-mail or fax to the enrolling site. Please check for errors, and submit any corrections in writing to the CTMU.

2.0 Data Submission

Data must be submitted electronically directly to the CTMU according to the following schedule:

FORM	TIME OF SUBMISSION
1. **S0012** Eligibility Checklist	At registration
2. **S0012** Breast Cancer Prestudy Form*	Within 2 weeks of registration
3. EPP Toxicity Form	Months 1, 2, 3, and every three months while on protocol therapy
4. EPP Follow-up Form*+	Every 3 months while on protocol treatment and every 6 months after completion of protocol treatment until 3 years after randomization
5. EPP Response/Progression Form*+	Every 3 months while on protocol treatment, every 6 months thereafter until progression/relapse
6. EPP Solid Tumor Evaluation Forms - Target Lesions+	Every 3 months until progression
7. EPP Solid Tumor Evaluation Forms - Non-Target Lesions+	Every 3 months until progression
8. EPP Chemotherapy/Immunotherapy/Hormonal Therapy Form*	Initial dose and date agent first administered within 2 weeks of start of therapy. Remainder of form at the completion of protocol therapy.
9. EPP Off-Treatment Form*	At the completion of all protocol therapy
10. EPP Notice of Secondary Malignancy Form*	Within 10 days of diagnosis
11. NCI/CTEP Secondary AML/MDS Report Form	Within 30 days of diagnosis of AML or MDS
1 2. EPP Death Form*	Within 7 days of knowledge of event

* These forms are to be submitted according to the above schedule for all patients who never started treatment.
+ If a patient is still alive after 3 years have elapsed after registration, no further follow-up is required.

3.0 EPP Adverse Event (AE) Reporting

A hyperlink to the CTEP home page that contains CTC version 2.0 for toxicity and adverse event reporting is available on the EPP web-site. EPP investigators are responsible for reporting adverse events according to the guidelines provided below, including notification to their local IRB. All reporting should be conducted within the time frames below, and completed forms should be submitted to the CTMU as outlined below. Once received, the CTMU will forward the forms to the SWOG, who will forward them to the appropriate authorities.

3.1 Explanation of terms used in adverse event reporting

Adverse event (AE) - An adverse event is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

To select the correct toxicity name for an adverse event, refer to the NCI's Common Toxicity Criteria (CTC) Version 2.0. The NCI has an available Index to the CTC Version 2.0 that provides help for classifying and locating toxicities. The CTC can be found on the Cancer Therapy Evaluation Program (CTEP) homepage at http://ctep.info.nih.gov/CTC3/ctc.html. If you need further assistance, please contact the CTMU.

Grade - The NCI Common Toxicity Criteria (CTC) Version 2.0 must be used to determine the grade (severity) of the adverse event.

Attribution - Attribution is defined as the determination of whether an adverse event is related to a medical treatment or procedure. Attribution categories are as follows:

• Unrelated	The adverse event is clearly NOT related to the commercial agent (s).
• Unlikely to be related	The adverse event is doubtfully related to the commercial l agent (s).
• Possibly related	The adverse event may be related to the commercial agent (s).
• Probably related	The adverse event is likely related to the commercial agent (s).
• Definitely related	The adverse event is clearly related to the commercial agent (s).

Expectedness - an adverse event is *unexpected* (unknown) if the type of event (or its specificity or severity) is not listed in the drug package insert or the Physician's Desk Reference (PDR). It is *expected* (known) if it has been reported previously by other physicians and is listed in the drug package insert or the PDR.

Commercial Agent - a commercial agent is any drug included in the protocol therapy that is not supplied under an Investigational New Drug Application (IND). In S0012, the commercial agents are Cyclophosphamide (Cytoxan), Doxorubicin and Filgrastim (G-CSF).

Investigational Agent - an investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In S0012, there are no investigational agents.

Treatment that contains both investigational and commercial agents should be reported according to the investigational guidelines. However, if the reaction is clearly a known reaction of the commercial agent involved, it should be reported according to the commercial agent guidelines.

