Phase III Trial Evaluating Letrozole As First-line Endocrine Therapy With or Without Bevacizumab for the Treatment of Postmenopausal Women with Hormone Receptor-Positive Advanced Breast Cancer: CALGB 40503 (Alliance)

Dickler, et al

DOI: 10.1200/JCO.2015.66.1595

Protocol Update #9
02/15/2015

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB 40503

ENDOCRINE THERAPY WITH OR WITHOUT ANTI-VEGF THERAPY: A RANDOMIZED, PHASE III TRIAL OF ENDOCRINE THERAPY ALONE OR ENDOCRINE THERAPY PLUS BEVACIZUMAB (NSC 704865; IND 7921) FOR WOMEN WITH HORMONE RECEPTOR-POSITIVE ADVANCED BREAST CANCER

Investigational agent supplied for this trial by the NCI: Bevacizumab (NSC 704865; IND 7921)
Commercial agent supplied for this trial by Novartis: Letrozole

[X]	**Update:**	[ ]	**Status Change:**
[ ]	Eligibility changes	[ ]	Activation
[ ]	Therapy / Dose Modifications / Study Calendar changes	[ ]	Closure
[ ]	Informed Consent changes	[ ]	Suspension / temporary closure
[ ]	Scientific / Statistical Considerations changes	[ ]	Reactivation
[ ]	Data Submission / Forms changes		
[X]	Editorial / Administrative changes		
[ ]	**Other :**		

No IRB approval of this amemdment is requited.
Please follow your local IRB guidelines.

UPDATES TO PROTOCOL:

- In keeping with new CTEP PIO requirements, the name of the lead group on the title page of the protocol has been updated from "Cancer and Leukemia Group B" to "Alliance for Clinical Trials in Oncology."
- The NCTN participating groups have been added to the lower left of the protocol cover page.

UPDATES TO THE MODEL CONSENT:

No changes have been made to the model consent form.

A replacement protocol and model consent document have been issued.
Please note that this study remains closed to new patient accrual.

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

ALLIANCE FOR CLINIAL TRIALS IN ONCOLOGY

CALGB 40503/CTSU 40503

ENDOCRINE THERAPY WITH OR WITHOUT ANTI-VEGF THERAPY: A RANDOMIZED, PHASE III TRIAL OF ENDOCRINE THERAPY ALONE OR ENDOCRINE THERAPY PLUS BEVACIZUMAB (NSC 704865; IND 7921) FOR WOMEN WITH HORMONE RECEPTOR-POSITIVE ADVANCED BREAST CANCER

Investigational agent supplied for this trial by the NCI: Bevacizumab (NSC 704865; IND 7921)
Commercial agent supplied for this trial by Novartis: Letrozole
Companion study: CALGB 70501

Study Chair
Maura Dickler, M.D.
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10021
Tel: 646-888-4560 Fax: 646-888-4555
dicklerm@mskcc.org

Alliance Breast Committee Vice-Chair
Lisa A. Carey, MD
Tel: 919-966-4431 Fax: 919-966-6735
lisa_carey@med.unc.edu

Correlative Science Co-Chair
Mary Ellen Moynahan, M.D.
Tel: 646-888-4561 Fax: 646-888-4555
moynaham@mskcc.org

PET Committee Co-Chair
Federico Innocenti, MD
Tel: 773-834-2452 Fax: 773-702-2770
finnocen@medicine.bsd.uchicago.edu

Cancer in the Elderly Co-Chair
Arti Hurria, M.D.
Tel: 626-256-4673 Fax: 626-301-8898
ahurria@coh.org

Breast Committee Co-Chairs

Clifford Hudis, M.D.
Tel: 646-888-4551
Fax: 646-888-4555
hudisc@mskcc.org

Eric P. Winer, M.D.
Tel: 617-632-3800
Fax: 617-632-1930
ewiner@partners.org

PET Committee Chair
Mark Ratain, M.D.
Tel: 773-702-4400
Fax: 773-702-3969
mratain@medicine.bsd.uchicago.edu

Breast Faculty Statistician
William T. Barry, Ph.D.
Tel: 919-681-5047
Fax: 919-688-9335
bbarry@jimmy.harvard.edu

PET Faculty Statistician
Kouros Owzar, Ph.D.
Tel: 919-681-8505
Fax: 919-681-8028
kouros.owzar@duke.edu

Staff Statistician
Connie Cirrincione, M.S.
Tel: 919-681-5404
Fax: 919-681-8028
connie.cirrincione@duke.edu

Data Coordinator
Elizabeth A. Delahunty
Tel: 919-668-9356 Fax: 919-668-9348
elizabeth.delahunty@duke.edu

Protocol Coordinator
Heather Becker
Tel: 773-834-2546 Fax: 312-345-0117
hpbecker@uchicago.edu

Participating NCTN Groups:
ECOG-ACRIN / ECOG-ACRIN Medical Research Foundation, Inc.
SWOG/SWOG

Alliance Protocol Operations Program Office
230 West Monroe Street, Suite 2050
Chicago, IL 60606
Tel: 773-702-9171 Fax: 312-345-0117
http://www.allianceforclinicaltrialsinoncology.org
Adverse Event Reporting
Chicago Office: 773-702-9860
NCI Investigative Drug Branch 301-230-2330

Alliance Statistics and Data Center
Hock Plaza
2424 Erwin Road, Suite 802
Durham, NC 27705
Tel: 919-668-9350
Data Operations Fax: 919-668-9348
Biostatistics Fax: 919-681-8028
CALGB Patient Registration: 919-668-9396
CALGB Patient Registration Fax: 919-668-9397

Alliance Pathology Coordinating Office
Innovation Centre
The Ohio State University
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967

CALGB 40503 Pharmacy Contact
Brenda Gebhart, RPH
Missouri Baptist Medical Center
Tel: 314-996-5012
bkg4029@bjc.org

CALGB 40503 Nursing Contact
Erica Fischer, RN, BSN, CBCN
Memorial Sloan-Kettering Cancer Center
Tel: 646-888-4867
fischere@mskcc.org

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

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

ECOG-ACRIN Study Co-Chair
Bryan P. Schneider, M.D.
Indiana Cancer Pavilion
Tel: 317-274-6473 Fax: 317-274-8002
bpschnei@iupui.edu

SWOG Study Co-Chair
Debasish Tripathy, M.D,
USC/Norris Comprehensive Cancer Center
Tel: 323-865-3900 Fax: 323-865-0061
tripathy@usc.edu

Document History	Effective Date:	Document History	Effective Date:
Activation	May 15, 2008	Update #05	June15, 2011
Update #01	August15, 2009	Update #06	December 16, 2011
Update #02	May 15, 2010	Update #07	September 15, 2013
Update #03	September 15, 2010	Update #08	September 01, 2014
Update #04	November 01, 2010	Update #09	February 15, 2015

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

ENDOCRINE THERAPY WITH OR WITHOUT ANTI-VEGF THERAPY: A RANDOMIZED, PHASE III TRIAL OF ENDOCRINE THERAPY ALONE OR ENDOCRINE THERAPY PLUS BEVACIZUMAB (NSC 704865; IND 7921) FOR WOMEN WITH HORMONE RECEPTOR-POSITIVE ADVANCED BREAST CANCER

Investigational agent supplied for this trial by the NCI: Bevacizumab (NSC 704865; IND 7921)
Commercial agent supplied for this trial by Novartis: Letrozole

Patient Eligibility

Histologic dx of primary or metastatic Stage IV or locally advanced, unresectable Stage IIIB cancer of the female breast (using AJCC criteria, 6th edition) not amenable to local therapy
No concurrent active secondary malignancy (see Section 4.1.2)

Tumors that are ER and/or PgR positive (see Section 4.1.3)

≥ 18 years of age

ECOG (Zubrod) PS ≤ 1; life expectancy ≥ 12 wk

Postmenopausal women are eligible for this trial (see Section 4.1.5)
Ovarian suppression required for premenopausal women who do not meet definition of postmenopausal in Section 4.1.5.2 (see Section 4.1.19)
Measurable/non-measurable disease by RECIST criteria (see Section 4.1.6)
Prior endocrine therapy not required, but is permitted in adjuvant setting; No prior endocrine therapy for metastatic disease unless initiated within 4 weeks prior to protocol registration (see Section 4.1.7)
No prior bevacizumab or anti-VEGF therapy
≥ 2 weeks since prior radiotherapy and all toxicities resolved
Prior adjuvant or neoadjuvant chemotherapy allowed (see Section 4.1.7.4); One prior chemotherapy regimen for metastatic disease allowed
Bisphosphonate therapy allowed (see Section 4.1.7.6)
No major surgery within 28 days of registration (see Section 4.1.8)
No history of abdominal fistula or intra-abdominal abscess within 6 months nor GI perforation within 12 months of registration (see Sections 4.1.9-4.1.11)
No history of significant bleeding within 6 months of registration
No clinically significant cardiovascular disease (see Section 4.1.12)
Pts on full dose anticoagulant must be on a stable dose of warfarin (see Section 4.1.13)
No arterial thrombotic events, TIA, CVA, acute MI or unstable angina within 6 months of registration
No known CNS metastases (see Section 4.1.14)
No known allergies to aromatase inhibitors or estrogen receptor modulators (see Section 4.1.15)
No serious, non-healing wound, ulcer or bone fx

Required Laboratory Values

Granulocytes ≥ 1000/µl
Platelet count ≥ 100,000/µl

Creatinine ≤ 2.0 mg/dl

Bilirubin ≤ 1.5 x ULN unless due to Gilbert's syndrome
Transaminases (ALT,AST) ≤ 2.5 x ULN
INR ≤ 1.6, unless on full dose warfarin (see Section 4.1.13)

Urine protein ≤ 1+ **or** UPC < 1 (see Section 4.20)

β-Hcg negative (in premenopausal

women

SCHEMA

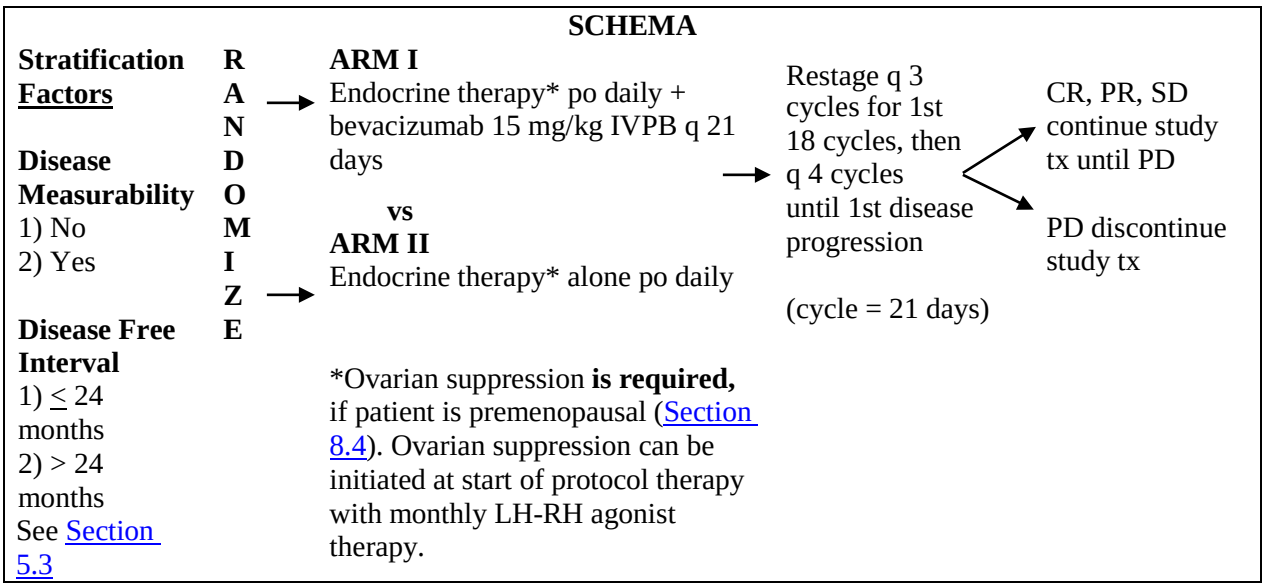
Stratification Factors
Disease Measurability
1) No
2) Yes
Disease Free Interval
1) ≤ 24 months
2) > 24 months
See Section 5.3
RANDOMIZE
ARM I
Endocrine therapy* po daily + bevacizumab 15 mg/kg IVPB q 21 days
vs
ARM II
Endocrine therapy* alone po daily
*Ovarian suppression is required, if patient is premenopausal (Section 8.4). Ovarian suppression can be initiated at start of protocol therapy with monthly LH-RH agonist therapy.
Restage q 3 cycles for 1st 18 cycles, then q 4 cycles until 1st disease progression
(cycle = 21 days)
CR, PR, SD continue study tx until PD
PD discontinue study tx

TABLE OF CONTENTS

SECTION PAGE

1.0 INTRODUCTION

Advanced breast cancer remains an incurable illness despite a number of effective treatments for metastatic disease. Although recent evidence suggests an improved prognosis for patients with recurrent breast cancer [1], more than 40,000 women are expected to die of metastatic disease this year [2]. New targeted treatments that delay disease progression would represent a significant advance in the care of women with breast cancer.

For postmenopausal women with hormone-receptor positive advanced stage disease, estrogen deprivation with a third generation aromatase inhibitor (AI) is more effective than tamoxifen for first-line endocrine treatment [3-5]. While this represents a significant advance for these patients, the major limitation of endocrine therapy remains the near universal development of resistance. Endocrine therapy resistance is also evident in the adjuvant setting where endocrine manipulations are only partially effective in reducing the death rate from breast cancer, even in populations of patients selected upon the basis of tumor estrogen receptor expression [6]. Although there are no proven clinical strategies to reverse or prevent endocrine therapy resistance, several pre-clinical investigations suggest opportunities for therapeutic intervention. This trial is designed to evaluate whether anti-VEGF therapy with bevacizumab can delay resistance and prolong progression-free survival when added to first-line endocrine therapy for hormone-receptor positive metastatic breast cancer.

1.1 Scientific rationale

1.1.1 Estrogen mediates angiogenesis

Several lines of evidence suggest that estrogen modulates angiogenesis through effects on endothelial cells under both physiological and pathological conditions [7]. The cyclical neovascularization of the female reproductive tract monthly in premenopausal women is one of the few active sites of angiogenesis in adult organisms under normal conditions, and suggests a potent angiogenic effect of estradiol.

Estradiol induces proliferation and migration of human umbilical vein endothelial cells in an in vitro cell culture model [8]. Expression of the estrogen receptor by endothelial cells has been detected in these model systems, suggesting a potential mechanism for estrogen regulation of endothelial function [9, 10]. In addition, endothelial cell proliferation and tube formation was inhibited by fulvestrant, a pure anti-estrogen, in this model. In the chick egg chorioallantoic membrane (CAM) assay, Gagliardi et al. demonstrated that both tamoxifen and fulvestrant inhibit angiogenesis in vivo in a dose-related manner [11]. Tamoxifen, clomiphene, and fulvestrant also significantly inhibited porcine pulmonary artery and human dermal microvascular endothelial cell growth that was stimulated by bFGF and VEGF in the CAM assay [12].

1.1.2 Estrogen regulates angiogenesis Through expression of VEGF

Vascular endothelial growth factor (VEGF) is the most potent and specific angiogenic factor identified to date and serves as a crucial regulator of both normal and pathologic angiogenesis [13]. The possibility that VEGF may be responsible for the angiogenic action of estradiol is suggested by several observations. Shweiki et al. demonstrated that VEGF is expressed in spatial and temporal proximity to the forming vasculature in ovarian follicles, the corpus luteum, for endometrial vessel repair, and for angiogenesis in embryonic implantation sites [14]. In ovariectomized rats, estradiol and tamoxifen (an estrogen agonist in the uterus) elevates uterine VEGF mRNA transiently, with a peak induction of 15-20 fold within one hour [15]. Induction of these VEGF transcripts

by estradiol in the rat uterus is selectively blocked by the pure antiestrogen fulvestrant in a dose-dependent manner [16].

Additional evidence to support a role for estrogen-mediated induction of VEGF has been demonstrated in the endometrium of mice treated with VEGF-targeted therapy. Heryanto et al. treated ovariectomized mice with SU5416, a VEGFR2 tyrosine kinase inhibitor, and a polyclonal anti-VEGF antibody [17]. After 24 hours of treatment with estradiol, these anti-VEGF therapies almost completely eliminated endometrial endothelial cell proliferation. In the baboon, Albrecht et al. showed that endometrial glandular epithelial and stromal cell VEGF mRNA and protein expression was decreased by ovariectomy and restored to normal by chronic administration of estrogen. Endometrial VEGF mRNA levels were increased within 2 hours of estradiol administration, with an 8-fold elevation compared with vehicle [18]. In addition, functional estrogen response elements have been described in the regulatory regions of the VEGF gene, supporting the hypothesis that estrogen may regulate VEGF expression by direct transcriptional actions of the ER [19, 20].

1.1.3 Estrogen and VEGF in breast cancer

Preclinical Data

Estrogen regulation of VEGF has been characterized in breast cancer cell lines. Estradiol significantly stimulated both growth rates and VEGF production in MCF-7 cells [21]. In the presence of estradiol, 4-hydroxytamoxifen (4OHT) inhibited VEGF production in this model, suggesting that 4OHT inhibits estrogen-stimulated angiogenesis in ER-positive breast cancer in vitro. Other investigators have demonstrated a biphasic increase of VEGF-A mRNA in MCF-7 cells in response to estradiol, which led to accumulation of the VEGF protein in culture medium [22]. In this in vitro model system, fulvestrant inhibited estradiol stimulation of VEGF, whereas tamoxifen induced VEGF mRNA expression. In the DMBA-induced rat model of mammary tumors, estradiol treatment 24 hours after ovariectomy increased VEGF mRNA in tumors within 2 hours, achieving peak levels in 6 to 8 hours [23]. In this model, VEGF protein levels increased after estradiol injection in 8 to 12 hours. This estrogen-induced VEGF expression was inhibited by anti-aromatase therapy. When these rats were given two injections (24 hours apart) of 4-hydroxyandrostenedione (formestane, a steroidal aromatase inhibitor) to reduce estrogen concentrations, a low level of VEGF mRNA was maintained for 96 hours. Injection of estradiol 2 hours after formestane treatment caused a rise in VEGF mRNA in 6-8 hours.

Clinical Evidence

In patients with breast cancer, several studies have shown that the degree of vascularization of the primary tumor is an independent predictor of survival, regardless of lymph node status [24-27]. Increased tumor cytosolic VEGF, as measured by ELISA, has been associated with decreased survival in patients previously treated with either adjuvant chemotherapy or endocrine therapy [28, 29].

VEGF may be an important target for hormone receptor-positive breast cancer. In patients with operable breast cancer, neoadjuvant antiestrogen therapy with tamoxifen reduced breast cancer angiogenesis in responding tumors as measured by microvessel count (MVC) [30]. A significant correlation was demonstrated between percentage change in MVC and percentage reduction in tumor volume.

1.1.4 Angiogenesis and endocrine therapy resistance

Preclinical data suggests that tumor angiogenesis may contribute to the development of endocrine therapy resistance. A second wave of angiogenesis that can support tumor regrowth may develop after initial vascular regression that follows hormone-ablation therapy for hormone-dependent tumors [31]. Using the Shionogi androgen-dependent murine tumor as a model of male mammary carcinoma, Jain et al. examined the mechanism of vascular regression following hormone withdrawal, the function of regressing vessels and the molecular determinants responsible for the second wave of angiogenesis and tumor regrowth. VEGF, expressed at high levels during the initial tumor growth, decreased to an almost undetectable level one week after castration. Tumor endothelial cells began to undergo apoptosis before neoplastic cells, and the regressing vessels in the tumor began to exhibit a normal phenotype. Two weeks after castration, a second wave of angiogenesis was associated with tumor regrowth, with a concomitant increase in VEGF expression.

Clinical data lends support to this hypothesis. In patients with metastatic breast cancer, response to first-line endocrine therapy was lower for patients who had high VEGF levels in tumor tissue by ELISA compared with patients who had low VEGF levels in a multivariate analysis (p=0.025) [32]. In addition, a trend towards shorter PFS (p=0.075) was also seen. In a randomized trial of tamoxifen in premenopausal women in the adjuvant setting, the benefits of tamoxifen for 2 years were significantly decreased in patients with high tumor-specific VEGFR2 expression by immunohistochemistry [33]. Further study is on-going to identify markers that may predict for responsiveness to tamoxifen therapy, including VEGF-A, VEGFR2, ERK phosphorylation, and HER2 [34-36].

Together, this data supports the hypothesis that estradiol modulates angiogenesis, and suggests one mechanism by which estrogen may promote breast tumor growth. The essential role of both estrogen and angiogenesis in the progression of breast cancer, in addition to the efficacy of endocrine therapy for hormone receptor-positive disease [3, 4, 37-43], suggests the need to further explore the combination of hormonal therapy and antiangiogenic therapy in breast cancer. In addition, VEGF has been recently validated as a therapeutic target in breast cancer, as bevacizumab, an anti-VEGF antibody, prolongs progression-free survival when added to first-line chemotherapy for metastatic disease [44]. Our proposed trial is designed to evaluate whether bevacizumab in combination with endocrine therapy prolongs progression-free survival in postmenopausal women receiving first-line endocrine therapy for hormone receptor-positive advanced breast cancer.