3.2 Procedures for reporting

Adverse event monitoring and reporting is a routine part of every clinical trial. Grade the severity of the event using the CTC v. 2.0. Then, determine whether the event is expected or unexpected (refer to Section 3.0 of the protocol, Drug Information) and if the adverse event is related to the medical treatment or procedure. With this information, determine whether an adverse event should be reported as an expedited report or as part of the routinely reported clinical data. All grades of all adverse events felt to be at least possibly treatment related, including those with separate reporting requirements described in the Table below, should be reported on the EPP Toxicity Form.

Expedited adverse event reporting requires submission of a written report to the CTMU. Telephone notification of NCI and CTMU may also be required. Telephone and written reports are to be completed within the timeframes specified in the table below. All expedited adverse event reports should also be submitted to the local Institutional Review Board (IRB).

Deaths are required to be reported via the EPP Death Form within 7 days of knowledge of the event. Any death more than 30 days after the patient's last study treatment or procedure which is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTC type and attribution assigned.

3.3 Expedited Adverse Event Reporting Requirements (based on NCI Guidelines: Expedited Reporting Requirements for NCI Commercial Agents, January 2001 Version)

	Grade 4 or 5 Unexpected Regardless of Attribution	rade 5 xpected with ttribution of ossible, Probable r Definite	Secondary AML/MDS[1]
ritten report to CTMU ithin 5 working days[2,3,4]	X	X	
CI/CTEP Secondary ML/MDS Report Form to TMU within 10 working ays[3]			X

1. Reporting for this AE required during or after treatment.
2. Use *Form FDA-3500* available on the EPP website.
3. Fax or mail reports to the CTMU, The EMMES Corporation, 11325 Seven Locks Road Suite 214, Potomac, MD 20854, Fax: 301-299-3991. The CTMU will submit the report to the SWOG and NCI, as required.
4. Grade 4 myelosuppression are not submitted via this form.

If a patient has been on more than one NCI-sponsored study, the NCI/CTEP Secondary AML/MDS Report form must be submitted for the most recent trial.

Remove patient names and identifiers such as social security number, address, etc., from reports and supporting documentation that may be forwarded to the IDB.

3.4 *Pregnancy occurring while the patient is on protocol therapy*

If a patient of an EPP investigator becomes pregnant while receiving protocol therapy, the CTMU Protocol Monitor (epppm@emmes.com) should be notified immediately.

Study Title: **S0012, A Randomized Comparison of Standard Doxorubicin And Cyclophosphamide Vs. Weekly Doxorubicin And Daily Oral Cyclophosphamide Plus G-CSF As Neoadjuvant Therapy For Inflammatory And Estrogen-Receptor Negative Locally Advanced Breast Cancer, Phase III**

Filgrastim (G-CSF) Drug Request Form

Requested by:

Pharmacist:_____

Institution:_____

Principal Investigator:_____

Phone #:_____

Ship To:

Name:_____

Address*:_____

_____

*** Please do not use P.O. Box numbers**

Fax:_____

					Check One	
Southwest Oncology Group	**Pt.**	**Pt. Initials**	**# Of Vials ****		**Initial**	**Re-order**
Protocol	**ID**	**(Last, First)**	**480 µg**		**(For this Pt.)**	**(For this Pt.)**

Reminder: See protocol section on drug formulation for instructions regarding amounts of drug to order

G-CSF will be shipped (refrigerated) on Monday through Thursday for next day delivery.

Shipment will be made on the same date the drug requested is received.

G-CSF can also be shipped on Friday, but ***only if the institution can guarantee*** receipt of Saturday delivery.

☐ **Check here if requesting Saturday delivery.**

_____ _____

Date of Drug Request Pharmacist Signature

Return Completed, Signed, and Dated form to:

Oncology Therapeutic Network (OTN)
Fax: 650-952-1588

RETURNED MEDICATION PACKING SLIP

Institution Name:

Address:

Principal Investigator: Phone No.:

Amgen Study No: Cooperative Group No.: **S0012**

Study Title: "A Randomized Comparison of Standard Doxorubicin And Cyclophosphamide Vs. Weekly Doxorubicin And Daily Oral Cyclophosphamide Plus G-CSF As Neoadjuvant Therapy For Inflammatory And Estrogen-Receptor Negative Locally Advanced Breast Cancer, Phase III"

Instructions:
Per FDA requirements, please retain a copy of this completed form for your files. Drug being returned for any reason should be sent, together with this original form, to Oncology Therapeutics Network OTN), 395 Oyster Point Boulevard, Suite 405, South San Fransisco, CA 94080. Questions may be directed to (800) 370-2508, Monday through Friday 8:00 am - 5:00 pm, Pacific Standard Time. Voice Mail is available at all other times.

Study in progress?

❑ Yes ❑ No

Study completed per protocol?

❑ Yes ❑ No

Reason drug returned? (Please check one)

❑ Drug Expired

❑ Unused drug being returned

Person Shipping Drug:_____

Drug being returned by:

❑ Fed Ex ❑ UPS ❑ US Mail

Date:_____ No. of cartons:_____

Data Manager's/Pharmacist's Signature:

_____ Date:_____

Return receipt requested: ❑ Yes ❑ No

Fax number:_____

DESCRIPTION OF RETURN SHIPMENT

Drug Name & Vial Description ___mcg/___ml/vial	Lot Number	Number of vials
Comments:		

TO BE COMPLETED BY AMGEN

Returned shipment received on_____ and checked by:_____
(Date) (Name)

19.4 ORAL TRIMETHOPRIM/SULFAMETHOXAZOLE DESENSITIZATION PROCEDURE

This is completed over six days with oral doses of TMP/SMX (trimethoprim/sulfamethoxazole, or "Bactrim"). One standard double-strength (DS) Bactrim tablet contains 160 mg of TMP and 800 mg of SMX. Desensitization is performed with solutions made from a standard oral suspension of TMP/SMX, which consists of 40 mg TMP and 200 mg SMX per 5 ml. The dilutions and final concentrations for TMP and SMX components are given below.

Day 1	1:100,000 dilution	(0.0004 mg SMX in 1 cc) (0.00008 mg TMP in 1 cc)
1 cc*		
2 cc*	QID	
4 cc		
8 cc		

* The first two doses should be administered in the clinic and patients should be observed for anaphylaxis for one hour after each of these two doses, with appropriate medications and equipment available for resuscitation. **Someone must be present who can observe and summon help if anaphylaxis occurs.**

Day 2	1:10,000 dilution	(0.004 mg SMX in 1 cc) (0.0008 mg TMP in 1 cc)
1 cc		
2 cc	QID	
4 cc		
8 cc		

Day 3	1:1,000 dilution	(0.04 mg SMX in 1 cc) (0.008 TMP in 1 cc)
1 cc		
2 cc	QID	
4 cc		
8 cc		

Day 4	1:100 dilution	(0.4 mg SMX in 1 cc) (0.08 TMP in 1 cc)
1 cc		
2 cc	QID	
4 cc		
8 cc		

Day 5	1:10 dilution	(4.0 mg SMX in 1 cc) (0.8 mg TMP in 1 cc)
1 cc		
2 cc	QID	
4 cc		
8 cc		

Day 6	Standard oral susp.	(40.0 mg SMX in 1 cc) (8.0 mg TMP in 1 cc)
1 cc 2 cc 4 cc 8 cc	QID	

Following Day 6, take one DS (double strength, 800 mg) tablet Bactrim every day until chemotherapy is complete. IT IS IMPORTANT TO NOT MISS A DAY.

If symptoms develop, the following interventions are recommended:

SYMPTOM	TREATMENT
Low grade fever, malaise, myalgia	Aspirin, Tylenol q 4 hrs as needed
Mild morbilliform eruption	Diphenhydramine hydrochloride 25-50 mg q 6-8 h prn; 50 mg hs
Raging fever and/or florid morbilliform rash: urgent care	Prednisone 60 mg daily for 3 days, then 40 mg daily for 3 days, then 20 mg daily for 3 days, then 10 mg daily for 3 days
Shortness of breath	Call 911

NOTE: Patients failing desensitization or intolerant to sulfa may receive pentamidine at the treating physician's discretion.

19.5 Clonal Hematopoiesis Assay Descriptions

Sample Processing:

High molecular weight DNA will be prepared from the blood and apheresis samples following Ficoll-gradient separation, according to standard proteinase K digestion and phenol/chloroform extraction methods. *(53)* Cells from each blood samples (a minimum of 20 - 30 x10^6 cells) will be frozen for variability according to standard methods. *(54)*

HUMARA assay: DNA samples from each of the 200 patients enrolled will be studied at the time points outlined previously. Clonality at the HUMARA locus will be assessed by PCR amplification according to Willman et al. using the primers described by Gale et al., and quantitated by the method of Delabesse et al. *(48, 55, 56)* Willman et al have performed mixing experiments which demonstrate that the percentage of clonal cells can be estimated with an error of ± 10%, and that a clonal population of cells can be detected if they constitute more than 10 percent of the cells in a polyclonal background. *(48)* Assays will performed in duplicate or triplicate.