1.2 Endocrine therapy as treatment for metastatic breast cancer: tamoxifen and third-generation aromatase inhibitors

The principal strategy for the treatment of hormone-sensitive breast cancer has been to block the action of estrogen at the level of the receptor or to reduce estrogen production. Tamoxifen is a selective estrogen-receptor modulator (SERM) that antagonizes the action of estrogen in breast tissue, and mimics the action of estrogen in others, such as the bone and uterus [45]. Aromatase inhibitors block estrogen synthesis by inhibiting aromatase, the enzyme responsible for the peripheral conversion of androgens to estrogen [46]. Clinical trials have demonstrated the efficacy of both tamoxifen and AI therapy (anastrozole, letrozole, and exemestane) in the treatment of postmenopausal women with hormone-sensitive advanced-stage disease [3-5, 38, 40, 41, 46].

Update #5
5/13/11

Endocrine therapy of breast cancer is undergoing rapid change as a consequence of results from large randomized trials reporting the benefits of steroidal and non-steroidal AIs for

adjuvant treatment of early-stage breast cancer [47-51]. These studies investigated an AI compared with tamoxifen as front-line endocrine therapy after surgery, as sequential therapy after 2-3 years of tamoxifen, or following completion of five years of adjuvant tamoxifen. Adjuvant AI therapy improves disease-free survival when administered *instead* of tamoxifen as initial endocrine therapy or *following* tamoxifen, and current recommendations support the incorporation of AIs into adjuvant therapy in postmenopausal women with hormone-receptor positive breast cancer [52]. Therefore, women with hormone-receptor positive metastatic breast cancer may have been exposed to a variety of therapies, including tamoxifen alone, tamoxifen followed by sequential use of an AI, or an AI alone. In clinical practice, the choice of first-line endocrine therapy for metastatic disease (AI or treatment with tamoxifen) will be influenced by prior hormone therapy in the adjuvant setting. We propose a trial design that will be flexible with regard to prior endocrine therapy and facilitate patient accrual over the coming years despite these changing practice patterns. The protocol includes guidelines for physicians as they choose first-line endocrine therapy of letrozole or tamoxifen for their patients with metastatic disease. The guidelines for study treatment are listed in Table A (Section 8.3). **For patients enrolled after local IRB approval of Update #5, endocrine therapy will consist of letrozole only.**

1.2.1 Rationale for ovarian suppression in premenopausal women

In premenopausal women with metastatic breast cancer, Klijn et al. demonstrated that tamoxifen plus ovarian suppression was more effective than treatment with either tamoxifen or ovarian suppression alone [53]. In that and other trials, ovarian suppression was started at the same time as tamoxifen. Anastrozole plus ovarian suppression improved response rates compared with tamoxifen plus ovarian suppression in pre/perimenopausal women as first-line endocrine therapy for metastatic disease [54]. Adjuvant therapy trials of aromatase inhibitors are presently underway in premenopausal women for the treatment of early stage breast cancer. Given the promising results of AI therapy in combination with ovarian suppression in metastatic disease, and on-going clinical investigations in early stage breast cancer, premenopausal women will be eligible for AI therapy on this trial after surgical or medical castration (e.g. oophorectomy or LH-RH agonist)

1.3 Anti-VEGF therapy with bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody that binds VEGF, the most potent and specific factor stimulating new blood vessel formation that has been identified to date [13]. It is recognized that angiogenesis is essential to the growth of solid tumors. The biologic effects of VEGF are mediated through binding and stimulation of two receptors on the surface of endothelial cells: VEGFR1 and VEGFR2. Increased levels of VEGF expression have been found in most human tumors, including tumors of the breast. Inhibition of VEGF using an anti-VEGF antibody blocks the growth of a number of human cancer cell lines in nude mice, including the breast cancer cell lines MCF-7 and MDA-MB-435 [55, 56].

1.3.1 Bevacizumab clinical studies for safety, dose and pharmacokinetics

Bevacizumab has been studied in several Phase I, II, and III clinical trials in more than 5000 patients in multiple tumor types. The following discussion summarizes bevacizumab's safety profile and presents some of the efficacy results pertinent to this particular trial. Please refer to the bevacizumab Investigator Brochure for descriptions of all completed Phase I, II, and III trials reported to date.

The pharmacokinetics (PK) of bevacizumab have been characterized in several phase I and phase II clinical trials, with doses ranging from 1 to 20 mg/kg administered weekly, every 2 weeks, or every 3 weeks [57]. The estimated half-life of bevacizumab is approximately 21 days (range 11-50 days). The predicted time to reach steady state was 100 days. The volume of distribution is consistent with limited extravascular distribution.

The maximum tolerated dose (MTD) of bevacizumab has not been determined; however, the dose level of 20 mg/kg was associated with severe headaches. The dose schedule of either 10 mg/kg q2w, or 15 mg/kg q3w is used in most phase 2 or 3 trials with only a few exceptions (e.g., the pivotal phase 3 trial in colorectal cancer, in which bevacizumab was given at 5 mg/kg q2w).

1.3.2 Bevacizumab clinical studies in advanced breast cancer

In patients with metastatic breast cancer, bevacizumab increased progression-free survival when added to weekly paclitaxel for the first-line treatment of metastatic disease [44]. In this trial, 715 women with chemotherapy-naïve measurable or non-measurable metastatic or locally advanced breast cancer were randomized to receive either weekly paclitaxel or paclitaxel combined with bevacizumab. Progression free survival was superior for patients receiving bevacizumab at 10.97 months compared to 6.11 months for the paclitaxel arm ($p<0.001$). In patients with measurable disease, the response rate for the combination was 34.3% vs 16.4% ($p<0.0001$) for paclitaxel alone. Overall survival data is immature, however to date no significant difference has been observed with the addition of bevacizumab (HR 0.84, $p=0.12$). Treatment was generally well tolerated with 13% grade 3 hypertension, 0.9% grade 3 proteinuria and 19.9% grade 3 neuropathy as the only statistically significant differences compared with patients treated with paclitaxel alone. There were no increased thromboembolic events or episodes of congestive heart failure in the combination arm. The increased peripheral neuropathy may have been secondary to the extended duration of paclitaxel therapy in the bevacizumab arm.

In addition, bevacizumab in breast cancer has been previously evaluated as a single agent and in combination with capecitabine. In a Phase I/II dose-escalation trial of single-agent bevacizumab in patients with metastatic breast cancer, objective responses were documented in 7 of 75 patients (9.3%, 6.7% confirmed) [57]. A randomized Phase III trial was performed in 462 women with anthracycline- and taxane-resistant metastatic breast cancer, evaluating chemotherapy (capecitabine) either alone or in combination with bevacizumab (15 mg/kg IV every 3 weeks) [58]. The addition of bevacizumab did not prolong progression-free survival (the primary end point), despite an improved overall response rate in the bevacizumab-treated arm (19.8% vs. 9.1%, $p=0.001$). This patient population was heavily pre-treated, raising the possibility that this trial could have failed to detect the potential activity of bevacizumab in this setting.

Preliminary results of a feasibility and safety study of combination letrozole and bevacizumab have been reported [59]. Eligible patients were candidates for therapy with an aromatase inhibitor for advanced stage disease, and prior non-steroidal AI use without progression was permitted. Forty-three women received letrozole 2.5 mg daily and bevacizumab 15 mg/kg IV every 3 weeks. Premenopausal patients were rendered postmenopausal prior to start of treatment. After a median of 13 cycles (range 1-71), the combination of letrozole and bevacizumab has proven to be been well tolerated. The most common Grade 2/3 toxicities were hypertension (19%/26%), headache (16%/7%), joint pain (19%/0%), proteinuria (14%/19%), and fatigue (19%/2%). There were four potentially treatment-related serious adverse events. Grade 3 hypertension

led to the hospitalization of two patients; one patient experienced grade 4 hyponatremia due to syndrome of inappropriate antidiuretic hormone (SIADH), which may have been exacerbated by the use of a diuretic to manage bevacizumab-related hypertension. One patient with known gastric varices secondary to pseudocirrhosis and portal hypertension was hospitalized with hematemesis; she underwent endoscopy with variceal banding, receiving blood product support and continued on study upon recovery. This patient eventually developed grade 3 thrombocytopenia and was taken off study. Nine patients discontinued therapy because of drug-related toxicities: grade 3 hypertension (n=3), grade 3 proteinuria (n=3), grade headache (n=1), grade 4 hyponatremia (n=1), and grade 3 thrombocytopenia associated with portal hypertension (n=1). Three patients withdrew from protocol therapy because of other reasons: one had definitive surgery with mastectomy after 17 cycles of study therapy for an initially unresectable locally advanced breast cancer at the time of study entry, and two withdrew informed consent. The median time to treatment failure was 10.4 months (95% confidence interval, 8.3-17.5) [60].

Efficacy was a secondary end point of the study. Bevacizumab was added to ongoing NSAI therapy in 84% of patients (median duration of prior AI therapy, 15 weeks [range, 1-216 weeks]). All 43 patients were evaluable for response. Four patients had partial response (PR) as best response on treatment, and there were no complete responses (CR), for a response rated of 9% (95% CI, 0.03-022). Twenty-nine patients had stable disease (SD) $\geq$ 24 weeks. Therefore, the clinical benefit rate (PR + SD $\geq$ 24 weeks) was 77% (95% confidence interval, 0.61-0.88). Six patients had stable disease for < 24 weeks, but discontinued study therapy for reasons other than progression. The remaining four patients had progressive disease as best response. Four patients with stable disease remain on study therapy at 38, 41, 44 and 53+ months. The median progression free survival was 17.1 months (95% confidence interval, 8.5-26.2).

For the breast cancer trials, bevacizumab was administered at 10 mg/kg IV every other week or 15 mg/kg every 3 weeks (5 mg/kg/week). The PK of these regimens is felt to be equivalent. Therefore, this study will administer bevacizumab at 15 mg/kg IV every 3 weeks to reduce the frequency of the required IV infusion.

1.3.3 Bevacizumab safety profile

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in phase III trials include congestive heart failure (CHF), gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). Risk factors for development of an ATE include a prior history of ATE or patient age 65 and older. [61]. The impact of other comorbid medical conditions or baseline functional status on the risk of bevacizumab-associated toxicity has not been rigorously studied and are included as secondary objectives 2.3.6 and 2.3.7 of this protocol. These and other safety signals are described in further detail in Section 10.5 and in the bevacizumab Investigator Brochure.

1.4 Protocol update #2

Since CALGB 40503 was activated on May 15, 2008, concerns have been raised that the placebo infusions presents several barriers to study activation at local institutions, patient enrollment and continued participation. In an effort to address concerns raised by local

investigators and to lessen barriers to enrollment, the protocol study design is being amended to an open-label trial design.
In the redesigned trial, patients will be randomized to endocrine therapy plus bevacizumab or endocrine therapy alone; the placebo infusion will be eliminated. Patients already enrolled on this study will be unblinded, notified of their treatment assignment, and reconsented to the new study design. The primary endpoint, stratification factors and the total number of patients enrolled in this study will not change. The details of implementing this change are described on the Update #4 cover page.

1.5 Rationale for the change in study design (protocol update #5)

Due to slow accrual to the randomized phase II tamoxifen trial (see Section 14.1), the CALGB Data and Safety Monitoring Board (DSMB) recommended closure to this portion of the study. The recommendation was based on slow accrual only, and was not due to a safety concern. The randomized phase III letrozole portion of this trial remains open, and the protocol will continue with every 6 month DSMB review.

1.6 Inclusion of women and minorities

The CALGB participating institutions will not exclude potential subjects from participating in this study solely on the basis of ethnic origin or socioeconomic status. This study of endocrine therapy is restricted to women due to the limited data regarding the efficacy of aromatase inhibitors in men. Every attempt will be made to enter all eligible patients into this protocol and therefore address the study objectives in a patient population representative of the entire breast cancer population treated by participating institutions.

2.0 OBJECTIVES

2.1 Primary objective

To compare the progression-free survival of letrozole therapy alone with the combination of letrozole therapy plus bevacizumab as first-line treatment in women with estrogen- and/or progesterone-receptor-positive advanced breast cancer.

2.2 Secondary objectives

2.2.1 To compare the proportion of patients receiving letrozole alone, who remain progression-free at 6 and 12 months, to those receiving letrozole plus bevacizumab.

2.2.2 To compare the incidence of objective response (CR + PR), in patients receiving letrozole with and without bevacizumab, as determined by RECIST criteria, excluding patients with non-measurable disease.

2.2.3 To compare the incidence of clinical benefit (CR + PR + stable disease ≥ 6 months) in patients receiving letrozole with and without bevacizumab.

2.2.4 To compare the duration of objective response in patients receiving letrozole with and without bevacizumab.

2.2.5 To compare the time to treatment failure in patients receiving letrozole with and without bevacizumab. Time to treatment failure is defined as the interval from randomization until progression, toxicity, withdrawn consent or going onto non-protocol therapy.

2.2.6 To compare the overall survival of patients receiving letrozole with and without bevacizumab, including the probability of survival until 36 months.

2.2.7 To compare toxicity levels between the bevacizumab arm and the arm without bevacizumab in both the letrozole-treated patients and in the tamoxifen-treated patients.

2.2.8 To compare progression-free survival and overall survival of all patients receiving endocrine therapy with and without bevacizumab (by combining both letrozole and tamoxifen patient subgroups).

2.3 Correlative study objectives

2.3.1 To compare baseline and changes in serial levels of circulating endothelial cells and circulating tumor cells in patients treated with endocrine therapy alone or endocrine therapy plus bevacizumab, and to explore the relationship of these markers with progression free survival.

2.3.2 To conduct proteomic analysis of longitudinal samples from patients with advanced-stage disease undergoing hormonal therapy to define new serum-based biomarkers related to disease activity.

2.3.3 To identify biologic correlates that will predict progression-free survival (PFS) and response to therapy.

2.3.4 To conduct pharmacogenomic assessment of candidate variants in the VEGF, CYP2D6, and CYP19 genes and evaluate their association with PFS and other study outcomes.

2.3.5 To identify SNPs associated with progression free survival in the genome-wide approach (GWAS).

2.3.6 To identify factors other than chronological age that predict the risk of grade 3, 4 or 5 toxicity with bevacizumab and endocrine therapy by means of functional age assessment measures. The factors to be studied include: a) functional status, b) comorbid medical conditions, c) cognitive function, d) psychological state, e) social support and f) nutritional status.

2.3.7 To perform an exploratory analysis of the ability of the other factors included in the functional age assessment (either individual or in combination), to predict the risk of grade 3, 4 or 5 toxicity.

2.3.8 To evaluate longitudinal changes in the parameters of the factors described in 2.3.6, while on therapy.

3.0 ON-STUDY GUIDELINES

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate.

Although they will not be considered formal eligibility (exclusion) criteria, physicians should recognize that the following may seriously increase the risk to the patient entering this protocol:

- Psychiatric illness which would prevent the patient from giving informed consent.

- Medical condition such as uncontrolled infection (including HIV) or uncontrolled diabetes mellitus, which, in the opinion of the treating physician, would make this protocol unreasonably hazardous for the patient.
- Inability to comply with study and/or follow-up procedures

4.0 ELIGIBILITY CRITERIA

4.1 Eligibility Requirements

All questions regarding eligibility criteria should be directed to the CALGB Study Chair. Please note that the Study Chair cannot grant waivers to eligibility requirements.

4.1.1 Histologic documentation

Histologic confirmation of invasive cancer of the female breast in either the primary or metastatic setting.

4.1.1.1 Stage: Stage IV disease or Stage IIIB disease (using AJCC criteria, 6th edition) not amenable to local therapy.

4.1.2 Patients may not have a "currently active" second malignancy other than non-melanoma skin cancers. Patients are not considered to have a "currently active" malignancy if they have completed therapy and are considered by their physician to be at less than 30% risk of relapse.

4.1.3 Hormone receptor status

Tumors (from either primary or metastatic sites) must express estrogen receptor (ER) and/or progesterone receptor (PgR) ≥ 1% cells will be considered positive.

4.1.4 Age ≥ 18 years of age.

4.1.5 Menopausal status

4.1.5.1 Postmenopausal women are eligible for this trial. Before study registration, menopausal status must be defined according to the criteria below.

4.1.5.2 Postmenopausal is defined as:

• Age ≥ 55 years and one year or more of amenorrhea

• Age < 55 years and one year or more of amenorrhea, with an estradiol assay < 20 pg/ml.

• For women age < 55 with prior hysterectomy but intact ovaries, with an estradiol assay < 20 pg/ml.

• Surgical menopause with bilateral oophorectomy (at least 28 days must elapse from surgery to time of study registration)

• Ovarian suppression on a LH-RH agonist

4.1.5.3 Premenopausal women who do not meet the postmenopausal criteria above are also eligible, but are required to undergo ovarian suppression (see Section 8.3). This can be initiated any time prior to or on day 1 of protocol therapy, regardless of chosen endocrine therapy, and will continue for the duration of protocol therapy.

4.1.6 Measurable/evaluable disease

Patients must have measurable or non-measurable disease by RECIST criteria, with radiologic scans within 28 days of study registration. See Section 7.0 (footnote D) for required baseline scans.

4.1.6.1 Measurable disease: lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 2.0 cm with conventional techniques or as ≥ 1.0 cm with spiral CT scan.

4.1.6.2 Non-measurable disease

All other lesions, including small lesions (longest diameter < 2.0 cm with conventional techniques or < 1.0 cm with spiral CT scan) and truly non-measurable lesions.

Lesions that are considered non-measurable include the following:

- Bone lesions
- Leptomeningeal disease
- Ascites
- Pleural/pericardial effusion
- Inflammatory breast disease
- Abdominal masses that are not confirmed and followed by imaging techniques
- Cystic lesions

4.1.7 Prior therapies

4.1.7.1 Prior endocrine therapy: Prior endocrine therapy is not required.

Update #5
5/13/11

• Prior endocrine therapy in the metastatic setting is not permitted (unless tamoxifen or an aromatase inhibitor was initiated within 4 weeks prior to registration to facilitate enrollment of patients who recently started first-line endocrine therapy for metastatic breast cancer). If prior letrozole therapy was initiated within the past 4 weeks, the patients should remain on letrozole as the study therapy. Patients who began therapy with tamoxifen, anastrozole or exemestane must switch to letrozole to be eligible to participate in this study.

• Prior endocrine therapy in the adjuvant setting is permitted. There is no time restriction for how long the patient must be on the adjuvant endocrine therapy, nor is there a time restriction for how long the patient needs to be off prior adjuvant endocrine therapy before beginning protocol therapy on 40503.

• Prior treatment with ovarian suppression is allowed in either the adjuvant or metastatic setting. If medical ovarian suppression is being administered it can be initiated any time prior to or at the start of protocol therapy, and continued throughout the duration of the trial. Surgical castration with bilateral oophorectomy must be performed at least 28 days prior to study registration (due to concerns of poor wound healing on bevacizumab).

4.1.7.2 Patients may not have received any prior anti-VEGF or VEGFR tyrosine kinase inhibitor therapy.

4.1.7.3 Prior radiotherapy must have been completed and all toxicities resolved at least two weeks prior to registration.

4.1.7.4 Chemotherapy in the adjuvant or neoadjuvant setting is permitted. At least twelve months prior to registration must have elapsed since the completion of adjuvant or neoadjuvant chemotherapy and all toxicities must have resolved. Taxane-related neurotoxicity must have resolved to sensory grade < 2 and no motor neuropathy of any grade is allowed.

4.1.7.5 Patients may have received one prior chemotherapy regimen for metastatic disease. The final dose of prior chemotherapy must have been administered at least 3 weeks prior to study registration.

4.1.7.6 Treatment with bisphosphonates is allowed and recommended as per ASCO guidelines [62].

4.1.8 Prior surgery

4.1.8.1 Patients must not have had a major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to study registration, and must have fully recovered from any such procedure.

4.1.8.2 Patients must not have anticipation of need for major surgical procedure during the course of the study.

4.1.8.3 Patients must not have had a core biopsy or other minor surgical procedure, within 7 days prior to study registration. Placement of a vascular access device is allowed within 7 days of registration.

4.1.9 Patients must not have a history of abdominal fistula, or intra-abdominal abscess within 6 months prior to study registration.

4.1.10 Patients with a history of GI perforation within 12 months prior to registration are not eligible.

4.1.11 Patients with a history of significant bleeding episodes (e.g., hemoptysis, upper or lower GI bleeding) within 6 months prior to registration are not eligible.

4.1.12 Cardiovascular status

Patients must not have clinically significant cardiovascular disease that includes the following:

4.1.12.1 Uncontrolled hypertension defined as systolic blood pressure >150 and/or diastolic blood pressure >90 mmHg on antihypertensive medications or any prior history of hypertensive crisis or hypertensive encephalopathy.

4.1.12.2 History of myocardial infarction or unstable angina within past 6 months.

4.1.12.3 New York Heart Association (NYHA) Grade 2 or greater congestive heart failure.

4.1.12.4 Symptomatic peripheral vascular disease.

4.1.12.5 Significant vascular disease (e.g., aortic aneurysm, aortic dissection) or arterial thrombotic events.

4.1.13 Full dose anticoagulation therapy is allowed for the treatment of prior conditions such as venous thromboses or atrial fibrillation, but not for the treatment of prior arterial thrombotic events. Patients on full dose anticoagulants must be on a stable dose of

warfarin and have an in-range INR (usually between 2 and 3) or be on a stable dose of LMW heparin. Patients receiving antiplatelet agents are eligible, as are patients on daily prophylactic aspirin or anticoagulation for atrial fibrillation.

4.1.14 CNS status

4.1.14.1 Patients may not have a history of stroke or transient ischemic attack within 6 months prior to study registration.

4.1.14.2 Patients with a history of seizures must be well controlled with standard medication.

4.1.14.3 Patients must not have known CNS metastases or leptomeningeal disease (screening with brain imaging is not required for asymptomatic patients).

4.1.15 Allergies

Update #5
5/13/11

In AI-treated patients: No known allergies to imidazole drugs, (e.g. clotrimazole, ketoconazole, miconazole, econazole, sulconazole, ticonazole, or terconazole) or compounds structurally similar to bevacizumab.

In tamoxifen treated patients: No know allergies to selective estrogen receptor modulators (e.g. tamoxifen, raloxifene or toremilfene) or compounds structurally similar to bevacizumab. **For patients enrolled after Update #5, endocrine therapy will consist of letrozole only and this criterion will no longer apply.**

4.1.16 ECOG (Zubrod) Performance Status ≤ 1.

4.1.17 No serious, non-healing wound, ulcer or bone fracture.

4.1.18 Life expectancy of ≥ 12 weeks.

4.1.19 Pregnancy status

All patients who are premenopausal (if not already receiving ovarian suppression therapy/surgical oophorectomy) must have a negative β-Hcg prior to starting on study treatment. Patients may not be pregnant or nursing at any time during the study. **Ovarian suppression is required in women of childbearing potential by the start of protocol therapy, and will continue for the duration of protocol therapy.**

4.1.20 Required initial laboratory data

Granulocytes	≥1,000/µl
Platelet count	≥100,000/µl
Creatinine	≤ 2.0 mg/dL
Bilirubin	≤ 1.5 x Upper limit of normal (ULN) unless due to Gilbert's syndrome
Transaminases (ALT, AST)	≤ 2.5 x ULN
INR	≤ 1.6, unless on full dose warfarin (see Section 4.1.13)
β-Hcg	Negative in premenopausal women as defined in section 4.1.5.3
Urine protein	≤ 1+* **or** UPC < 1

* Patients discovered to have ≥ 2 + proteinuria at baseline must undergo a 24-hour urine collection that must demonstrate < 1 g of protein/24 hr, or UPC ratio < 1 to allow participation in the study (See Appendix III).

5.0 REGISTRATION/RANDOMIZATION AND STRATIFICATION

5.1 Registration requirements

5.1.1 Informed consent

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

5.1.2 CALGB patient registration/randomization

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

Confirm eligibility criteria (Section 4.0). Complete the Registration Worksheet. Access the Web-based Patient Registration system via the Patient Registration tab on the CALGB Member Web site at www.calgb.org. If the study does not appear on the list of studies in the Patient Registration system, the registration must be performed by the CALGB Registrar via phone or fax. If the registering CRA requires assistance, he/she may consult the on-line help file at the bottom of the screen or call the IS Help Desk at 1-888-44CALGB. If further assistance is required, the registering CRA may call the CALGB Registrar (919)-668-9396, Monday-Friday, 9 AM – 5 PM, Eastern Time. Enter the following information:

- CALGB patient ID #, if applicable
- Study
- Name of group (CALGB)
- Name of institution where patient is being treated
- Name of treating physician
- Treating physician's NCI investigator number
- Name of person in contact with the patient record (responsible contact)
- Protocol IRB approval date
- Date of signed consent
- Treatment Start Date
- Date (of) HIPAA authorization signed by the patient
- Patient's initials (L, F, M)
- Patient's Social Security #, date of birth, hospital ID #
- Patient's gender
- Patient's race
- Patient's ethnicity
- ECOG (Zubrod) performance status
- Patient's height (cm) and weight (kg)
- Type of insurance (Method of Payment)
- Patient's postal code
- Disease, type and stage, if applicable
- Companion studies patient has consented to
- Eligibility criteria met (no, yes)

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

After registration is complete, the patient may be randomized. The patient is randomized according to the stratification factors indicated in Section 5.3, which must be entered to obtain a treatment assignment.

The Main Member Institution and registering institution will receive a Confirmation of Randomization. Please check for errors. Submit corrections in writing to the data coordinator at the CALGB Statistical Center, Data Operations, Hock Plaza, 2424 Erwin Rd, Ste 802 Hock Plaza, Durham, NC 27705, or fax to 919-668-9397.

5.2 Registration to companion studies

5.2.1 Registration to substudies described in Appendices I and II

There are two substudies within CALGB 40503. These correlative science and pharmacogenomic studies **must be offered to all patients** enrolled on CALGB 40503 (although patients may opt to not participate). These substudies do not require separate IRB approval. The substudies included within CALGB 40503 are:

- Correlative Science Studies, CALGB 150605 (Appendix I)
- Pharmacogenomic Studies, CALGB 60605 (Appendix II)

If a patient answers "yes" to "My specimens may be used for the research described above." question #1 in the model consent, they have consented to participate in the substudy described in Appendix I. The patient should be registered to CALGB 150605 at the same time they are registered to the treatment trial (40503). Samples should be submitted per Sections 6.2, 6.2.1, 6.2.3 and 6.2.4.

If a patient answers "yes" to "My specimen may be used for the genetic research described above.", question #2 in the model consent, they have consented to participate in the substudy described in Appendix II. We strongly encourage the collection of a pre-therapy sample and registration to CALGB 60605 at the same time the patient is registered to the treatment trial (40503) however, patients may be registered to 60605 within 60 days of registration to 40503. At that time the whole blood sample may be collected after consent has been obtained, and submitted per Section 6.2.2.

If registering to 60605 within 60 days of registration to 40503, proceed to the online registration screen. Select "register a patient". Enter the patient number from CALGB 40503. Confirm the selection. Choose the companion "CALGB 60605" and continue the process as indicated. Once the sequence is completed and the patient is registered; you will receive a confirmation of registration to CALGB 60605.

•CALGB 70501, "Collection of patient reported symptoms and performance status via the internet", is a separate companion protocol available to CALGB institutions. All patients enrolled or enrolling to CALGB 40503 should be approached and invited to participate in CALGB 70501. Registration should occur simultaneously with the CALGB registration/randomization to CALGB 40503; however, registrations to CALGB 70501 may take place later. Please note the CALGB 70501 participants must be registered prior to receiving therapy on scheduled clinic visit/cycle #2 of 40503.

5.3 Stratification factors

Disease measurability: 1) No
2) Yes

Update #5
5/13/11

Disease free interval (months from initial diagnosis to first progression)
1) $\leq$ 24 months
2) > 24 months

6.0 DATA AND SPECIMEN SUBMISSION

6.1 Data submission:

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

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

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

Form		Submission Schedule
	CALGB 40503 Registration Worksheet	
C-1548	40503 On-study Form	
C-816	CALGB Baseline Solid Tumor Evaluation Form	Submit within 2 weeks of registration
Report*	Operative and Pathology Reports w/ER/PgR results	
C-1549	Pre-existing Conditions Form	
C-1552	CALGB 40503 Treatment Form	Submit every other cycle until the discontinuation of protocol therapy
C-1554	CALGB 40503 Adverse Event Form	
C-1862	CALGB 40503 Supplemental Adverse Event Form	
C-817	Follow-up Solid Tumor Measurement Form	Submit q 3 cycles for 1st 18 cycles, then q 4 cycles until the discontinuation of protocol therapy
S-045	CALGB: 40503 Letrozole/Tamoxifen Medication Calendar	Submit every other cycle while on tamoxifen or letrozole (see Section 8.5).
C-1556	CALGB 40503 Treatment Summary Form (All Patients)	Submit at completion or discontinuation of protocol treatment
C-1555	CALGB: 40503 Follow-up Form	Submit at the discontinuation of protocol therapy, then q 6 months for 2 years, then annually until death or for a maximum of 5 years from study entry; submit at 1st disease progression**; submit at new primary (see Section 6.4); submit at death
C-1742	CALGB: Confirmation of Lost to Follow-up Form	Follow form instructions
C-1776	CALGB: 40503 Functional Age Assessment Measure (Healthcare Professional Questionnaire)	For pts who have agreed to participate, submit prior to beginning study treatment; at restaging 1 (after 3 cycles), 2 (after 6 cycles) and then every other restaging time point (#4, 6, 8 etc.); and on the last day of treatment or up to 1 month later, but prior to the start of a new treatment
C-1777	CALGB: 40503 Functional Age Assessment Measure (Patient Questionnaire)	

- Please use "CALGB Remarks Addenda" (C-260) if additional comments are necessary or additional writing space is needed.
- Submit copies of all required reports to confirm eligibility and restating results.

** At 1st disease progression submit documentation (scan reports etc.) upon which disease progression was determined.

- The Treatment Form and Medication Calendar should be completed for each cycle, but can be submitted every other cycle. The Adverse Event Form and Supplemental Adverse Event Form may cover 2 cycles.

  Common Terminology Criteria for Adverse Events (CTCAE): This study will utilize the Common Terminology Criteria for Adverse Events version 3.0 for routine toxicity reporting on study forms.

6.2 Specimen submission for correlative studies:

All participating institutions must ask patients for their consent to participate in the correlative substudies planned for CALGB 40503, although patient participation is optional. Rationale and methods for the scientific components of these studies are described in Appendices I and II. For patients who consent to participate, blood and tissue will be collected as follows for these studies:

Correlative study	Sample type	Volume and tube	Prior to treatment	Follow-up samples	Ship to	Testing Investigator/ institution
Circulating tumor cells	Blood	2 x 10 mL CellSave	Yes	Yes (see Section 6.2.4)	UCSF	Hope Rugo, John Park UCSF
Circulating endothelial cells	Blood	5 mL EDTA (lavender)	Yes	Yes (see Section 6.2.4)	UCSF	Hope Rugo, John Park UCSF
Proteomics	Blood	10 mL SST (red/grey)	Yes	Yes (see Section 6.2.1)	PCO	Reid Townsend Washington University
Proteomics	Blood	8 mL CPT	Yes	Yes (see Section 6.2.1)	PCO	Reid Townsend Washington University
Pharmacogenomics	Blood	10 mL EDTA (lavender)	Yes	No	PCO	Federico Innocenti University of Chicago
Tissue markers	Tumor block	-	Yes	No	PCO	Torsten Nielsen University of British Columbia
PIK3CA mutation analysis	Tumor block	-	Yes	No	PCO	Mary Ellen Moynahan MSKCC
VEGF mRNA analysis	Tumor block	-	Yes	No	PCO	Matthew Ellis Washington University

USE OF THE ALLIANCE BIOSPECIMEN MANAGEMENT SYSTEM (BioMS) IS MANDATORY AND ALL SPECIMENS MUST BE LOGGED AND SHIPPED VIA THIS SYSTEM.

BioMS is a web-based system for logging and tracking all biospecimens collected on Alliance trials. Authorized individuals may access BioMS at the following URL: http://bioms.allianceforclinicaltrialsinoncology.org using most standard web browsers (Safari, Firefox, Internet Explorer). For information on using the BioMS system, please refer to the 'Help' links on the BioMS web page to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55BIOMS. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55BIOMS.

After logging collected specimens in BioMS, the system will create a shipping manifest.

This shipping manifest must be printed and placed in the shipment container with the specimens.

Note: Kits containing the CellSave tubes for the circulating tumor cells (CTC) and **the CPT tubes** for the proteomics are provided in a kit for this study. The kits should be ordered from the Alliance Pathology Coordinating Office, by calling the phone number below, at the time that the 40503 protocol is submitted for local IRB approval. Unused CellSave and CPT tubes should be returned to the Alliance PCO in the kit box.
All specimens (except the blood for circulating tumor and endothelial cells, see Section 6.2.4) should be sent to the following address:

Alliance Pathology Coordinating Office
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967

6.2.1 Whole blood submission for proteomics

For patients who have agreed to participate, plasma will be used for the analysis of novel biomarkers and serum will be used to define new serum based biomarkers described in Appendix I, Part B.

Collect two samples, as described below, prior to the initiation of study treatment, then two samples on day 1 of Cycle 2 (wk 4), day 1 of Cycle 3 (wk 7) and day 1 of Cycle 4 (wk 10).

For patients receiving bevacizumab, the blood should be drawn before the infusion has been started, during weeks 4, 7 and 10.

Draw 10 mL of peripheral venous blood in an SST (red/grey) tube and gently invert 5 times to mix clot activator with blood. Let blood for up to one hour. Observe a dense clot. Centrifuge at 1300g for 10 minutes and refrigerate until shipped.

Draw 8 mL in a CPT tube (DO NOT centrifuge) and refrigerate until shipped with the SST tube on cold refrigerant pack by overnight mail to the Alliance PCO. The samples should be shipped the same day that the blood is drawn.

6.2.2 Whole blood submission for pharmacogenomics

For patients who have agreed to participate, germ line DNA will be extracted from the whole blood sample and used to investigate polymorphisms.

It is strongly encouraged that the sample be collected prior to the initiation of study treatment, however sample collection and registration to this pharmacogenetic substudy may take place within 60 days of registration to the treatment trial (see Section 5.2.1).

Draw 10 mL of peripheral venous whole blood in an EDTA (lavender) Vacutainer tube, refrigerated until shipped on cold refrigerant pack by overnight mail to the Alliance PCO. The sample should be shipped the same day that the blood is drawn.

6.2.3 Tissue submission for markers, and PIK3CA mutation analysis

For patients who have agreed to participate, formalin fixed paraffin embedded tissue will be used for luminal subtyping and efficacy of endocrine therapy and to evaluate the impact of PIK3CA mutations of the efficacy of bevacizumab.

Submit one formalin fixed paraffin embedded tissue block with representative primary tumor. Blocks must be labeled with the institutional surgical pathology number, CALGB study number and CALGB patient ID number.

In instances where the tissue block cannot be released due to institutional or governmental policy, please contact the Alliance PCO at 614-293-7073, to obtain instructions for obtaining four (4) 2.0 mm tissue cores (punches). Punches will be taken from tumor rich areas (2) as well as from benign areas (2). The cores will be used for incorporation into the tissue micro array (TMA) and extraction of RNA, and DNA.

6.2.4 Whole blood submission for circulating tumor and endothelial cells

For patients who have agreed to participate, peripheral blood will be used for the evaluation of circulating tumor cells and circulating endothelial cells as early markers of time to progression and response in patients with ER and/or PgR positive, metastatic breast cancer.

Collect three samples prior to the initiation of study treatment, then two samples on day 1 of Cycle 2 (wk 4), day 1 of Cycle 3 (wk 7) and day 1 of Cycle 4 (wk 10).

For patients receiving bevacizumab, the blood should be drawn before the infusion has been started, during weeks 4, 7 and 10.

Collect 10 mL of peripheral whole blood into each of two CellSave tubes (fill tubes completely), prior to initiation of study treatment, (and one CellSave tube at the other collection time points), and 5 mL into one EDTA lavender top tube at each collection time point. The tubes must be shipped at ambient temperature the **same day that the blood is drawn** by overnight carrier **"For Saturday Delivery"**.

Prior to shipping each sample:
Alert Dr. Scott and Eddie Sosa via
• FAX: 415-476-8218
OR
• Email: jscott@cc.ucsf.edu and sosae@cc.ucsf.edu

Ship samples, with the 40503 Blood Specimen Submission Form (C-1550), to Dr. Scott at the Park Laboratory:
Janet Scott, PhD
c/o Park Laboratory
2340 Sutter Street, 4th Floor, Room S471
San Francisco, CA 94115
Tel: 415-514-3969

6.3 Submission of CT, MRI and bone scan or PET/CT images

The complete CT (or MRI) and bone scans or PET/CTs **must** be submitted to the Imaging Core Lab in the preference of digital DICOM format. Alternatively, films are acceptable if digital DICOM images are not available. The raw data of the entire study should be saved until the scan is accepted by the Imaging Core Lab. Imaging data should be de-identified using institutional procedures to remove patient name and medical record number while preserving the CALGB subject ID number and protocol number.

De-identified imaging data sets may be submitted to the Imaging Core Lab by 1) FTP transfer or 2) Mail/Shipment.

6.3.1 FTP transfer

Any FTP software can be used to initiate access to the secure FTP Server of the Imaging Core Lab. The standard FTP access will be provided separately through the specific trial email CALGB 40503@ImagingCorelab.com, by request of participating sites before their first data submission.

Once you have access to the main data directory of the FTP server at Imaging Core Lab, create a folder using the CALGB40503 Subject Identifier assigned to the image data set you wish to FTP (upload). Then create another Sub-Folder with the Date of the exam (YYYYMMDD) followed by the abbreviation of the study performed (CT, bone). Upload (copy) the imaging files to the appropriate sub-directory. (The imaging files can be exported as a single file series).

Once the upload is complete, the institution must send an e-mail to the Imaging Core Lab at **CALGB40503@ImagingCorelab.com** to inform them that the study has been uploaded from their institution.

6.3.2 Mail/shipment:

If FTP data transfers cannot be achieved, the de-identified digital DICOM images (burned to a CD) or films should be mailed to the Imaging Core Laboratory. Please submit only one patient's images per CD, with the patient's CALGB subject ID number, study types (e.g., CT baseline, bone follow-up), date of scans and name of submitting institution.

Mail these data to:
CALGB Imaging Core Laboratory
Attn: CALGB 40503
Wright Center of Innovation
The Ohio State University
Room #414, 395 W. 12th Ave.
Columbus, OH 43210
Phone: 614/293-2788
Fax: 614/293-9275

The Imaging Core Lab will acknowledge receipt of the imaging data sets via email confirmation to the institution within 1 business day of receipt.

If there are any difficulties or questions on the data transmission, please contact the imaging data submission, please contact the Imaging Core Laboratory at CALGB40503@ImagingCorelab.com or by Telephone at 1-614-293-2788.

6.4 Follow-up

Follow all patients enrolled on this study, including those who do not receive any protocol therapy, for first distant progression. Thereafter follow for survival for a maximum of 5 years from study registration. During the entire follow-up period, report 1) DCIS and 2) any new primary of invasive cancer regardless of site.

Except for DCIS, do not report as a new primary any *in situ* carcinoma or squamous/basal cell skin cancer. Note that lobular carcinoma *in situ* (LCIS) is **not** considered a breast cancer event and should not be reported.

Follow patients for toxicity while receiving any protocol therapy.

7.0 REQUIRED DATA

Pre-Study Testing Intervals

To be completed within 14 DAYS before registration:
- All bloodwork; urine for proteinuria
- History and physical

To be completed within 28 DAYS before registration:
- Any X-ray, scan of any type or ultrasound, which is utilized for tumor measurement per protocol (see footnote D).

Tests & Observations	Prior to Registration	Prior to Initiation of Therapy	Day 1 of each cycle	Time of Restaging**	Post Treatment Follow up
History and Progress Notes	X		E		I
Physical Examination	X		E		I
Pulse, Blood Pressure	X		G		I
Weight③	X		E		
Performance Status	X		E		
TUMOR MEASUREMENTS	X			X	
ER, PgR Status	X♦				
Drug Toxicity Assessment			E		
Laboratory Studies					
CBC, Differential, Platelets	X♠		F*f*		
Serum Creatinine/BUN	X♠		F*f*		
Serum Electrolytes, Ca++	X♠		F*f*		
SGOT, SGPT, Bilirubin	X♠		F*f*		
INR or PT	X♠		F+*f*		
Urine protein	X♠#		H*f*#		
β-HCG	X♠*				
Estradiol	X♠&				
Staging					
CT/spiral CT – chest abd/pelvis (preferred), or MRI †	A			A	
Bone Scan †	A			A	
Treatment Record					
40503 Letrozole/Tamoxifen Medication Calendar		X	E		
Companion Studies					
Whole blood		B	B		
Paraffin embedded tissue block(s)		C			
Functional Age Assessment Questionnaires		D		D	D

③ The dose of bevacizumab need not change unless the calculated dose changes by 10%.

♠ Pre-registration labs may be used for day 1 of cycle 1 tests if obtained within 14 days prior to day 1 of Cycle 1.

f For subsequent cycles, labs and urine protein may be obtained up to 5 days before study treatment or on the day of tx.

Urine protein should be checked before **every other cycle** and the results should be available before treatment.

♦ Determination of ER, PgR status required prior to registration as described in Sections 4.1.3.

\+ PTor INR only required for pre-registration labs unless pt is on full dose warfarin; required each cycle for pts on full dose warfarin.

* For women of child bearing potential (see Section 4.1.5.3).

& If < age 55 and one or more years of amenorrhea with intact ovaries. Not required for pts receiving regular injections of an LHRH agonist pre-study.

** Restage every 3 cycles for the first 18 cycles, then every 4 cycles until first disease progression.

† **All scans must be forwarded to CALGB Imagining Core Lab. See Section 6.3 for instructions.**

A Baseline scans are required for all patients and can include: 1) a CT, spiral CT or MRI **and** bone scan (option 1), or 2) a PET/CT providing the following criteria are met: the PET/CT is performed with **IV contrast**, and the CT is of **diagnostic quality** and is acquired with 5mm or less slice thickness (option 2). **Response assessment should include assessment of all measurable and non-measurable sites and use the same imaging method utilized at baseline.**
B For consenting patients, draw and ship blood per Section 6.2 of the protocol.
C For consenting patients, ship tissue block(s) after registration, as described in Sections 6.2 and 6.2.3.
D For patients who have agreed to participate, see Section 8.5 and Appendix V, for instructions regarding assessment administration.
E Every cycle for the first 19 cycles, then every other cycle (i.e. 21,23, 25 etc.).
F Every cycle for the first 9 cycles, then every other cycle (i.e. 11, 13, 15 etc.) until cycle 24 (approximately month 18). After cycle 24, labs taken every fourth cycle (i.e. 28, 32, 36, etc.).
G Required **every** cycle for patients on **Arm I**. For patients on **Arm II,** required every cycle for the first 19 cycles, then every other cycle. For patients on Arm I who have discontinued bevacizumab, obtain every other cycle.
H Required for patient on **Arm I only**. UPC ratio can be discontinued at the time that bevacizumab is discontinued.
I After completion of all study therapy patients should be followed every 6 months for 2 years, then annually until 5 years from study entry or death, whichever occurs first.