Microsatellite instability assay: Microsatellite instability will be assessed at multiple chromosomal loci: 7q31 (D7S522 marker), 5q31 (Mfd27 marker), 17p12 (Mfd41 marker), 8p22 (LPL marker), 11q23(D11S939 marker) and the BAT loci (25, 26 and 40). (57-63) Although the microsatellite assay is a general assay for genomic instability, we have chosen highly polymorphic microsatellites from regions known to be associated with t-MDS/AML since these markers may also provide information about loss of heterozygosity in these genomic regions. The PCR assays will be done in duplicate according to published methods. *(52)*

Detection of MLL gene rearrangements and *RAS* mutations: In cases where the HUMARA or microsatellite repeat assays are positive for clonal hematopoiesis, sensitive reverse-transcriptase PCR assays, using RNA from banked specimens, will be used to detect MLL fusion transcripts commonly reported in AML with 11q23 abnormalities. *(64, 65)* *RAS* mutations will be performed according to published methods. *(31, 42)*

19.6 Post-Surgical Treatment Data Collection.

All post-surgical treatment must be documented on the Southwest Oncology Group Follow-Up Form (Form #61519). This information may be included in the "Notes" section of the form. If more space is needed, an additional sheet may be attached to the Follow-Up Form and the additonal sheet must include the study number, patient's initials and patient number.

Record each chemotherapy agent given and include doses and dates, if available. Record any radiation therapy with a description of the sites irradiated, dates and doses, if available.

S9719 Biologic

Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer: Pilot Study to Evaluate Incidence

Study Coordinators:
M Slovak, C Willman, W Stock, K Albain, S Bearman

Statisticians:
S Green, D Lew

Data Coordinator:
K Rantala

Date Activated:
10/15/1997

Date Closed:
2/15/2001

Objectives

To estimate the incidence of early genetic damage, defined by the presence of clonal hematopoiesis using a general clonality assay, the HUMARA (human androgen receptor assay), in pretreatment blood and bone marrow, apheresis, and two sequential post-treatment specimens in breast cancer patients enrolled in S9623.

To detect genetic damage following dose-intensive adjuvant regimens for breast cancer by screening these samples for the presence of defective DNA mismatch repair mechanisms and loss of heterozygosity using microsatellite instability assays.

To estimate the incidence of MLL (myeloid lymphoid leukemia) gene fusion transcripts in cases where either the HUMARA or microsatellite repeat assays are positive for clonal hematopoiesis, a finding commonly reported in t-AML with 11q23 abnormalities.

To determine the frequency of RAS gene mutations (H-, K-, and N-RAS) following dose-intensive adjuvant regimens for breast cancer.

Patient Population

Patients must be enrolled on S9623 and registered to this biologic study before beginning protocol treatment on S9623.

A pretreatment sample of peripheral blood, and bone marrow when available, must be collected.

Accrual Goals

One hundred patients per arm from S9623 will be accrued on this study.

Summary Statement

This study closed along with S9623 on February 15, 2001 with a total of 29 registrations. The study will now be done as an option on S0012.

Registration by Institution

Institutions	Total Reg	Institutions	Total Reg
Columbia River CCOP	7	Harris Methodist/San Antonio, U of TX	1
Northwest CCOP	3	Henry Ford Hosp	1
Oregon Hlth Sci Univ	3	LSU-Shreveport	1
Loyola University	2	N Colorado Med Ctr/Colorado, U of	1
St Francis/Stormont/Kansas, U of	2	Salem Hospital/Oregon Hlth Sci Univ	1
Arizona, U of	1	St Anthony Hospital/Colorado, U of	1
Carilion Medical Ctr/Loyola University	1	St Lukes/Mt States/Utah, U of	1
City of Hope Med Ctr	1	Sutter Hlth CRG-East/Davis, U of CA	1
Columbia University	1	Total (17 Institutions)	29