8.0 TREATMENT PLAN

Patients will be randomized with equal probability to receive endocrine therapy alone or endocrine therapy and bevacizumab. The choice of endocrine therapy (letrozole or tamoxifen) will be up to the treating physician (see Section 8.3 for recommendations). **For patients enrolled after local IRB approval of Update #5, endocrine therapy will consist of letrozole only.** Endocrine therapy may begin up to 4 weeks prior to study registration (see Section 4.1.7.1). Starter supplies of letrozole may be ordered (see Section 10.6), but starter supplies may not be used until the patient has been registered.

Update #5 5/13/11

All premenopausal patients must be treated with ovarian suppression prior to the start of protocol therapy (see Section 8.3).

Protocol therapy will begin within 14 days of registration/randomization to allow time for the study drug supplies to arrive at the study sites.

Protocol therapy will continue until first disease progression or unacceptable toxicity.

8.1 Arm I: Endocrine therapy and bevacizumab

Endocrine therapy orally, once daily plus bevacizumab 15 mg/kg IVPB every 21 days. One cycle = 3 weeks.

8.2 Arm II: Endocrine therapy alone

Endocrine therapy orally, once daily alone.
One cycle = 3 weeks.
Patients should receive study therapy on schedule however, minor deviations (± 5 days) owing to holidays, patient scheduling conflicts, inclement weather, etc. are permitted. These minor changes in schedule should be recorded on the CALGB 40503 Treatment Form (C-1552).

8.3 Physician choice of endocrine therapy: letrozole or tamoxifen

Physicians will choose first-line endocrine therapy for their patients with either letrozole or tamoxifen. Guidelines are provided in Table A, and suggestions are based on prior adjuvant endocrine therapy.
For patients enrolled after local IRB approval of Update #5, endocrine therapy will consist of letrozole only and Table A will no longer apply.

Update #5 5/13/11

TABLE A: Options for Endocrine Therapy

Prior adjuvant endocrine therapy	***Choice of first-line endocrine therapy on protocol***
None (endocrine-therapy naïve)	Premenopausal: tamoxifen or letrozole Postmenopausal: letrozole
Tamoxifen only	Letrozole
Sequencing of tamoxifen and non-steroidal AI (letrozole or anastrozole)	Re-introduction of tamoxifen
Sequencing of tamoxifen and steroidal AI (exemestane)	Letrozole (class switch) or re-introduction of tamoxifen
AI only (anastrozole, letrozole or exemestane)	Tamoxifen
Tamoxifen and both steroidal and non-steroidal AIs	Tamoxifen or (re-introduce) letrozole

TABLE B: Endocrine Therapy and Dose

Update #5
5/13/11

Endocrine Therapy	**Dose Route**
Letrozole	2.5 mg orally daily
Tamoxifen	20 mg orally daily

For patients enrolled after local approval of Update #5, endocrine therapy will consist of letrozole only.

8.4 Ovarian suppression in premenopausal patients

All premenopausal patients must undergo ovarian suppression either medically, using an LHRH-agonist, or surgically by oophorectomy. Ovarian suppression can begin any time prior to or at the start of protocol therapy, in combination with tamoxifen or letrozole. If medical ovarian suppression is planned, treatment with an LHRH-agonist must be initiated by the start of protocol therapy. The LHRH-agonist can be goserelin acetate or leuprolide acetate (see Table C). The LHRH-agonist should be administered monthly however every 3-week administration is permitted to coincide with bevacizumab administration. If surgical oophorectomy is planned, the procedure must be performed at least 28 days prior to study registration to allow for adequate wound healing.

Ovarian radiation is not permitted to induce ovarian suppression in preparation for this protocol treatment.

Women are considered **postmenopausal** if they fall into one of the categories listed below. All other women will require ovarian suppression.

Postmenopausal is defined as:

- Age ≥ 55 and one year or more of amenorrhea.
- Age < 55 and one year or more of amenorrhea, with an estradiol assay < 20 pg/ml.
- For women age < 55 with prior hysterectomy but intact ovaries, with an estradiol assay < 20 pg/ml.
- Surgical menopause with bilateral oophorectomy (at least 28 days must elapse from surgery to time of study registration).
- Ovarian suppression on a LHRH-agonist.

TABLE C: LHRH Agonists

LHRH-agonist	Dose/Route of administration
Goserelin acetate	3.6 mg subcutaneously every month (or at time of bevacizumab administration q 3 weeks)
Leuprolide acetate	7.5 mg intramuscular every month (or at time of bevacizumab administration q 3 weeks)

8.5 Adherence

Since patients will take oral medications at home without direct supervision, we will monitor drug compliance until the discontinuation of tamoxifen/AI using medication calendars. At study entry the patient will receive a CALGB: 40503 Letrozole/Tamoxifen Medication Calendar (S-045), and be instructed on how to take the medication and how to use the calendar. The patient will bring the calendar for the previous 3 weeks with her to each 3-weekly clinic visits where it will be reviewed. The patient will then be given a new calendar for the coming 3 weeks. If a patient cites reasons other than drug side effects for not taking the required oral medication, the reasons for missing the doses will be reviewed and the importance of taking all dosages on schedule will be reinforced with her.
Patients who discontinue bevacizumab early, but continue on an letrozole or tamoxifen should bring the calendar with them to each staging clinic visit and receive enough calendars until the next scheduled visit.

8.6 Assessments Required

All patients who understand and are able to follow directions in English (as the assessment instruments are only available in English), and have agreed to participate, will take part in the functional age assessments. Please see Section 3.0 of Appendix V for instruction regarding assessment administration. Nurses and/or CRAs who will administer the assessments must contact Dr. Hurria (626-256-4673) to review the assessment measures via telephone conference, prior to performing the first patient assessment.

9.0 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

9.1 Endocrine therapy

There will be no dose reductions for endocrine therapy. Endocrine therapy will be held for > grade 3 hepatic function impairment. If endocrine therapy is held for hepatic toxicity, hold bevacizumab. Resume endocrine therapy at the previous dose when hepatic toxicity resolves to ≤ grade 2. If endocrine therapy is held for > 3 weeks (1 cycle), permanently discontinue protocol therapy. Endocrine therapy may be permanently discontinued at the discretion of the treating physician for other endocrine related toxicity. If endocrine therapy is permanently discontinued, permanently discontinue bevacizumab.

9.2 Bevacizumab

Bevacizumab dose is always 15 mg/kg. Bevacizumab may be held or permanently discontinued for toxicity as described below, but the dose is not reduced.
If bevacizumab is held for > 6 weeks for toxicity, permanently discontinue bevacizumab. If bevacizumab is permanently discontinued or held for toxicity, continue endocrine therapy.

9.2.1 Hypersensitivity or infusion reaction

The initial bevacizumab dose should be administered over a minimum of 90 minutes. If no hypersensitivity or infusion reactions occur, the second dose should be administered

over a minimum of 60 minutes. If no hypersensitivity or infusion reactions occur with the second dose, the third and subsequent doses should be administered over a minimum of 30 minutes. If infusion–related reactions occur, subsequent bevacizumab infusions should be administered over the shortest period that is well-tolerated. Patients may receive premedication with antihistamines prior to bevacizumab if they have previously experienced allergic reactions (grade 1 or 2).

Grade 3 or 4 hypersensitivity reactions: permanently discontinue bevacizumab. Continue endocrine therapy.

9.2.2 Hypertension

- Blood pressure ≤ 160/100:

Continue bevacizumab and consider adding or adjusting antihypertensive medications as appropriate for blood pressure > 130/80.

Consider initiating antihypertensive medications in antihypertensive-naïve patients when blood pressure > 130/80.

- **Blood pressure > 160/100:** hold bevacizumab until blood pressure ≤ 160/100 and add or adjust antihypertensive medications. Continue endocrine therapy.
- **Grade 4 hypertension (life-threatening e.g., hypertensive crisis):** permanently discontinue bevacizumab. Continue endocrine therapy.
- Signs and symptoms suggestive of reversible posterior leukoencephalopathy syndrome (RPLS) such as confusion, headache, seizures, cortical blindness: hold bevacizumab for up to 6 weeks. Suspected RPLS should be investigated with MRI as described in Section 10.5. If diagnosis of RPLS is confirmed, bevacizumab should be permanently discontinued. If RPLS is ruled out via MRI, resume bevacizumab when the signs and symptoms have completely resolved. If bevacizumab is held or permanently discontinued to rule out RPLS, continue endocrine therapy.

9.2.3 Hemorrhage/bleeding

- Grade 3 or 4 bleeding: permanently discontinue bevacizumab. Continue endocrine therapy.

9.2.4 Thrombosis

- Grade 3 or asymptomatic grade 4 thrombosis: hold bevacizumab.

  For patients receiving anticoagulation with warfarin or warfarin derivatives use the following guidelines. If the planned duration of full-dose anticoagulation is < 2 weeks, bevacizumab should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is ≥ 2 weeks, bevacizumab may be resumed during anticoagulation therapy provided all of the following are met:

- The patient must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin or be on a stable dose of low molecular weight heparin prior to resuming bevacizumab.
- The patient must not have pathologic conditions that carry a high risk of bleeding (e.g., tumor involving major blood vessels).
- The patient must not have had bleeding events while on study. Continue endocrine therapy while bevacizumab is held.

- Symptomatic grade 4 or recurrent/worsening venous thrombolic events after resumption of bevacizumab: permanently discontinue bevacizumab. Continue endocrine therapy.

9.2.5 Arterial thromboembolic events including angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event:

- Grade 2 arterial thrombotic events not present at baseline or worsened since the initiation of treatment: permanently discontinue bevacizumab. Continue endocrine therapy.
- **Grade 3 or 4 arterial thrombotic events:** permanently discontinue bevacizumab. Continue endocrine therapy.

9.2.6 Congestive Heart Failure (Left ventricular systolic dysfunction)

- Grade 3 or 4: permanently discontinue bevacizumab. Continue endocrine therapy.

9.2.7 Dose modifications for GI perforation and wound dehiscence

- **For any grade GI perforation, GI leak or intra-abdominal fistula:** Permanently discontinue bevacizumab. Continue endocrine therapy.
- **For wound dehiscence requiring medical or surgical intervention:** Permanently discontinue bevacizumab. Continue endocrine therapy.

9.2.8 Proteinuria

See Appendix III for information regarding the calculation of UPC (urine protein to creatinine) ratio.

- **For proteinuria of ≥ 2+:** Confirm total urine protein with a 24-hour urine collection or urine protein to creatinine (UPC) ratio. For 2+ proteinuria, the scheduled dose of bevacizumab may be given while awaiting the results of the 24-hour collection or UPC ratio. For > 2+ proteinuria, hold bevacizumab while awaiting results of the 24-hour urine collection or UPC ratio. Endocrine protocol therapy may be continued while bevacizumab is on hold.
- If monitoring protein with UPC no confirmation is necessary.
- **If urine protein is ≥ 2 g/24 hours or UPC ratio ≥ 2.0:** Hold bevacizumab until urine protein recovers to < 2g/24 hours or UPC < 2.0, Continue endocrine therapy. If bevacizumab is delayed more than 8 weeks due to proteinuria, permanently discontinue bevacizumab. Continue endocrine therapy.
- For patients who were on the bevacizumab arm and have discontinued bevacizumab therapy, UPC ratio for these patients may also be discontinued **IF** the proteinuria is ≤ grade 1.
- Grade 4 or nephrotic syndrome: permanently discontinue bevacizumab. Continue endocrine therapy.

9.2.9 Other unspecified bevacizumab-related adverse events

- **Grade 3 or 4:** permanently discontinue bevacizumab therapy. Continue endocrine therapy.

For patients who require surgery while on study, it is recommended that bevacizumab be held for > 60 days prior to surgery, if possible. Therapy with bevacizumab may be

re-initiated after ≥ 28 days and the patient has fully recovered (60 days following high risk procedures such as liver resection, thoracotomy, or neurosurgery).

Patients who have an ongoing bevacizumab-related grade 4 or serious adverse event at the time of permanent discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible.

9.3 Dose modification for obese patients

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

10.0 DRUG FORMULATION, AVAILABILITY, AND PREPARATION

10.1 Qualified Personnel Handling Agents

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

10.2 Discarding Unused Agents

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

10.3 Dosage Rounding for Bevacizumab

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

10.4 Weight & Dose Calculation for Bevacizumab

It is not necessary to change the doses of bevacizumab due to changes in weight unless the calculated dose changes by ≥10%.

10.5 Bevacizumab (NSC #704865, IND #7921)

All investigators who receive a copy of the protocol should also obtain a copy of the Investigator's Brochure (IB). IB's are available from the Pharmaceutical Management Branch, CTEP, DCTD, NCI and may be obtained by emailing the IB Coordinator (ibcoordinator@mail.nih.gov) or by calling the IB Coordinator at 301-496-5725.
Bevacizumab is a recombinant humanized anti-VEGF monoclonal antibody, consisting of 93% human and 7% murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding complementarity-determining regions. Bevacizumab blocks the binding of vascular endothelial growth factor (VEGF) to its receptors resulting in inhibition of angiogenesis.

Availability
Bevacizumab (NSC 704865) will be provided free of charge by Genentech and distributed by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI).
Once the study has converted to the open label design, (see protocol update #4) a supply of bevacizumab may be ordered by as follows: Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. **For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.**

Prior to the conversion to the open label design, continue to order patient specific supplies per the original blinded study design.
Open label bevacizumab will be supplied as a clear to slightly opalescent, sterile liquid ready for parenteral administration. Each 400 mg (25 mg/mL – 16 mL fill) glass vial contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP.
Prior to conversion to the open label study design, bevacizumab will continue to be supplied as a clear to slightly opalescent, sterile liquid ready for parenteral administration. Each 100 mg (25mg/mL – 4mL fill) glass vial contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP.

NOTE: At the time of disease progression, ALL remaining clinical supplies of bevacizumab should be returned to PMB (see "Drug Returns" below).

Drug Returns: **Only unopened clinical supplies should be returned to the PMB.** When it is necessary to return study drug (e.g., sealed vials remaining when a patient completes therapy, sealed vials remaining when a patient permanently discontinues protocol treatment or expired vials recalled by the PMB), investigators should return the study drug to the PMB using the NCI Return Drug List available on the CTEP home page (http://ctep.cancer.gov) or by calling the PMB at 301-496-5725.
Drug Accountability: The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of all drugs received from the PMB using the NCI Drug Accountability Record Form (DARF) available on the CTEP home page (http://ctep.cancer.gov) or by calling the PMB at 301-496-5725.
Note: Supplies for the open label study design are study specific (one DARF for the study). Supplies for the blinded study design continue to be patient specific, requiring a separate DARF for each patient as long as the blinded design is in effect.

Storage and Stability
Bevacizumab is shipped on blue ice for next day delivery. On receipt, bevacizumab should be stored in a refrigerator (2^{o} to 8^{o} C) and should remain refrigerated until just prior to use. Do not freeze. Do not shake. Shelf-life studies of bevacizumab are continuing. Investigators will be notified when lots have expired. The sterile single use vials contain no antibacterial preservatives; therefore, vials should be discarded eight hours after initial entry. Solutions diluted for infusion may be stored in the refrigerator for up to 8 hours.

Preparation
Vials contain no preservative and are intended for single use only. **Place the calculated dose in 100 mL of 0.9% Sodium Chloride for Injection.** Once diluted in 0.9% Sodium Chloride for Injection, the bevacizumab solution must be administered within 8 hours.

Administration
Bevacizumab is administered as a continuous intravenous infusion. The initial dose should be administered over a minimum of 90 minutes. If no adverse reactions occur after the initial dose, the second dose should be administered over a minimum of 60 minutes. If no adverse reactions occur after the second dose, all subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, all subsequent infusions should be administered over the shortest period that was well tolerated.
To ensure complete delivery of bevacizumab, the IV infusion line must be flushed with 0.9% Sodium Chloride for Injection. Please note that this flush is not included in the

infusion times. The following are two recommended methods for flushing the bevacizumab IV infusion line:

- When the bevacizumab infusion is complete, add an additional 50mL of 0.9% Sodium Chloride for Injection to the bevacizumab infusion bag. Continue the infusion until a volume equal to that of the volume contained in the tubing has been administered.
- Replace the empty bevacizumab infusion bag with a 50mL bag of 0.9% Sodium Chloride for Injection and infuse a volume equal to the volume contained in the tubing.

Toxicities

Hypertension: Hypertension has been commonly seen in bevacizumab clinical trials to date and oral medications have been used to manage the hypertension when indicated. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) [63, 64]. RPLS may include signs and symptoms of headache, altered mental function, seizures, and visual disturbances/ cortical blindness and requires treatment, which should include control of hypertension, management of specific symptoms, and discontinuation of bevacizumab.

Reversible posterior leukoencephalopathy syndrome (RPLS) or similar leukoencephalopathy syndrome: RPLS or clinical syndromes related to vasogenic edema of the white matter have been recently reported in association with bevacizumab therapy. These syndromes have been seen in < 1% of patients to date. Clinical presentations are variable and may include altered mental status, seizure and cortical visual deficit. HTN is a common risk factor and was present in most (though not all) patients on bevacizumab who developed RPLS. MRI scans are key to diagnosis and typically demonstrate vasogenic edema (hyperintensity in T2 and FLAIR images and hypointensity in T1 images) predominantly in the white matter of the posterior parietal and occipital lobes; less frequently, the anterior distributions and the gray matter may also be involved. RPLS should be in the differential diagnosis in patients presenting with unexplained mental status change, visual disturbance, seizure or other CNS findings. RPLS is potentially reversible, but timely correction of the underlying causes, including control of BP and interruption of the offending drug, is important in order to prevent progression to irreversible tissue damage.

Proteinuria: Proteinuria ranging from asymptomatic abnormal urinalysis to nephrotic syndrome, has been described in 10% or more of patients receiving bevacizumab. Proteinuria is managed with dose modifications as described in Section 9.2.7.

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer (CRC) trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials. In the phase III pivotal trial in metastatic CRC, there was a slightly higher rate of **venous TE** events that was not statistically significant in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%). There was also a higher rate of **arterial TE** events (3% vs. 1%) such as myocardial infarction, transient ischemia attack, cerebrovascular accident/stroke and angina/unstable angina**.** A pooled analysis of the rate of arterial TE events from 5 randomized studies (1745 patients) showed that treatment with chemotherapy plus bevacizumab increased the risk of having an arterial TE event compared with chemotherapy alone (3.8% vs. 1.7%, respectively) [65]. Furthermore, subjects with certain baseline characteristics (age ≥ 65 years and/or a history of a prior arterial TE event) may be at higher risk of experiencing such an event. See the bevacizumab Investigator Brochure for additional information on risk factors.

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin ≤ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similar retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events, and similar data with respect to metastatic colorectal cancer patients were presented at ASCO 2005 [66]. Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Gastrointestinal perforation: Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory process and gastrointestinal perforation to bevacizumab has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, and breast) and may be higher in incidence in some tumor types.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. In an analysis of pooled data from two trials in metastatic colorectal cancer, patients undergoing surgery 28-60 days before study treatment with 5-FU/LV plus bevacizumab did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone [67]. Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of bevacizumab is 21 days. Bevacizumab should be discontinued in patients with severe wound healing complications.

Hemorrhage: Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight phase I, II, and III clinical trials in multiple tumor types (bevacizumab Investigator Brochure, October 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Tumor-associated hemorrhage – was observed in phase I and phase II bevacizumab studies. Six serious events, of which 4 had fatal outcome, were observed in a phase II trial of patients with non-small cell lung cancer receiving bevacizumab. These events occurred suddenly and presented as major or massive hemoptysis in patients with either squamous cell histology and/or tumors located in the center of the chest in close proximity to major blood vessels. In five of these cases, these hemorrhages were preceded by cavitation and/or necrosis of the tumor. Tumor-associated hemorrhage was also seen rarely in other tumor types and locations, including central nervous system (CNS) bleeding in a patient with hepatoma with occult CNS metastases and continuous oozing of blood from a thigh sarcoma with necrosis.

Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen. There have also been less common events

of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Congestive heart failure: CHF has been reported in bevacizumab clinical trials and may be increased in incidence in patients with prior exposure to anthracyclines or prior irradiation to the chest wall. In a phase III trial (AVF2119g) of capecitabine with or without bevacizumab for metastatic breast cancer, 7 subjects (3.1%) who received capecitabine plus bevacizumab developed clinically significant CHF compared with 2 subjects (0.9%) treated with capecitabine alone; of note, all subjects in this trial had had prior anthracycline treatment. In addition, 2 subjects had a left ventricular ejection fraction < 50% at baseline and 2 others had prior left chest wall irradiation. A recently published phase II study in subjects with refractory acute myelogenous leukemia reported 5 cases of cardiac dysfunction (CHF or decreases to <40% in left ventricular ejection fraction) of 48 subjects treated with sequential cytarabine, mitoxantrone, and bevacizumab. All but one of these subjects had significant prior exposure to anthracyclines as well [68]. Other studies are ongoing in this patient population.

Osteonecrosis of the jaw: There are several reports in the literature suggesting that bevacizumab increases the likelihood of osteonecrosis of the jaw in patients receiving bisphosphonates, and that bevacizumab may cause osteonecrosis of the jaw in patients not receiving bisphosphonates. This effect is thought to be related to inhibition of angiogenesis.

For a comprehensive list of adverse events and potential risks (CAEPR), see Section 15.3. Also refer to the bevacizumab Investigator's Brochure for additional information about toxicities as well as information about the production of bevacizumab for clinical trial use.

10.6 Letrozole

Please refer to the FDA-approved package insert for letrozole for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Availability

Letrozole tablets will be provided for this trial, free of charge, by Novartis. Letrozole should be ordered using the 40503 Drug Shipment Request, which is available on the CALGB web site on the 40503 study page under Supplemental materials. Initial or starter supply orders must be accompanied by the study specific Form FDA 1572, also available on the 40503 study page. Please allow 7-10 business days for drug delivery. Letrozole supplies are not patient specific.

Letrozole is supplied as 2.5 mg, dark yellow, film-coated tablets in bottles of 30 tablets.

Storage and Stability

Intact bottles of letrozole should be stored at room temperature.

Administration

Letrozole will be taken orally, at a dose of 2.5 mg once daily without regard to meals.

Institutions can dispense the letrozole using their preferred procedure (i.e. dispense letrozole in the original container(s) or dispense the desired number of tablets from the original container). Returns should be counted and may be given back to the same patient. Returns CANNOT be given to a different patient.

Toxicities

The most common adverse events reported in recent trials with letrozole in advanced breast cancer include hot flashes and nausea. Of concern, primarily with long-term use of

aromatase inhibitors including letrozole, are effects on lipid metabolism with subsequent cardiovascular events and on bone resorption. Other less frequent adverse events include bone and muscle pain.

10.7 Tamoxifen citrate

For patients enrolled after local approval of Update #5, endocrine therapy will consist of letrozole only.

Update #5 5/13/11

Please refer to the FDA-approved package insert for tamoxifen citrate for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Availability

Tamoxifen is available in 10 mg and 20 mg tablets for oral administration. Tamoxifen is commercially available and will not be supplied by the Pharmaceutical Management Branch (PMB) for this study.

Storage and Stability

Tamoxifen should be stored at room temperature.

Administration

Tamoxifen will be administered orally, at a dose of 20 mg daily without regard to meals.

Toxicities

The most common adverse events reported in recent trials with tamoxifen include hot flashes, nausea and vaginal discharge. Tamoxifen is also associated venous thrombosis and pulmonary embolism. As a result of tamoxifen's estrogenic effect on the endometrium, endometrial hyperplasia and endometrial cancer have been observed. In contrast, the estrogenic effect of tamoxifen is protective in bone (less osteoporosis and fewer fractures compared to aromatase inhibitors), and tamoxifen also reduces cholesterol.

Drug Interactions

Tamoxifen is extensively metabolized by CYP isoforms. In particular CYP2D6 catalyzes the formation of endoxifen and 4-hydroxytamoxifen, both of which are significantly more potent than tamoxifen. Inhibition of CYP2D6 activity, as a result of variant polymorphisms or concomitant administration of CYP2D6 inhibitors, is associated with decreased endoxifen levels. SSRIs are increasingly used to manage hot flashes, including hot flashes secondary to tamoxifen. Improvement in symptoms may result from decreased generation of endoxifen, and the potential exists for decreased effectiveness of tamoxifen. Among SSRIs studied, venlafaxine had the least effect on endoxifen levels. Paroxetine was the most potent inhibitor of CYP2D6, resulting in the lowest endoxifen concentrations.

10.8 Leuprolide acetate

Please refer to the FDA-approved package insert for leuprolide for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Availability

The preferred regimen of leuprolide for ovarian suppression in this study I leuprolide depot of 7.5 mg IM every month. Leuprolide depot is available as a kit or prefilled dual-chamber syringe. The leuprolide in these single dose preparations is present as lyophilized microspheres. Leuprolide is commercially available and will not be supplied by the Pharmaceutical Management Branch (PMB) for this study.

Storage and Stability
Intact kits and syringes should be stored at room temperature. Once reconstituted using the diluent provided in the kit or syringe, leuprolide suspension is stable for 24 hours.

Preparation
Reconstitute the microspheres with the diluent provided in the kit or release the diluent in the syringe into the microspheres. Gently shake the reconstituted product to yield a uniform suspension.

Administration
Leuprolide 7.5 mg is administered via intramuscular injection every 4 weeks.

Toxicities
Common toxicities are mostly related to the effects of decreased estrogen. Hot flashes are seen in more than 50% of patients. Mood disorders, dizziness and sleep disorders are also common. Decreased libido occurs somewhat less. Also related to decreased estrogen is vaginitis. Back or joint pain may occur and decreases in bone mineral density are seen with chronic administration (e.g., > 6 months). Nausea/vomiting, anorexia, headaches and weakness are also seen. Injection site reactions occur infrequently.

10.9 Goserelin acetate

Please refer to the FDA-approved package insert for goserelin acetate for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Availability
The preferred regimen of goserelin of ovarian suppression in this study is goserelin implant subcutaneously every month. Goserelin implant is available as a prefilled syringe containing 3.6 mg and intended for administration every 4 weeks. Goserelin is commercially available and will not be supplied by the Pharmaceutical Management Branch (PMB) for this study.

Storage and Stability
Intact syringes should be stored at room temperature.

Administration
Goserelin 3.6 mg implant is administered by subcutaneous injection into the upper abdominal wall, every 4 weeks.

Toxicities
Common toxicities are mostly related to the effects of decreased estrogen. Hot flashes are seen in more than 50% of patients. Mood disorders, dizziness and sleep disorders are also common. Decreased libido occurs somewhat less. Also related to decreased estrogen is vaginitis. Back or joint pain may occur and decreases in bone mineral density are seen with chronic administration (e.g., > 6 months). Nausea/vomiting, anorexia, headaches and weakness are also seen. Injection site reactions occur infrequently.

10.10 Collaborative agreement provisions

The bevacizumab supplied by CTEP, DCTD, NCI used in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA) between Genentech Inc. (hereinafter referred to as "Collaborator" and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in

the “Intellectual Property Option to Collaborator” (at http://ctep.cancer.gov/industry) contained within the terms of award, apply to the use of bevacizumab in this study:

1. Bevacizumab may not be used for any purpose outside the scope of this protocol, nor can it be transferred or licensed to any party not participating in the clinical study. Collaborator’s data for bevacizumab are confidential and proprietary to Collaborator and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient’s family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For this clinical protocol in which there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data.”):
    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group Office for Cooperative Group studies for immediate delivery to Collaborator for advisory review and comment prior to submission for publication. Collaborator will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator’s confidential and proprietary data, in addition to Collaborator’s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator for courtesy review as soon as

possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release.
Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:
Regulatory Affairs Branch, CTEP, DCTD, NCI
Executive Plaza North, Suite 7111
Bethesda, Maryland 20892
FAX 301-402-1584
Email: anshers@ctep.nci.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator. No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

11.0 ANCILLARY THERAPY

11.1 Supportive Care

Patients should receive full supportive care, including transfusions of blood and blood products, epoetin, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on Form C-1552.

11.2 Therapy Exceptions

Treatment with hormones or other chemotherapeutic agents may not be administered except for steroids given for adrenal failure or chronic non-cancer related diseases, hormones administered for non-disease-related conditions (e.g., insulin for diabetes), and intermittent use of dexamethasone as an antiemetic in solid tumor protocols.

11.3 Palliative Radiation Therapy

Palliative radiation therapy may not be administered during protocol therapy. The need for palliative radiation therapy will be considered evidence of progressive disease, and patients will be taken off study. Irradiate a symptomatic lesion, or one that may produce disability (e.g., unstable femur) prior to study initiation, provided other measurable or non-measurable disease is present.

11.4 Bisphosphonates

Patients with bone metastases should receive intravenous bisphosphonates according to ASCO guidelines. Bisphosphonate therapy can be initiated at any time during protocol therapy.

11.5 Anticoagulants

Warfarin or low molecular weight heparin may be used for thrombosis as described in Section 9.2.4.

11.6 Recommendations for the management of hypertension and proteinuria

11.6.1 Hypertension

Encourage patients to start home blood pressure monitoring if blood pressure (BP) is consistently elevated above baseline/pre-treatment levels. Consider starting or adjusting anti-hypertensive medications if BP > 130/80. A suggested starting agent is an angiotensin converting enzyme (ACE) inhibitor, titrating up based on close BP

monitoring. If BP is inadequately controlled with a single agent, a second agent such as an angiotensin receptor blocker (ARB) can be added. If the combination of an ACE inhibitor and an ARB are not effective, consider the addition of HCTZ or a calcium channel blocker.

11.6.2 Proteinuria

Adequate control of hypertension may help prevent or reduce proteinuria. If UPC ratio starts to rise ≥ 1, consider the addition of an angiotensin converting enzyme (ACE) inhibitor. If hypertensive, consider treating as above in Section 11.6.1.

11.7 CALGB policy concerning the use of growth factors

11.7.1 Epoetin (EPO)

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

11.7.2 Filgrastim (G-CSF) and sargramostim (GM-CSF)

G-CSF/GM-CSF may be used at the discretion of the treating physician, however myelosuppression and therefore the need for G-CSF/GM-CSF are not anticipated with the treatments used in this study.
If filgrastim/pegfilgrastim or sargramostim are used, they must be obtained from commercial sources.

12.0 CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE:

A treatment cycle is defined as one 21 day period. Patients should be restaged every 3 cycles (+/- 1 week) for the first 18 cycles of therapy, which is equivalent to 54 weeks or one year of protocol therapy. Thereafter patients should be restaged every 4 cycles until first disease progression.

12.1 Target lesions

All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

12.1.1 Complete response: Disappearance of all target lesions.

12.1.2 Partial response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.

12.1.3 Progression (PD): At least a 20% increase in the sum of the LD of target lesions taking as references the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

12.1.4 Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started. Patients having a documented response with no reconfirmation of the response will be listed with stable disease.

12.2 Non-target lesions

All other lesions (or sites of disease) not included in the "target disease" definition should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent."

12.2.1 Complete response (CR): Disappearance of all non-target lesions.

12.2.2 Non-complete response (non-CR)/Non-progression (non-PD): Persistence of one or more non-target lesion.

12.2.3 Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

12.3 Cytology and histology

If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.
These techniques can be used to differentiate between PR and CR in rare cases.
The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

12.4 Evaluation of best overall response

The best overall response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see Section 12.7).

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note:

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration" on the Treatment Summary Form (C-1556) under "other." Every effort should be made to document the objective progression even after discontinuation of treatment.
- Conditions that may define "early progression, early death and inevaluability" are study specific and should be clearly defined in each protocol (depending on treatment duration, treatment periodicity).

For example: Conditions that may define early death include patients that have died without documentation of disease progression and before it was time to conduct the first tumor reassessment. Inevaluable patients have received protocol treatment (regardless of how much was received) and did not have any follow-up assessment completed before initiation of alternative treatment.

- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

12.5 Guidelines for evaluation of measurable disease

12.5.1 Clinical Lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

12.5.2 Chest X-ray: Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

12.5.3 Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to the chest, abdomen, and pelvis. Head & neck and extremities usually require specific protocols.

12.5.4 Ultrasound (US) should not be used to measure tumor lesions that are clinically not easily accessible when the primary endpoint of the study is objective response evaluation. It is a possible alternative to clinical measurements of superficial palpable nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

12.5.5 Endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques can be useful to confirm complete pathological response when biopsies are obtained.

12.6 Guidelines for evaluation of non-measurable disease

12.6.1 Bone only disease: Since bone lesions are not considered measurable, patients with bone only disease will be evaluated for progression only. Progression is defined as a bone event requiring intervention (surgery/radiation), or the occurrence of a pathologic fracture or the appearance of new lytic lesions or other new bone destruction thought to be related to cancer by x-ray, MRI or CT scan. Changes in bone scan should not be used to define progression. Disease “hot spots” should be evaluated radiographically by x-ray, MRI or CT scan to ascertain the presence of bone destruction versus a healing reaction. The appearance of new lesions on bone scan may constitute progressive disease if associated with clinical symptoms suggestive of disease progression.

12.6.2 Other non-measurable disease: In patients without measurable lesions, progression will be defined as development of new lesions or ‘unequivocal progression’ of existing non-measurable lesions. Unequivocal progression is determined based on the treating physician’s judgment of the absence of beneficial effect from therapy. The following

guidelines will be used in making that determination: there must be either clear evidence of an increase in the area(s) of disease involvement or a magnitude estimated to be at least as great as that required for progression of target lesions, or significant deterioration in the patient's condition that is directly attributable to disease.

12.7 Duration of response

12.7.1 Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

12.7.2 Duration of stable disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

13.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

13.1 Duration of treatment

13.1.1 CR, PR, or SD: Continue treatment until the appearance of disease progression.

13.1.2 Disease progression: At first disease progression, protocol therapy will be discontinued. Document details of progression on the on the Follow-up Solid tumor Measurement Form (C-817).

13.2 Extraordinary Medical Circumstances

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

- Notify the Study Chair.
- Document the reason(s) for discontinuation of therapy on the CALGB 40503 Treatment Summary Form.
- Continue to follow the protocol requirements in Section 6.4.

14.0 STATISTICAL CONSIDERATIONS

14.1 Study design

This trial consists of a randomized phase III and randomized phase II screening trial with a total target accrual of 442 patients. Patients on this trial will receive either letrozole or tamoxifen at their physicians' discretion. Letrozole patients will be enrolled into a randomized Phase III trial of letrozole alone versus letrozole with bevacizumab whose primary endpoint is PFS. Tamoxifen patients will be enrolled into a randomized Phase II screening trial of tamoxifen alone versus tamoxifen with bevacizumab whose primary endpoint is toxicity. Approximately 80% of the patients are anticipated to receive letrozole. All patients will be randomized with equal allocation to receive endocrine therapy alone or with bevacizumab within strata defined by type of endocrine therapy (letrozole or

tamoxifen), measurable versus non-measurable disease, and disease-free interval (≤ 24 months versus >24 months). The protocol will be closed to accrual of both letrozole and tamoxifen patients when 352 letrozole patients have been accrued, or if the letrozole trial is stopped early for futility, regardless of how many tamoxifen patients have been accrued. While accruing the 352 letrozole patients, we anticipate that about 90 tamoxifen patients will be accrued, although this number will be capped at 150, even if the trial is still accruing letrozole patients.

Update #5: Closure of accrual to the phase II tamoxifen trial

The target accrual of letrozole patients is 352, unless the letrozole trial is stopped early for futility. With an anticipated accrual rate of 16 letrozole patients per month, accrual would be finished in 22 months. This is the sample size on which statistical power is calculated. With 7 months of additional follow-up for progression after accrual closes, the anticipated total duration of this trial is 29 months.

Thus the anticipated total sample size of 442 patients (352 letrozole + 90 tamoxifen) would be accrued at the rate of 20 patients per month over 22 months. This is the sample size on which statistical power is calculated. However, the maximum permitted sample size to this protocol is 502 patients (352 letrozole + 150 tamoxifen). With 7 months of additional follow-up for progression after accrual closes, the anticipated total length of this trial is 29 months.

Justification for estimate of the duration of progression-free survival for the control arm: First-line hormonal therapy trials for patients with metastatic breast cancer have typically excluded patients who received adjuvant tamoxifen therapy within 12 months of study entry. In addition, these participants received adjuvant hormonal therapy with tamoxifen only (these trials predated use of AIs in the adjuvant setting). At the present time, the vast majority of women with hormone-receptor positive early stage breast cancer will receive some form of adjuvant hormonal therapy with either tamoxifen and/or an aromatase inhibitor. To provide flexibility in this setting, this trial will include patients who relapse while receiving adjuvant hormonal therapy or who have had prior exposure to AIs. Therefore, median PFS from prior first-line hormone therapy trials for metastatic disease would likely overestimate the median control arm PFS for this study. In general, median PFS for tamoxifen in these first-line trials has ranged from 5.6 - 8.3 months, and median PFS of AI therapy has ranged from 8.2 - 11.1 months [3, 4, 40 69]. Using this information, we chose the median PFS of the control arm to be 6 months.

14.2 Endpoints for patients receiving letrozole

The primary endpoint of the letrozole trial is PFS defined as the interval from randomization until disease progression or death, whichever occurs first. Patients who discontinue protocol therapy due to severe toxicity or withdrawn consent will continue to be followed for progression and death. Secondary endpoints include: objective tumor response as defined by RECIST criteria for those patients with measurable disease, the probability of being progression-free at 6- and 12-months, site of progression, treatment-related toxicity, time-to-treatment-failure, duration of tumor response, overall survival (OS), and the probability of surviving until 36 months.

14.3 Sample size and power calculations for patients receiving letrozole

We assume a median PFS for the letrozole control arm of 6 months. A 50% improvement in median PFS to 9 months for the letrozole + bevacizumab arm would be considered clinically meaningful. The null hypothesis then is that the hazard ratio of the two treatment arms is 1.0; the alternative hypothesis is that the hazard ratio of the control to the experimental regimen is 1.5. Power calculations assume exponential PFS, 22 months of

accrual at a rate of 16 patients per month (for a total of 352 patients) and 7 additional months of follow-up. Using a one-sided Type I error rate of 0.025, the log rank test has at least 90% power to detect an arm difference in PFS medians of 6 versus 9 months.

14.4 Interim analyses of letrozole data

Interim analyses of PFS will be conducted on a semiannual basis to coincide with the semiannual meetings of the Data and Safety Monitoring Board (DSMB). Under the alternative hypothesis, the number of events expected at the end of the study is 274. The first formal interim analysis will be conducted for the first DSMB meeting after which at least 50% of the events have occurred (137 events). We expect this to occur by about 17 months after the trial starts accruing; this 17-month estimate does not include the 4 initial months of the trial during which approval by individual institutional IRBs is obtained. We anticipate two interim analyses before the final analysis. If this timing holds, only the first interim analysis will occur while the trial is still accruing.

Futility boundaries will be based on testing the alternative hypothesis at a one-sided 0.005 alpha level, as recommended by Freidlin and Korn [70]. Specifically, Z-score futility boundaries will be calculated as -2.576 + log (1.5)*sqrt(n/4), where n is the total number of observed events. For illustrative purposes we give the boundaries that would be used under the specific assumptions of the previous paragraph. Thus, the Z-score boundaries would be:

Interim number	**1**	**2**	**3**
Percent information	0.50	0.79	1.00
Expected number of events	137	217	274
Futility boundary	−0.21	0.40	1.9577

If the true hazard ratio is 1.0, then the probability of stopping early to fail to reject the null is 0.68. If the true hazard ratio is 1.5 then there is only a 0.008 chance of erroneously stopping the trial early. The Type I error after considering the futility boundaries is 0.025 and the power for a hazard ratio of 1.5 is 0.91.

Interim analyses of toxicity will be presented to all meetings of the Data and Safety Monitoring Board (i.e., toxicity monitoring will start before PFS monitoring). Maximum tolerated differences between arms in toxicity rates will be used to trigger a careful review of the toxicity data with consideration given to modifying the therapy or closing the trial. Specifically, a toxicity review will be triggered if the observed difference between arms in rate of grade 3+ stroke, proteinuria, thrombosis, hypertension is greater than 0.10, 0.10, 0.20, or 0.25, respectively. P-values from the chi-square test (with one-sided alpha of 0.05) will be used to help with interpretation of the data, but they will not trigger a data review, since significant differences between the arms in these four types of toxicities are expected. A toxicity review will also be triggered if the chi-square test has a p-value < 0.05 for the test of an arm difference in rate of all other Grade 3+ toxicities combined.

14.5 Data analysis of letrozole data

All analyses of PFS will be intention-to-treat, such that patients will be analyzed in the arm to which they were randomized and those patients who withdraw for toxicity, who withdraw consent for continued treatment, or who start non-protocol therapy will continue to be followed for PFS. Patients who withdraw consent to be followed will be censored. The only patients who will not be used in the analyses are patients who cancel before starting treatment. The trial will not over-accrue in order to make up for the loss of power caused by early withdrawals, since adequate power is still obtained even if the trial has 10% less events than the number powered on above. Specifically, if as many as 28

progressions were to be prematurely censored in the Phase III, the log-rank test would achieve a power of 0.88.

The final test of the arm effect on PFS will be made with the score test from the stratified proportional hazards model using a one-sided alpha of 0.025, where the strata are measurable/non-measurable disease and disease-free interval. Any efficacy claim will be based solely on this primary statistical analysis. In secondary analyses, we will compare the arms on the proportion of patients who are progression-free at 6 months and at 12 months. Only patients with a documented time of progression of 28 weeks or less will be called progressed at 6 months; only patients with a documented time of progression of 55 weeks or less will be considered progressed at 12 months. The chi-square test for a difference in proportions (one-sided alpha of 0.025) has at least 86% power to detect a difference in proportions of 0.25 versus 0.40 (at 12 months) and about 69% power to detect a difference of 0.50 versus 0.63 (at 6 months). These proportions are those that would be expected if the exponential distributions under the null and alternative hypotheses hold.

Other secondary analyses will also be conducted at the time of the final primary analysis. We will use the proportional hazards model to compare the arms on time-to-treatment-failure. Time-to-treatment-failure is defined as the interval from randomization until first disease progression, early termination of protocol therapy due to toxicity or withdrawn consent, or beginning non-protocol therapy, whichever occurs first. Using only those patients with measurable disease, the logistic and proportional hazards models will be used in the final analysis to test for arm differences in response rate and response duration. Treatment-related toxicity rates by Type, Grade, and arm will be tabulated. In secondary analyses of time-to-event variables, treatment arm will be assessed using a 1-sided alpha of 0.025; in secondary analyses of other variables, treatment arm will be assessed using a 2-sided alpha of 0.05.

As another secondary analysis, we will examine in an exploratory way whether prior systemic therapy (chemotherapy or endocrine therapy) influences drug response or PFS. Specifically, the interaction between arm and prior therapy (yes/no) will be tested with the proportional hazards model, controlling for the covariates listed above. We will also perform Kaplan-Meier analyses within the four subgroups defined by arm-by-prior therapy.

All analyses of PFS will use investigator-determined response as the clinical endpoint. Radiologic images will be collected and stored for the potential future analysis of independent-determination of response. Patients without measurable disease are to be excluded from any independent analysis, and patients with incomplete imaging or pertinent medical information available for independent review will be censored at the last date with complete information. For the planned group sequential design, total accrual of 352 letrozole subjects would result in 274 events under the alternative hypothesis. It is anticipated that approximately 20% of events would be excluded from any independent determination of response. Under an assumption data are missing completely at random, the sample size would yield 219 events such that there would be 85% power for a single-stage analysis using a one-sided alpha = 0.025.

Finally, to analyze overall survival, the letrozole data will be pooled with the tamoxifen data (see Section 14.7).

14.6 Data analysis of tamoxifen data

Update #5
5/13/11

The primary objective of this trial is to estimate adverse events rates, especially for stroke, proteinuria, thrombosis, hypertension. Interim analyses of toxicity will be presented to all meetings of the Data Safety and Monitoring Board (i.e., toxicity monitoring will start before PFS monitoring). Maximum tolerated differences between arms in toxicity rates will be used to trigger a careful review of the toxicity data with consideration given to modifying the therapy or closing the trial. Specifically, a toxicity review will be triggered if

the observed difference between arms in rate of grade 3+ stroke, proteinuria, thrombosis, hypertension is greater than 0.10, 0.10, 0.20, or 0.25, respectively. P-values from the chi-square test (with one-sided alpha of 0.05) will be used to help with interpretation of the data, but they will not trigger a data review, since significant differences between the arms in these four types of toxicities are expected. A toxicity review will also be triggered if the chi-square test has a p-value < 0.05 for the test of an arm difference in rate of all other Grade 3+ toxicities combined.

14.7 Data analysis of overall survival in the letrozole patients

Update #5
5/13/11

Overall survival will be evaluated during interim monitoring using the letrozole cohort only. The overall survival of the treatment and control arms will be descriptively compared with a Kaplan-Meier analysis at each DSMB meeting and at the time of the final analysis of PFS. The arms will also be compared by calculating the hazard ratio and its 90% confidence interval.

14.8 CDUS reporting

The CALGB Statistical Center, Data Operations will submit quarterly reports to CTEP by electronic means using the Clinical Data Update System (CDUS).

14.9 Correlative science statistical plan

14.9.1 Evaluation of circulating tumor cells (CTC) and circulating endothelial cells (CEC)

Update #5
5/13/11

The anticipated accrual to this trial is 382 patients, with early termination of the tamoxifen cohort. We conservatively assume that 75% of patients will have CTC count data. With this sample size, we have an expected number of progression events of about 225 and > 80% power (two-sided alpha of 0.05) for detecting subgroup hazard ratios as small as 1.5 even if the sample size of one subgroup is twice as large as the other. (Subgroup is defined by a given dichotomization of CTC count.)

Combining the data from all arms, we will use descriptive statistics and proportional hazards regression modeling to examine whether circulating tumor cells (CTC) and circulating endothelial cells (CEC) measured at baseline predict PFS. We will address the independent prognostic effects of CTC and CEC by including treatment arm and letrozole/tamoxifen indicator in the model as well as known prognostic factors such as patient age, estrogen-receptor status, measurable/non-measurable disease, number of sites of metastases, presence of visceral metastases, and disease-free interval. We will also use the model to test the interaction of CTC and CEC with treatment arm. Since the form of the relationship of baseline CTC and CEC measurements with PFS is not known, we will consider a variety of functional forms, including untransformed values, logarithms, and cut points. In particular, we will try to replicate results from previous studies that reported testing CTC cutpoints of < 1 versus > 2 and < 4 versus > 5. No previously tested cutpoints for CEC are reported in the literature. We will use regression trees and loess plots within test/validation samples to explore other cutpoints.

Including interaction terms in the model will allow us to examine whether the association of CTC and CEC with PFS differs depending upon treatment arm. In theory, elevated CTC counts may indicate a subgroup of patients with active angiogenesis that is more likely to benefit from bevacizumab than a subgroup patients with non-elevated CTC counts. To replicate results from a previous study in which an interaction of arm and CTC was found [71], we will categorize CTC at baseline and at

9-weeks into 1 or less cells versus 2 or more cells and test whether these two categorical variables interact with treatment arm. This analysis will then be repeated by categorizing CTC at less than 4 cells versus 5 or more cells. These tests are exploratory and have limited statistical power. We will also estimate the median PFS and its 90% confidence interval within arm-by-subtype.

The longitudinal aspect of CTC and CEC levels will be more difficult to analyze and potentially more interesting. We will develop a multivariate proportional hazards model that relates various functional forms of CTC and CEC levels to the hazard of progression. The goal is to decide whether and how levels of CTCs and CECs and changes in those levels predict progression. We will analyze similarly for response with logistic regression. Finally, in theory, patients receiving bevacizumab may have changes in CTCs and CECs that are different from those in endocrine-only patients. The mixed linear model will be used to test for arm differences in changes in CTCs and CECs across time.

14.9.2 Proteomic analysis of longitudinal samples

We plan a case control design to study longitudinal proteomic analysis on serum from selected patients to define new biomarkers that correlate with disease activity. These initial analyses will focus on letrozole-treated patients as the effect of tamoxifen and letrozole on normal serum components may differ. In a pilot set of 15 patients responding to endocrine therapy alone serial samples will be analyzed for proteins that exhibit a decrease over time. Using plots of protein levels against time, responding patients will be compared with a similar number of non-responding patients that are matched for disease site and disease burden. Proteins of interest will be those that show discordant changes in these two groups (i.e. a decrease in responders and an increase in non-responders) with a pattern that can be reproduced in several patients. As controls we will also measure CEA, CA27-29 and HER2 ECD in these same samples to help define response and to compare the pattern of expression from the novel biomarker with established biomarkers. The case control design is essential because we anticipate that there will also be generic changes in serum protein components as a result of estrogen deprivation effects on normal tissues. From a sample size of 176 patients receiving letrozole without bevacizumab we expect that about 70% will have laboratory data, and that about 70% of these will have measurable disease. This results in roughly 85 patients, approximately 15 of whom will be responders and so we expect to be able to do this pilot analysis only once. We will also be able to supplement this analysis with samples from the experimental arm. Since there is a different therapeutic approach with these patients, the change in serum protein biomarkers could well be distinct justifying a separate analysis of samples from the two treatment arms. Once a list of potential protein biomarkers has been defined, they will be identified through MS and targeted for assay development so that we will be able to study these proteins in other databases. Since this is an exploratory or pilot exercise power size calculations are not applicable. The statistical analysis will largely involve statistical graphics to plot trends across time.

14.9.3 Luminal subtyping and efficacy of endocrine therapy

Update # 5
5/13/11

The purpose of this aim is to examine the association between progression free survival (PFS) and breast cancer subtype determined by immunohistochemistry. We anticipate that about 75% of the 382 patients accrued to this trial will contribute laboratory data to this correlative study; thus the total sample size for this correlative study could be as large as 286. About 225 progressions/deaths could be expected in these 286 patients if

the median PFS of the two treatment arms are 6 and 9. We assume that about 50% of the patients will be LumA and 50% poor prognosis subtypes. Given these assumptions, we have at least 86% power (two-sided alpha of 0.05) to detect a PFS hazard ratio of about 1.5 for comparing the LumA subtype to the other subtypes combined.

While median PFS with 80% confidence interval will be estimated in each of the subtypes, for purposes of statistical inference, our sample size of about 225 events will require that we compare the LumA tumors to the other tumors combined. We will use a stratified proportional hazards model to correlate subtypes with PFS (stratified on randomization stratification factors). We will address independent prognostic effects by including treatment arm in the model along with prognostic factors such as patient age, estrogen-receptor status, number of sites of metastases, line of therapy, and presence of visceral metastases. In addition, we hypothesize that luminal A subtype tumors exhibit greater benefit from the addition of bevacizumab than the LumB and misdiagnosed ER-subtypes (as a combined category). We will evaluate this hypothesis by estimating median PFS and its confidence interval within arm-by-subtype, and by incorporating and an arm by subtype interaction term in the multivariate model.

To determine the best cutpoint of Ki67 that best predicts PFS, we will consider two different methods. We will use regression tree analysis (with PFS as the endpoint) on the entire dataset, and generate bootstrap samples to examine the amount of variance in this cutpoint. Second, we will randomly select 50% of the patients in which to do the regression tree analysis, and try to validate this cutpoint in the remaining 50% of the cases. The resulting dichotomized Ki67 will be crosstabulated with breast cancer subtype, with the expectation that Ki67 redefines as poor prognosis a proportion of LumA tumors and places them in the LumB category. The effect is to improve the predictive value of the test in placing patients into group that benefit from endocrine therapy and the addition of bevacizumab (LumA) and a group that do not (LumB). These associations will be tested with the chi-square test. Finally, in order to explore the degree to which the best Ki67 cutpoint depends on subtype, we may also try to define cutpoints within each subtype separately.

We will try to validate Ki67 cutpoints reported in the literature by testing these cutpoints in PFS models, and comparing our results to those of the literature Makrestsov reports a tri-chotomization of Ki67 into < 10%, between 10 and 30%, and > 30%. They also report Ki67 dichotomization by cutting at 30% [72].

For those patients who have had prior early stage disease, we will try to acquire any of the original tumor blocks of the early stage tumor for luminal subtyping or for other marker analyses. We will examine the discordance rate between the early stage and advanced stage tumors with respect to luminal subtype and its constituent markers, as well as with respect to Ki67 and VEGF status.

14.9.4 The impact of PIK3CA mutations on the efficacy of bevacizumab

The purpose of this study is to examine the association between progression free survival (PFS) and PIK3CA (mutated vs. unmutated). Data from each of the arms and endocrine therapies (letrozole/tamoxifen) will be combined for this analysis. We anticipate that about 75% of the 382 patients in this clinical trial will contribute laboratory data to this correlative study; thus the total sample size could be as large as 286. About 225 progressions/deaths could be expected in these 286 patients if the median PFS of the two treatment arms are 6 and 9 months. We assume that about 70% of the patients will be unmutated (wildtype) and 30% mutated. We hypothesize that the median PFS according to arm and PIK3CA may show the following type of pattern.

	PIK3CA Not mutated	PIK3CA Mutated	
Bevacizumab	8	11	9
Control	6	4	6
	7.5	7.5	7.5

That is, within the bevacizumab arm, the patients with mutations will have better PFS than patients without mutations. Within the control arm, the patients with mutations will have worse PFS than patients without mutations. We will evaluate this hypothesis by estimating median PFS and its confidence interval within arm-by-PIK3CA subgroups. We will test the arm by PIK3CA interaction in a stratified proportional hazards model that includes known prognostic factors such as patient age, estrogen-receptor status, number of sites of metastases, line of therapy, and presence of visceral metastases. Randomization stratification factors will form the strata.

We will correlate PIK3CA with a number of other biologic endpoints, such as CTC, CEC, VEGF, CD31, AR, and Luminal subtype. Thus, we expect mutated PIK3CA patients to have higher CTCs due to increased invasiveness, higher CECs due to increased angiogenesis, higher VEGF expression due to the direct effect of increased pAkt, and higher CD31 due to the direct effect of increased VEGF. We expect more PIK3CA mutations in the Luminal B subtype than in the Luminal A subtype. The association of PIK3CA with dichotomous markers will be assessed with contingency tables and the chi-square test. The association of PIK3CA with continuous markers such as VEGF will be assessed by using the logistic regression model to examine the functional form of the association. We will consider a variety of functional forms through the use of cutpoints, loess plots and restricted cubic splines.

We expect about 40% of the PIK3CA mutated patients to be mutated at exon 9, 50% at exon 20, and 10% at other sites. Since only about 30% of the 330 patients (~100 patients) in this correlative study will be mutated, the sample size at each site within arm will be small. Therefore, as a purely descriptive analysis, we will calculate median PFS with its 80% confidence interval according to arm and mutation site (unmutated, exon 9, exon 20, other exon).

14.10 Pharmacogenomic studies

14.10.1 Candidate gene approach

Primary objective: The primary objective of the pharmacogenetics portion of this study is to investigate if the improvement in PFS due to bevacizumab depends on the VEGF gene. This will be investigated in the framework of a two-way multiplicative log-linear Cox model with factors drug (P=control or B=bevacizumab) and VEGF gene (1=CT/TT or 2=CC). For the purpose of this analysis the two control arms are combined and the two bevacizumab arms are combined as no interaction between endocrine therapy) with either the drug or genotype factor is expected.

It is expected that a total of N=382 patients will be accrued to the study. For the pharmacogenetic studies, it is expected that 324 patients (85% of 382) will provide consent and usable samples. The primary analysis population will consist of those patients classified as “genetic” Europeans on the basis of their genome-wide SNP

Update #5
5/13/11

profiles. The expected sample size for the primary pharmacogenetic analyses is expected to be 275 (85% of 324).

The putative prevalence rates for groups 1 and 2 are [illegible] respectively. For the purpose of these power calculations, it will be assumed that the distribution of PFS in each drug arm consists of two-component (the genotype groups) mixtures of exponential distributions. More specifically, the PFS survival function, at time t>0, of the control arms is given by P[T>t] = [illegible] while that of the bevacizumab arm is given by P[T>t] = [illegible]. The four rate parameters are chosen so as to satisfy 0.5= [illegible] and 0.5= [illegible], where M_P=6 and M_B=9 months (as assumed in the clinical protocol).

Under the assumed model, the four hazard rates are given by [illegible]. The effect size for the interaction is given by b_{12}=log[[illegible]. Given the sample size only large interactions will be detectable with reasonable power. Given that this an hypothesis generating analysis, a type I error rate of 0.2 will be employed. For example, if [illegible] then the power at the two-sided 0.2 level is 0.68 (based on 10,000 simulations).

Secondary objectives:

The impact of adjusting for baseline VEGF for the PKG analysis will be considered. The above questions will also be considered in the context of other genotypes in additional candidate genes (i.e., among them, CYP2D6, CYP19, and KDR) of putative importance. The pharmacogenetic analysis related to tamoxifen and letrozole will be conducted as a secondary exploratory analysis of gene-toxicity, gene-response, and gene-PFS relationships: the association between CYP2D6 (tamoxifen) and CYP19 (letrozole) variants and clinical phenotypes (systemic toxicity, response, and PFS) will be performed by construction of hazard ratios or other appropriate non-parametric techniques. An *unadjusted* two-sided level of 0.05 will be used for all secondary/exploratory analyses. Additional genes or variants of interest might also be explored as new information relevant to the study emerges.

14.10.2 Genome-wide association approach

Primary objective: The primary objective for this genome-wide association study (GWAS) is to identify single-nucleotide polymorhphisms (SNPs) associated with progression-free survival (PFS).

Other endpoints of interest are other relevant clinical endpoints such as adverse events (e.g., proteinuria, hypertension, and other common side effects of study drugs), overall survival (OS) and progression-free survival (PFS). The clinical definitions for these endpoints will coincide with those of specified in the CALGB 40503 protocol.

Additionally, we will seek to identify potential SNP by bevacizumab interactions with respect to outcome, and seek to develop prediction models for the outcomes based on SNPs adjusted for important clinical and demographics co-variables.

We will also validate results found in other CALGB studies (e.g., 80303, 40101 and 90401) and perform a risk analysis by comparing the 40503 SNP data to SNP data from controls (patient thought not to have cancer). The latter will be obtained from public databases.

Pre-processing: For pre-processing (quality control and genotype calls) the Illumina chips, we will use the commercial program Bead Studio, developed by Illumina.

Although Illumina does not provide a Linux port of Bead Studio, one can run the software on VMWARE, running on a Linux host. A two CPU dual core (four cores) AMD Operation Socket F workstation, with 16GB of RAM, will be available for this purpose. The statistical analyses will be carried out on a Linux server with 8 dual core Operation Socket F CPUs (16 cores) with 64GB of RAM (expandable to 128GB if needed).

Analyses to assess genotyping quality and population stratifications

Initial quality studies will be conducted to identify SNPs that have generated sufficiently poor quality genotype data that they should be removed from analyses. Call rate, patterns of missing data, and departures from Hardy-Weinberg equilibrium (HWE) will be assessed using an exact test will all be scrutinized to identify markers that will not be used in analysis. In general, SNPs with call rates < 95% and those with highly significant departures from HWE ($p<10^{-7}$) will not be included in analyses. Non-random patterns of missing data are sometimes encountered in data generated on high-throughput genotyping platforms; the most common non-random missing data problem is that heterozygous genotypes are more likely to be assigned as missing than either homozygous genotype. We will perform analyses using blind duplicates as well as analyses assessing the relationship between heterozygous call rates and missing data to identify any SNPs in which data are clearly not missing at random. Depending on the number and degree of difficulty observed, we will either remove problematic SNPs from analysis, or assign quality scores to reflect the extent of the non-random missing data.

Additional preliminary quality control analyses will be conducted to insure that the sample does not include duplicated samples or closely related individuals. These analyses can be rapidly conducted using PLINK [73]. Duplicated samples (or unrecognized identical twins) will be reduced to a single sample for further analyses. Although we do not expect to have closely related individuals included in this sample, only one member of any set of first-degree relatives will be included in subsequent analysis. For each sample, we will also generate a gender call based on the SNPs on the X chromosome and study the missingness patterns for the SNP on the Y and XY chromosomes in order to convincingly determine that all samples are from female patients.

Population structure that is not appropriately recognized and accommodated can lead to both false positive and false negative results in association studies. We will conduct studies using structure [74] to estimate ancestry proportions using 10,000 SNPs chosen for having no pairwise LD with unrelated individuals from the HapMap CEU, YRI and CHB+JPT samples used to model the ancestral populations. Substantial previous research has shown this to be a rapid and effective approach to defining historical geographic ancestry. Although self-identified race/ethnicity is usually highly correlated with estimated historical geographic ancestry, there are often a few individuals who appear to be misclassified with self-defined labels, and it is the genetically defined ancestry that is critical to correctly accommodate to insure robust results from association studies.

Each individual will then have estimates of European, African and Asian ancestry. For individuals with high ancestry proportion for a single group (> 98%), we will conduct further analyses with eigenstrat [75] using all SNPs to determine whether there are additional important sources of variation among individuals leading to detectable stratification by allele frequencies (reflecting, for example, differences in ethnic make-up within individuals of European descent from different U.S. cities from which

subjects for the trial were obtained). Primary analyses, described below, will be conducted within groups defined by historical geographic ancestry. Secondary analyses will be conducted using logistic regression with ancestry proportions (and any additional stratification indentified using eigenstrat) as covariates.

Feature discovery

The association between the genotype call (say AA, AB or BB) for each autosomal SNP and PFS will be carried out using a univariable Cox model. Let $lam_0[t]$,$lam_1[t]$ and $lam_2[t]$, denote the hazard rate at time t conditional on having 0, 1 or 2 copies of the B allele.

For the power calculations we will assume that the SNPs satisfy HWE and that the PFS distribution for the control arm is expressible as a mixture of exponential laws of the form $exp(-lam_{1*}t)=(1-q)^2exp[-lam_{1,0*}t]+2*q*(1-q)exp[-lam_{1,1*}t]+q^2exp[-lam_{1,2*}t]$ where q denotes the relative frequency of the B allele and lam_1 the exponential hazard rate specified in the clinical protocol. Similarly for arm 2, we will assume a mixture distribution of the form $exp(-lam_2*t)=(1-q)^2exp[-lam_{2,0}*t]+2*q*(1-q)exp[-lam_{2,1}*t]+q^2exp[-lam_{2,2}*t]$.

We will power the study for the additive genetic model with no drug interaction. In other words, for some D>1, $exp(-lam_1*t)=(1-q)^2exp[-lam_{1,0}*t]+2*q*(1-q)exp[-lam_{1,0}*D*t]+q^2exp[-lam_{1,0}*D^2*t]$ and $exp(-lam_2*t)=(1-q)^2exp[-lam_{2,0}*t]+2*q*(1-q)exp[-lam_{2,0}*D*t]+q2exp[-lam_{2,0}*D^2*t]$for some $0<lam_{2,0}<lam_{1,0}$.

Update #5 5/13/11

According to the clinical protocol $lam_1=log(2)/6$ and $lam_2=log(2)/9$. It is expected that a total of N=382 patients will be accrued to the study. For the pharmacogenetic studies, it is expected that 324 patients (85% of 382) will provide consent and usable samples. The primary analysis population will consist of those patients classified as “genetic” Europeans on the basis of their genome-wide SNP profiles. The expected sample size for the primary pharmacogenetic analysis is expected to be 275 (85% of 324). While these 275 patients will comprise the primary patient population for this proposed pharmacogenetic study, we plan to genotype all patients who provide consent and usable samples. Specifically, in two CALGB GWAS studies (80303 and 40101) some of the signals observed in the primary patient population were also observed in the African American patients.

A feature (SNP) will be considered significant if the corresponding nominal unadjusted two-sided P-value is less than 0.05/K, where K is number of features which pass the pre-processing step. Needless to say, this approach may be conservative. It does however guarantee strict type I error control. It is expected that these samples will be genotyped on the Illumina 610Quad platform. The power, at the two-sided 0.05/600000 level (i.e., assuming K=600,000 autosomal SNP markers pass through the pre-processing step), is illustrated in Table 1 the Cox statistics, coding the genotypes AA,AB and BB as 0, 1 and 2 is used. Each case is based on 10,000 simulation replicates. In addition to the additive model, we provide power calculations for the recessive (i.e., $lam_0=lam_1$,lam_2-lam_0*D) and dominant (i.e., $lam_1=lam_2=lam_0*D$) models if the test based on the additive model is used.

These analyses may require additional candidate SNP genotyping or the sequencing of specific genes. In addition, we may use the DNA collected to conduct genome-wide association with (GWAS) to identify novel candidates, or as next generation sequencing platforms become more cost effective consider whole-genome sequencing.

Table 1. Power illustration

Hazard Ratio (D)											
q	**Model**	**2.0**	**2.1**	**2.2**	**2.3**	**2.4**	**2.5**	**2.6**	**2.7**	**2.8**	**2.9**
0.1	additive	0.20	0.31	0.41	0.53	0.63	0.72	0.80	0.86	0.90	0.94
	recessive	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	dominant	0.09	0.13	0.20	0.27	0.36	0.44	0.52	0.59	0.67	0.74
0.20	additive	0.63	0.76	0.87	0.93	0.96	0.98	0.99	1.00	1.00	1.00
	recessive	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	dominant	0.20	0.28	0.38	0.49	0.59	0.69	0.77	0.83	0.87	0.92
0.30	additive	0.83	0.92	0.96	0.98	0.99	1.00	1.00	1.00	1.00	1.00
	recessive	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	dominant	0.15	0.23	0.32	0.42	0.52	0.60	0.69	0.76	0.82	0.87
0.40	additive	0.90	0.95	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00
	recessive	0.00	0.00	0.01	0.01	0.02	0.02	0.03	0.04	0.05	0.07
	dominant	0.06	0.10	0.15	0.21	0.27	0.35	0.42	0.50	0.57	0.63
0.50	additive	0.88	0.94	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00
	recessive	0.02	0.03	0.05	0.08	0.11	0.15	0.20	0.26	0.31	0.37
	dominant	0.01	0.02	0.04	0.05	0.07	0.10	0.13	0.16	0.20	0.24
0.60	additive	0.80	0.90	0.96	0.98	0.99	1.00	1.00	1.00	1.00	1.00
	recessive	0.06	0.11	0.16	0.24	0.31	0.40	0.50	0.57	0.66	0.72
	dominant	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.02	0.02	0.03
0.70	additive	0.61	0.74	0.85	0.92	0.96	0.98	0.99	0.99	1.00	1.00
	recessive	0.10	0.17	0.25	0.33	0.44	0.54	0.62	0.71	0.77	0.83
	dominant	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.80	additive	0.24	0.35	0.46	0.58	0.68	0.77	0.84	0.89	0.92	0.95
	recessive	0.07	0.12	0.18	0.26	0.35	0.42	0.51	0.60	0.68	0.74
	dominant	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.90	additive	0.01	0.01	0.02	0.03	0.05	0.07	0.09	0.12	0.16	0.20
	recessive	0.00	0.01	0.01	0.02	0.03	0.05	0.06	0.09	0.11	0.15
	dominant	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

14.10.3 Submission of molecular data

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

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

Additionally, the lab will also provide the complete results from any quality control measurers carried out. If a sample had to be redone (e.g., defective or poor quality array), the lab will provide all replicate idat files and add an appropriate column to the supplementary table. The molecular data generated for this aim may not be shared with

other investigators or used for any analysis not specified in the protocol until a formal approval fro the CALGB Statistical Center is obtained.

14.10.4 Secondary objectives

Other clinical endpoints such as overall-survival and toxicity are of interest. The definitions for these will coincide with those of the clinical protocol. Note that due to small sample size, we are primarily focusing on finding prognostic features. From a pharmacogenetic point of view, what is of greater interest is to validate existing or find novel predictive markers. This will be done in the context of multiplicative two-way ANOVA log-linear Cox (logistic) for censored (binary) outcomes.

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

The Illumina HuamaHap610 Quad contains 4,300 SNPs in regions with common copy number variants (CNVs). Given the complex structure of CNVs, it is not always clear how to define the genotype of a CNV. Instead of categorizing copy numbers into genotypes, we will estimate relative genomic abundance probe intensities. This approach allows for the consideration of other CNVs beyond deletions, including duplications and combinations of both. For notational brevity, we shall refer to these as CNV markers.

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

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

Secondary relevant clinical endpoints include other adverse events (e.g., proteinuria, hypertension, and other common side effects of study drugs) and overall survival. For censored time-to-event outcomes, the stratified log-rank rtest will be primarily used for assessment of significance.

A risk analysis will be carried out by comparing the genotypic distributions of the SNPs from the CALGB 40503 data to those form controls (thought to not have cancer). The SNP data from the controls will be obtained from public databases.

In addition to conduction analyses on all features directly assessed on the high-throughput platform used in these studies, we will also interrogate all additional HapMap SNPs that are not in strong pairwise LD with any genotyped SNP, but for which there is sufficient multi-locus LD to SNPs on the high-throughput platform. Testing UNtyped Alleles (TUNA) is a robust approach for conducting such analyses that provides inexpensive in silico follow up to the initial analysis and allows us to more efficient design any followu up genotyping studies [76, 77]. For example, use of Illumina HumanHap300 enables direct testing of 270K-450K SNPs, and indirect testing of 750K-1.5M additional SNPs (i.e., these SNPs are so highly correlated with SNPs that are directly tested for association that testing them would provide little additional information). The ranges given above, bracket the expectations for different human populations, with European populations at the high end of the range, and populations of recent African descent at the lower end. Use of TUNA enables interrogation of an additional 100K-250K SNPs that are neither on the platform nor highly correlated with

any individual SNP on the platform. Note that use of TUNA will facilitate comparisons to genome wide association studies on potentially related phenotypes (e.g., clinical trials of the same or related drugs) conducted using other high-throughput platforms or candidate gene studies utilizing SNPs not directly genotyped on the high-throughput platform chosen for our studies.

Finally, we note that the methodology field for the analysis of genome-wide SNP data is in its infancy. We will consider the employment of "newer" methods if they are deemed to be statistically sound and enable us to better interrogate, and more importantly, understand the data.

14.10.5 Statistical software

The R statistical environment [78] and Bioconductor [79] packages will be used for all of the primary statistical analyses relating features to phenotypes. Specialized statistical genetics software, including PLINK [73] structure [74], eigenstrat [75] and TUNA [76] [77] will be used for some of the quality or secondary analyses, and R will be used for logistic regression analyses allowing for ancestry covariates.

14.11 Correlative Science Study

Understanding patient characteristics of risk of toxicity to bevacizumab and endocrine therapy and understanding longitudinal changes in physical state, comorbid medical conditions and psychological state while on treatment

The purpose of this study is to examine the associations between the occurrence of grade 3, 4, or 5 toxicity and clinical factors collected from patients assessments taken at baseline and during treatment. The primary comparisons of interest are in baseline assessments of patients receiving bevacizumab in conjunction with hormonal therapy. We anticipate that at least 90% of the 221 patients accrued to this arm of the trial will provide viable assessment information at baseline. We estimate that the overall rate of grade 3, 4, or 5 toxicity in patients receiving bevacizumab will be 40%. This estimate was based upon Dr. Dickler's data from a Memorial Sloan Kettering Cancer Center pilot study, that evaluated the feasibility of letrozole with bevacizumab.

To account for multiple comparisons in the primary objective, we will apply a Bonferroni correction to a two-sided Type I error of 0.05. Thus, to consider 5 primary factors of interest, each test will be conducted using $\alpha = 0.01$. For the continuous factors [MOS Physical Functioning, Karnofsky Performance Status Rated Healthcare Professional, Timed "Up and Go", OARS Physical Health Section], we will use logistic regression to determine the odds ratio of toxicity. With 198 patients, and a two-sided $\alpha = 0.01$, a univariate test will have 90% power to detect a odds ratio of 1.97 between one standard deviation change in the continuous factor. Under the assumption of normality and that the 50-th percentile of the population has a 40% probability of having a toxicity, this odds ratio equates to the 84-th percentile having a 57% probability of toxicity, and the 16-th percentile having a 25% probability of toxicity. The OARS MFAQ (IADL) will be treated as a binary predictor, and we will use Fisher's Exact test to test for differences in the patient subgroups. An approximate power calculation is based on the assumption that the factor will dichotomize patients into equally sized groups (N = 99 for each group). For a two-sided $\alpha = 0.01$, there will be 90% power to detect a difference in the overall rate of toxicity of 27% versus 53% in the subgroups defined by the binary factor.

As a secondary objective, an exploratory analysis will examine the associations of baseline levels of additional factors [Karnofsky Performance Status Rated by Patient, Number of falls in last 6 months, Patient reports number and names of medications, herbs, or vitamins,

Blessed Orientation-Memory-Concentration Test, Hospital Anxiety and Depression Scale, % Unintentional Weight Loss in last 6 months, Body Mass Index, MOS Social Activity Limitation Scale, Medical Outcomes Study (MOS) Social Support Survey Subscale] to grade 3, 4, or 5 toxicity in patients receiving bevacizumab. Logistic regression will be used for univariate analyses of all continuous factors, and Fishers Exact test will be used for all binary factors. All tests will use an uncorrected two-sided Type I error of 0.05. We will also build a prognostic model for toxicity from all baseline factors obtained by patient assessments using multivariate logistic regression. All factors with univariate p-values less than 0.2 will be considered, including the potential for interactions of interest.

Additionally, since the relationship between continuous factors and toxicity are not known for this patient cohort, we will apply receiver operator characteristic (ROC) curves to consider a variety of cut points for binary classification. The Youden index will be used to identify the optimal cut point for maximizing the sensitivity and specificity of a predictor on the additive scale. To compare the associations of each factor to toxicity among patients receiving therapy with and without bevacizumab (N = 442), the treatment x factor interactions will be tested in logistic regression models using two-sided Type I error rates of 0.05.

The relationship between toxicity and profiles of assessment factors over time will be analyzed through univariate longitudinal models. The goal is to examine whether and how changes in assessment scores correlate to the occurrence of toxicity during treatment. These analyses will be exploratory in nature, and consider a variety of covariance structures in modeling the profiles of assessed factors over the course of treatment.

15.0 ADVERSE EVENT REPORTING (AER)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. Investigators are required to notify the Investigational Drug Branch (IDB), the CALGB Central Office, the Study Chair, and their Institutional Review Board if a patient has a reportable serious adverse event. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting beginning October 1, 2010. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov). All reactions determined to be "reportable" in an expedited manner must be reported using the NCI Adverse Event Expedited Reporting System (AdEERS).

CALGB requires investigators to route all AdEERS reports through the CALGB Central Office for CALGB-coordinated studies.

15.1 CALGB 40503 reporting requirements:

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: AdEERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent.

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

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

AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

AdEERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

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

March 2005

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

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

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions (see below).
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

15.2 Additional instructions or exclusions from AdEERS expedited reporting requirements for phase 2 and 3 trials utilizing an agent under a CTEP IND:

- All adverse events reported via AdEERS (i.e., serious adverse events) should also be forwarded to your local IRB.
- For the purposes of expedited adverse event reporting, the CAEPR (which includes expected adverse events) for bevacizumab may be found in Section 15.3 below. Expedited adverse events for endocrine therapy may be found in the package inserts. **Note**: The ASAEL column of the bevacizumab CAEPR has been replaced with the specific protocol exceptions to expedited reporting (SPEER) list. This list now includes "expected" severity grades in addition to event terms.
- CALGB 40503 uses a drug under a CTEP IND. The reporting requirements for investigational agents under a CTEP IND should be followed for all agents (any arm) in this trial.
- Deaths occurring greater than 30 days after the last dose of treatment, that are due to disease progression, do not require AdEERS.
- AdEERS reports should be submitted electronically to the CALGB Central Office (calgb@uchicago.edu). Faxed copies of the AdEERS paper template, available at the AdEERS web page, will be accepted (312-345-0117), but electronic submission is preferred.
- Reporting of cases of secondary AML/MDS is to be done using the NCI/CTEP Secondary AML/MDS Report Form. New primary malignancies should be reported using study form C-1001.
- The reporting of adverse events described above is in addition to and does not supplant the reporting of adverse events as part of the report of the results of the clinical trial, e.g., study summary forms or cooperative group data reporting forms (see Section 6.0 for required CALGB forms).

15.3 Comprehensive adverse events and potential risks list (CAEPR) for bevacizumab (rhuMAb VEGF, NSC 704865)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 3540 patients.* Below is the CAEPR for bevacizumab (rhuMAb VEGF).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.3, August 1, 2013[1]

Adverse Events with Possible Relationship to Bevacizumab (rhuMAb VEGF) (CTCAE 4.0 Term) [n= 3540]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
		Blood and lymphatic system disorders - Other (renal thrombotic microangiopathy)	
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
CARDIAC DISORDERS			
		Acute coronary syndrome[2]	
	Cardiac disorders - Other (supraventricular arrhythmias)[3]		***Cardiac disorders - Other (supraventricular arrhythmias)[3] (Gr 3)***
		Heart failure	
		Left ventricular systolic dysfunction	
		Myocardial infarction[2]	
		Ventricular arrhythmia	
		Ventricular fibrillation	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
	Colitis		***Colitis (Gr 3)***
	Constipation		***Constipation (Gr 3)***
	Diarrhea		***Diarrhea (Gr 3)***
	Dyspepsia		***Dyspepsia (Gr 2)***
		Gastrointestinal fistula[4]	
	Gastrointestinal hemorrhage[5]		***Gastrointestinal hemorrhage[5] (Gr 2)***
	Gastrointestinal obstruction[6]		
		Gastrointestinal perforation[7]	
		Gastrointestinal ulcer[8]	
	Ileus		
	Mucositis oral		***Mucositis oral (Gr 3)***
	Nausea		***Nausea (Gr 3)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue (Gr 3)***
	Infusion related reaction		***Infusion related reaction (Gr 2)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr 3)***
	Pain		***Pain (Gr 3)***
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		***Allergic reaction (Gr 2)***
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[9]		***Infection[9] (Gr 3)***
		Infections and infestations - Other	

		(necrotizing fasciitis)	
	Infections and infestations - Other (peri-rectal abscess)		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
		Injury, poisoning and procedural complications – Other (anastomotic leak)[10]	
	Wound complication		***Wound complication (Gr 2)***
	Wound dehiscence		***Wound dehiscence (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 3)***
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 3)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 3)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
	Cardiac troponin I increased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 3)***
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 3)***
	White blood cell decreased		***White blood cell decreased (Gr 3)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 3)***
	Dehydration		***Dehydration (Gr 3)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 3)***
	Musculoskeletal and connective tissue disorder - Other (bone metaphyseal dysplasia)[11]		
	Myalgia		***Myalgia (Gr 3)***
	Osteonecrosis of jaw[12]		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 3)***
		Intracranial hemorrhage	
		Ischemia cerebrovascular[2]	
	Peripheral sensory neuropathy[13]		
		Reversible posterior leukoencephalopathy syndrome	
	Syncope		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Hematuria		***Hematuria (Gr 3)***
	Proteinuria		***Proteinuria (Gr 2)***
		Renal and urinary disorders - Other (Nephrotic Syndrome)	
		Urinary fistula	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
Reproductive system and breast disorders -			

Other (ovarian failure)[14]			
		Vaginal fistula	
	Vaginal hemorrhage		***Vaginal hemorrhage (Gr 3)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Allergic rhinitis		***Allergic rhinitis (Gr 3)***
		Bronchopleural fistula	
		Bronchopulmonary hemorrhage	
	Cough		***Cough (Gr 3)***
	Dyspnea		***Dyspnea (Gr 2)***
	Epistaxis		***Epistaxis (Gr 3)***
	Hoarseness		***Hoarseness (Gr 3)***
		Respiratory, thoracic and mediastinal disorders - Other (nasal-septal perforation)	
		Respiratory, thoracic and mediastinal disorders - Other (tracheo-esophageal fistula)	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Pruritus		***Pruritus (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
	Urticaria		***Urticaria (Gr 2)***
VASCULAR DISORDERS			
Hypertension			***Hypertension (Gr 3)***
	Thromboembolic event		***Thromboembolic event (Gr 3)***
		Vascular disorders - Other (arterial thromboembolic event)[2,15]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]The risks of arterial thrombosis such as cardiac or CNS ischemia are increased in elderly patients and in patients with a history of diabetes.

[3]Supraventricular arrhythmias may include supraventricular tachycardia, atrial fibrillation and atrial flutter.

[4]Gastrointestinal fistula may include: Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Gastric fistula, Gastrointestinal fistula, Rectal fistula, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[5]Gastrointestinal hemorrhage may include: Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Intra-abdominal hemorrhage, Oral hemorrhage, Rectal hemorrhage, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[6]Gastrointestinal obstruction may include: Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Rectal obstruction, Small intestinal obstruction, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[7]Gastrointestinal perforation may include: Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Jejunal perforation, Rectal perforation, Small intestinal perforation, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[8]Gastrointestinal ulcer may include: Duodenal ulcer, Esophageal ulcer, Gastric ulcer, and other sites under the GASTROINTESTINAL DISORDERS SOC.

[9]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[10]Anastomotic leak may include Gastric anastomotic leak; Gastrointestinal anastomotic leak; Large intestinal anastomotic leak; Rectal anastomotic leak; Small intestinal anastomotic leak; Urostomy leak; Vaginal anastomotic leak

[11]Metaphyseal dysplasia was observed in young patients who still have active epiphyseal growth plates.

[12]Cases of osteonecrosis of the jaw (ONJ) have been reported in cancer patients in association with bevacizumab treatment, the majority of whom had received prior or concomitant treatment with i.v. bisphosphonates.

[13]Increased rate of peripheral sensory neuropathy has been observed in trials combining bevacizumab and chemotherapy compared to chemotherapy alone.

[14]Ovarian failure, defined as amenorrhea lasting 3 or more months with follicle-stimulating hormone (FSH) elevation (≥30 mIU/mL), was increased in patients receiving adjuvant bevacizumab plus mFOLFOX compared to mFOLFOX alone (34% vs. 2%). After discontinuation of bevacizumab, resumption of menses and an FSH level <30 mIU/mL was demonstrated in 22% (7/32) of these women. Long term effects of bevacizumab exposure on fertility are unknown.

[15]Arterial thromboembolic event includes visceral arterial ischemia, peripheral arterial ischemia, heart attack and stroke.

Also reported on bevacizumab (rhuMAb VEGF) trials but with the relationship to bevacizumab (rhuMAb VEGF) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (idiopathic thrombocytopenia purpura); Bone marrow hypocellular; Disseminated intravascular coagulation; Hemolysis

CARDIAC DISORDERS - Atrioventricular block complete; Atrioventricular block first degree; Cardiac arrest; Myocarditis; Pericardial effusion; Restrictive cardiomyopathy; Right ventricular dysfunction

EAR AND LABYRINTH DISORDERS - Ear and labyrinth disorders - Other (tympanic membrane perforation); Hearing impaired; Tinnitus; Vertigo

ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism

EYE DISORDERS - Blurred vision; Cataract; Dry eye; Extraocular muscle paresis; Eye disorders - Other (blindness); Eye disorders - Other (conjunctival hemorrhage); Eye disorders - Other (corneal epithelial defect); Eye disorders - Other (floaters); Eye disorders - Other (ischemic CRVO); Eye disorders - Other (macular pucker); Eye disorders - Other (transient increased IOP > or =30 mm Hg); Eye disorders - Other (vitreous hemorrhage); Eye pain; Keratitis; Optic nerve disorder; Photophobia; Retinal detachment; Retinal tear; Retinopathy; Watering eyes

GASTROINTESTINAL DISORDERS - Ascites; Chelitis; Colonic stenosis; Dry mouth; Dysphagia; Enterocolitis; Esophageal pain; Esophageal stenosis; Flatulence; Gastrointestinal disorders - Other (peritonitis); Oral pain; Pancreatitis; Proctitis; Rectal mucositis; Rectal stenosis;Typhlitis

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Edema face; Edema limbs; Edema trunk; Facial pain; Fever; Flu like symptoms; Gait disturbance; Injection site reaction; Localized edema; Multi-organ failure; Sudden death NOS

HEPATOBILIARY DISORDERS - Cholecystitis; Gallbladder necrosis; Gallbladder obstruction; Hepatic failure; Hepatic necrosis

INFECTIONS AND INFESTATIONS - Infections and infestations - Other (aseptic meningitis)

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Arterial injury; Bruising; Burn; Dermatitis radiation; Fracture

INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood antidiuretic hormone abnormal; CD4 lymphocytes decreased; CPK increased; Carbon monoxide diffusing capacity decreased; Electrocardiogram QT corrected interval prolonged; Forced expiratory volume decreased; GGT increased; INR increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased; Weight gain

METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Chest wall pain; Fibrosis deep connective tissue; Generalized muscle weakness; Head soft tissue necrosis; Joint effusion; Muscle weakness lower limb; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (aseptic necrotic bone); Musculoskeletal and connective tissue disorder - Other (myasthenia gravis); Musculoskeletal and connective tissue disorder - Other (polymyalgia rheumatica); Neck pain; Pain in extremity; Pelvic soft tissue necrosis; Soft tissue necrosis lower limb

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain

NERVOUS SYSTEM DISORDERS - Arachnoiditis; Ataxia; Central nervous system necrosis; Cerebrospinal fluid leakage; Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Dysgeusia; Dysphasia; Encephalopathy; Extrapyramidal disorder; Facial nerve disorder; Hydrocephalus; Leukoencephalopathy; Memory impairment; Nervous system disorders - Other (increased intracranial pressure); Paresthesia; Peripheral motor neuropathy; Pyramidal tract syndrome; Seizure; Somnolence; Tremor; Vasovagal reaction

PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Libido decreased; Psychosis

RENAL AND URINARY DISORDERS - Bladder spasm; Chronic kidney disease; Cystitis noninfective; Renal and urinary disorders - Other (dysuria); Renal and urinary disorders - Other (ureterolithiasis); Renal hemorrhage; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract obstruction; Urinary tract pain

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Erectile dysfunction; Irregular menstruation; Pelvic pain; Vaginal discharge

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Hypoxia; Nasal congestion; Pulmonary fibrosis; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (dry nares); Respiratory, thoracic and mediastinal disorders - Other (pulmonary infarction)

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Nail loss; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Photosensitivity; Purpura;

Rash acneiform; Skin and subcutaneous tissue disorders - Other (diabetic foot ulcer); Skin and subcutaneous tissue disorders - Other (skin breakdown/ decubitus ulcer); Skin hyperpigmentation; Skin induration; Skin ulceration; Stevens-Johnson syndrome

VASCULAR DISORDERS - Flushing; Hot flashes; Hypotension; Lymphocele; Phlebitis; Vasculitis

Note: Bevacizumab (rhuMAb VEGF) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

16.0 REFERENCES

1. Giordano, S.H., et al., *Is breast cancer survival improving?* Cancer, 2004. **100**(1): p. 44-52.
2. Jemal, A., et al., *Cancer statistics, 2005.* CA Cancer J Clin, 2005. **55**(1): p. 10-30.
3. Mouridsen, H., et al., *Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the International Letrozole Breast Cancer Group.* J Clin Oncol, 2001. **19**(10): p. 2596-606.
4. Nabholtz, J.M., et al., *Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group.* J Clin Oncol, 2000. **18**(22): p. 3758-67.
5. Paridaens, R., et al., *First line hormonal treatment (HT) for metastatic breast cancer (MBC) with exemestane (E) or tamoxifen (T) in postmenopausal patients (pts): A randomized phase III trial of the EORTC breast group.* Proceedings Am Soc Clin Oncol, 2004. **22**(14S).
6. Early Breast Cancer Trialists' Collaborative Group, *Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and a 15-year survival: an overview of the randomised trials.* Lancet, 2005. **365**: p. 1687-1717.
7. Losordo, D.W. and J.M. Isner, *Estrogen and angiogenesis: A review.* Arterioscler Thromb Vasc Biol, 2001. **21**(1): p. 6-12.
8. Morales, D.E., et al., *Estrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model.* Circulation, 1995. **91**(3): p. 755-63.
9. Venkov, C.D., A.B. Rankin, and D.E. Vaughan, *Identification of authentic estrogen receptor in cultured endothelial cells. A potential mechanism for steroid hormone regulation of endothelial function.* Circulation, 1996. **94**(4): p. 727-33.
10. Kim-Schulze, S., et al., *Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells.* Circulation, 1996. **94**(6): p. 1402-7.
11. Gagliardi, A. and D.C. Collins, *Inhibition of angiogenesis by antiestrogens.* Cancer Res, 1993. **53**(3): p. 533-5.
12. Gagliardi, A.R., B. Hennig, and D.C. Collins, *Antiestrogens inhibit endothelial cell growth stimulated by angiogenic growth factors.* Anticancer Res, 1996. **16**(3A): p. 1101-6.
13. Ferrara, N. and T. Davis-Smyth, *The biology of vascular endothelial growth factor.* Endocr Rev, 1997. **18**(1): p. 4-25.
14. Shweiki, D., et al., *Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis.* J Clin Invest, 1993. **91**(5): p. 2235-43.
15. Hyder, S.M., et al., *Uterine expression of vascular endothelial growth factor is increased by estradiol and tamoxifen.* Cancer Res, 1996. **56**(17): p. 3954-60.
16. Hyder, S.M., et al., *Selective inhibition of estrogen-regulated gene expression in vivo by the pure antiestrogen ICI 182,780.* Cancer Res, 1997. **57**(13): p. 2547-9.

17. Heryanto, B., K.E. Lipson, and P.A. Rogers, *Effect of angiogenesis inhibitors on oestrogen-mediated endometrial endothelial cell proliferation in the ovariectomized mouse.* Reproduction, 2003. **125**(3): p. 337-46.

18. Albrecht, E.D., et al., *Acute temporal regulation of vascular endothelial growth/permeability factor expression and endothelial morphology in the baboon endometrium by ovarian steroids.* J Clin Endocrinol Metab, 2003. **88**(6): p. 2844-52.

19. Hyder, S.M., et al., *Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor.* Cancer Res, 2000. **60**(12): p. 3183-90.

20. Mueller, M.D., et al., *Regulation of vascular endothelial growth factor (VEGF) gene transcription by estrogen receptors alpha and beta.* Proc Natl Acad Sci U S A, 2000. **97**(20): p. 10972-7.

21. Takei, H., E.S. Lee, and V.C. Jordan, *In vitro regulation of vascular endothelial growth factor by estrogens and antiestrogens in estrogen-receptor positive breast cancer.* Breast Cancer, 2002. **9**(1): p. 39-42.

22. Ruohola, J.K., et al., *Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells.* Mol Cell Endocrinol, 1999. **149**(1-2): p. 29-40.

23. Nakamura, J., et al., *Estrogen regulates vascular endothelial growth/permeability factor expression in 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors.* Endocrinology, 1996. **137**(12): p. 5589-96.

24. Weidner, N., et al., *Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma.* Journal of the National Cancer Institute, 1992. **84**: p. 1875-1887.

25. Weidner, N., et al., *Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma.* N Engl J Med, 1991. **324**(1): p. 1-8.

26. Hansen, S., et al., *The prognostic value of angiogenesis by Chalkley counting in a confirmatory study design on 836 breast cancer patients.* Clin Cancer Res, 2000. **6**(1): p. 139-46.

27. Obermair, A., et al., *Vascular endothelial growth factor (VEGF) in human breast cancer: correlation with disease-free survival.* Int J Cancer, 1997. **74**(4): p. 455-8.

28. Linderholm, B., et al., *Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment.* J Clin Oncol, 2000. **18**(7): p. 1423-31.

29. Linderholm, B., et al., *Vascular endothelial growth factor is of high prognostic value in node-negative breast carcinoma.* J Clin Oncol, 1998. **16**(9): p. 3121-8.

30. Marson, L.P., et al., *The effect of tamoxifen on breast tumour vascularity.* Breast Cancer Research and Treatment, 2001. **66**(1): p. 9.

31. Jain, R.K., et al., *Endothelial cell death, angiogenesis, and microvascular function after castration in an androgen-dependent tumor: role of vascular endothelial growth factor.* Proc Natl Acad Sci U S A, 1998. **95**(18): p. 10820-5.

32. Manders, P., et al., *Vascular endothelial growth factor is associated with the efficacy of endocrine therapy in patients with advanced breast carcinoma.* Cancer, 2003. **98**(10): p. 2125-32.

33. Ryden, L., et al., *Tumor-Specific Expression of Vascular Endothelial Growth Factor Receptor 2 but Not Vascular Endothelial Growth Factor or Human Epidermal Growth Factor Receptor 2 Is Associated With Impaired Response to Adjuvant Tamoxifen in Premenopausal Breast Cancer.* J Clin Oncol, 2005. **23**(21): p. 4695-4704.

34. Svensson, S., et al., *ERK phosphorylation is linked to VEGFR2 expression and Ets-2 phosphorylation in breast cancer and is associated with tamoxifen treatment resistance and small tumours with good prognosis.* 2005. **24**(27): p. 4370.

35. Ryden, L., et al., *Tumor-specific VEGF-A and VEGFR2 in postmenopausal breast cancer patients with long-term follow-up. Implication of a link between VEGF pathway and tamoxifen response.* Breast Cancer Res Treat, 2005. **89**(2): p. 135-43.

36. Landberg, G., et al., *Cyclin I is expressed in human breast cancer and closely associated with VEGF and KDR expression.* Breast Cancer Res Treat, 2005. **89**(3): p. 313-6.

37. Dombernowsky, P., et al., *Letrozole, a new oral aromatase inhibitor for advanced breast cancer: double-blind randomized trial showing a dose effect and improved efficacy and tolerability compared with megestrol acetate.* J Clin Oncol, 1998. **16**(2): p. 453-61.

38. Mouridsen, H., et al., *Phase III Study of Letrozole Versus Tamoxifen as First-Line Therapy of Advanced Breast Cancer in Postmenopausal Women: Analysis of Survival and Update of Efficacy From the International Letrozole Breast Cancer Group.* J Clin Oncol, 2003. **21**(11): p. 2101-2109.

39. Bonneterre, J., et al., *Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma.* Cancer, 2001. **92**(9): p. 2247-58.

40. Howell, A., et al., *Comparison of Fulvestrant Versus Tamoxifen for the Treatment of Advanced Breast Cancer in Postmenopausal Women Previously Untreated With Endocrine Therapy: A Multinational, Double-Blind, Randomized Trial.* J Clin Oncol, 2004. **22**(9): p. 1605-1613.

41. Osborne, C.K., et al., *Double-Blind, Randomized Trial Comparing the Efficacy and Tolerability of Fulvestrant Versus Anastrozole in Postmenopausal Women With Advanced Breast Cancer Progressing on Prior Endocrine Therapy: Results of a North American Trial.* J Clin Oncol, 2002. **20**(16): p. 3386-3395.

42. Paridaens, R., et al., *Promising Results with Exemestane in the First-Line Treatment of Metastatic Breast Cancer: A Randomized Phase II EORTC Trial with a Tamoxifen Control.* Clin Breast Cancer, 2000. **64**: p. 167.

43. Kaufmann, M., et al., *Exemestane is superior to megestrol acetate after tamoxifen failure in postmenopausal women with advanced breast cancer: results of a phase III randomized double-blind trial. The Exemestane Study Group.* J Clin Oncol, 2000. **18**(7): p. 1399-411.

44. Miller, K., et al. *A randomized phase III trial of paclitaxel versus paclitaxel plus bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer: a trial coordinated by the Eastern Cooperative Oncology Group (E2100).* in *28th Annual San Antonio Breast Cancer Symposium 2005*. 2005. San Antonio, Texas: Breast Cancer Research and Treatment.

45. Osborne, C.K., *Tamoxifen in the Treatment of Breast Cancer.* N Engl J Med, 1998. **339**(22): p. 1609-1618.

46. Simpson, E.R. and M. Dowsett, *Aromatase and its inhibitors: significance for breast cancer therapy.* Recent Prog Horm Res, 2002. **57**: p. 317-38.

47. Baum, M., et al., *Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial.* Lancet, 2002. **359**(9324): p. 2131-9.

48. Goss, P.E., et al., *A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer.* N Engl J Med, 2003. **349**(19): p. 1793-802.

49. Coombes, R.C., et al., *A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer.* N Engl J Med, 2004. **350**(11): p. 1081-92.

50. Thurlimann, B.J., et al., *BIG1-98: randomized double-blind phase III study to evaluate letrozole (L) vs. tamoxifen (T) as adjuvant endocrine therapy for postmenopausal women with receptor-positive breast cancer.* Proceedings Am Soc Clin Oncol, 2005. **23**(16S).

51. Jakesz, R., et al., *Benefits of switching postmenonpausal women with hormone-sensitive early breast cancer to anastrozole after 2 years adjuvant tamoxifen: combined results from 3,123 women enrolled in the ABCSG Trial 8 and the ARNO 95 trial.* Br Can Res Treat, 2004. **88**: p. S7.

52. Winer, E.P., et al., *American Society of Clinical Oncology Technology Assessment on the Use of Aromatase Inhibitors As Adjuvant Therapy for Postmenopausal Women With Hormone Receptor-Positive Breast Cancer: Status Report 2004.* J Clin Oncol, 2005. **23**(3): p. 619-629.

53. Klijn, J.G., et al., *Combined treatment with buserelin and tamoxifen in premenopausal metastatic breast cancer: a randomized study.* J Natl Cancer Inst, 2000. **92**(11): p. 903-11.

54. Milla-Santos, A., et al., *A randomized trial of goserelin (Zoladex) + tamoxifen versus goserelin + anastrozole (Arimidex) in pre/perimenopausal patients with hormone dependent advanced breast cancer.* Breast Cancer Res Treat, 2002. **76 supplement 1**.

55. Pham, C.D., et al., *Magnetic resonance imaging detects suppression of tumor vascular permeability after administration of antibody to vascular endothelial growth factor.* Cancer Invest, 1998. **16**(4): p. 225-30.

56. Borgström, P., et al., *Importance of VEGF for breast cancer angiogenesis in vivo: implications from intravital microscopy of combination treatments with an anti-VEGF neutralizing monoclonal antibody and doxorubicin.* AntiCancer Research, 1999. **19**: p. 4203-4214.

57. Cobleigh, M.A., et al., *A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer.* Seminars in Oncology, 2003. **30**(5, Suppl 16): p. 117-124.

58. Miller, K.D., et al., *Phase III trial of capecitabine (Xeloda^{R}) plus bevacizumab (Avastin^{TM}) versus capecitabine alone in women with metastatic breast cancer previously treated with an anthracycline and a taxane.* Breast Cancer Res Treat, 2002. **76**: p. S37.

59. Traina, T., et al., *Letrozole with bevacizumab is feasible in patients with hormone receptor-positive metastic breast cancer.* ASCO Annual Meeting Proceedings, 2006. **24**: p. 133.

60. Traina, T., et al., *A feasibiity trial of letrozole in combination with bevacizumab in patients with metastatic breast cancer.* In press Journal of Clinical Oncology.

61. Scappaticci, F.A., et al., *Arterial thromboembolic events in patients with metastatic carcinoma treated with chemotherapy and bevacizumab.* J Natl Cancer Inst, 2007. **99**(16): p. 1232-9.

62. Hillner, B.E., et al., *American Society of Clinical Oncology 2003 Update on the Role of Bisphosphonates and Bone Health Issues in Women With Breast Cancer.* J Clin Oncol, 2003. **21**(21): p. 4042-4057.

63. Ozcan, C., S.J. Wong, and P. Hari, *Reversible posterior leukoencephalopathy syndrome and bevacizumab.* N Engl J Med, 2006. **354**(9): p. 980-2; discussion 980-2.

64. Glusker, P., L. Recht, and B. Lane, *Reversible posterior leukoencephalopathy syndrome and bevacizumab.* N Engl J Med, 2006. **354**(9): p. 980-2; discussion 980-2.

65. Skillings J, J.D., Kabbinavar F, Bergsland, Holmgren E, Holden S, Hurwitz H, Scappaticci, *Arterial thromboembolic events in a pooled analysis of 5 randomized, controlled trials of bevacizumab with chemotherapy.* 2005 ASCO Annual Meeting Proceedings, 2005. **23**(16S): p. 196s.

66. Hambleton J, S.J., Kabbinavar F, Bergsland E, Holmgren E, Holden SN, Hurwitz H, Scappaticci, *Safety of low-dose aspirin in a pooled analysis of 3 randomized, controlled trials of bevacizumab with chemotherapy in patents with metastatic colorectal cancer.* 2005 ASCO Annual Meeting Proceedings, 2005. **23**(16S): p. 259s.

67. Scappaticci, F.A., et al., *Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab.* J Surg Oncol, 2005. **91**(3): p. 173-80.

68. Karp, J.E., et al., *Targeting vascular endothelial growth factor for relapsed and refractory adult acute myelogenous leukemias: therapy with sequential 1-beta-d-arabinofuranosylcytosine, mitoxantrone, and bevacizumab.* Clinical Cancer Research, 2004. **10**(11): p. 3577-85.

69. Bonneterre, J., et al., *Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the Tamoxifen or Arimidex Randomized Group Efficacy and Tolerability study.* J Clin Oncol, 2000. **18**(22): p. 3748-57.

70. Freidlin, B. and E.L. Korn, *A comment on futility monitoring.* Controlled Clinical Trials, 2002. **23**(4): p. 355-66.

71. Cristofanilli, M., et al., *Circulating tumor cells, disease progression, and survival in metastatic breast cancer.* New England Journal of Medicine, 2004. **351**(8): p. 781-91.

72. Makretsov, N.A., et al., *Hierarchical clustering analysis of tissue microarray immunostaining data identifies prognostically significant groups of breast carcinoma.* Clinical Cancer Research, 2004. **10**(18 Pt 1): p. 6143-51.

73. Purcell, S., et al., *PLINK: a tool set for whole-genome association and population-based linkage analyses.* Am J Hum Genet, 2007. **81**(3): p. 559-75.

74. Falush, D., M. Stephens, and J.K. Pritchard, *Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies.* Genetics, 2003. **164**(4): p. 1567-87.

75. Price, A.L., et al., *Principal components analysis corrects for stratification in genome-wide association studies.* Nat Genet, 2006. **38**(8): p. 904-9.

76. Nicolae, D.L., *Testing untyped alleles (TUNA)-applications to genome-wide association studies.* Genet Epidemiol, 2006. **30**(8): p. 718-27.

77. Nicolae, D.L., *Quantifying the amount of missing information in genetic association studies.* Genet Epidemiol, 2006. **30**(8): p. 703-17.

78. Team, R., *R: A language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing., 2006.

79. Gentleman, R.C., et al., *Bioconductor: open software development for computational biology and bioinformatics.* Genome Biol, 2004. **5**(10): p. R80.

APPENDIX I

CORRELATIVE SCIENCE STUDIES FOR CALGB 40503

Part A: Correlative Science Investigators:

Hope Rugo, M.D.
John Park, M.D. University of California, San Francisco

Part B: Correlative Science Investigators:

Reid R. Townsend, M.D. Washington University

Part C Correlative Science Investigators:

Torsten Nielsen, M.D., Ph.D., University of British Columbia

Part D Correlative Science Investigators:

Mary Ellen Moynahan, M.D. Memorial Sloan Kettering Cancer Center

PART A

Evaluation of Circulating Endothelial Cells (CECs) and Circulating Tumor Cells (CTCs) as Early Markers of Time to Progression and Response in Patients with Estrogen and/or Progesterone Receptor Positive Metastatic Breast Cancer Receiving Endocrine Therapy Alone or Endocrine Therapy Plus Bevacizumab

1.0 BACKGROUND

The investigators hypothesize that baseline levels and changes in serial levels of circulating endothelial cells (CECs) and circulating tumor cells (CTCs) will predict PFS and serve as early markers of response or resistance to endocrine therapy and endocrine therapy plus bevacizumab.

1.1 Circulating tumor cells (CTCs)

"Micrometastasis" (MM) was originally a theoretical concept: CTCs were presumed to exist because cancers spread hematogenously, but by definition CTCs were not detectable by standard clinical methods. Like subatomic particles in the early 20th century, these hypothetical entities became detectable with the advent of increasingly sensitive methodologies. Yet, the biological and clinical meanings of CTCs have not been fully determined.

New and emerging technologies now make it possible to sensitively detect CTCs/MM in peripheral blood (PB) and/or bone marrow (BM) of patients with cancer. In principle, these CTC technologies can be applied to many aspects of cancer management, including detection, staging/prognosis, assessment of treatment response, surveillance during remission, validation of novel therapies, and isolation of tumor cells for molecular analysis. For example, CTC detection may be of particular value in the initial management of breast cancer by better identifying those patients at risk of metastasis, thus allowing more judicious use of chemotherapy. Indeed, multiple studies have now shown that detection of CTCs in marrow by immunocytochemistry (ICC) is strongly prognostic of recurrence and death [1-3].

A number of studies have suggested that the presence of CTCs in patients with metastatic disease is associated with poor survival [4-6] and disease burden[7-9]. Recent studies [10, 11] have quantified circulating tumor cells (CTCs) in late stage breast cancer to evaluate prognosis and monitor response to therapy. These studies have found high sensitivity in detecting CTCs using immunomagnetic bead enrichment of peripheral blood samples. The threshold for detection is often only a few cells/ml of peripheral blood, however many metastatic patients have > 100 CTC/ml [10]. Using a newly developed technology for automated ICC (Cell Search System), the presence of at least 5 or more CTCs per 7.5 ml of blood at the start of treatment and first follow-up in a study of 177 patients with metastatic disease correlated with a shorter median progression free and overall survival [12]. In a multivariate analysis, CTCs were the most significant independent predictor of progression free and overall survival. Two or more cells were detected at start of treatment in 61% of patients. It may be that evaluation of CTCs will have particular value in the setting of larger randomized trials to enable early determination of effect. In this trial, CTCs may also indicate early evidence of a differential anti-tumor effect from the addition of bevacizumab to hormone therapy. CTCs will be quantified using the Cell Search System, a fully validated commercial test.

Studies of targeted agents in the treatment of breast cancer have been complicated by the lack of target identification to individualize therapy. The success of specific targeting has been demonstrated in the treatment of HER-2 positive cancers with trastuzumab [13].

Evaluating potential targets is particularly difficult in metastatic disease, where novel therapeutics undergo initial testing and generally meet with success or failure - due in part to the difficulty in obtaining initial and serial tissue for study. CTCs provide a readily accessible source of tumor cells for comparison of markers pre- and post-therapy. It is not yet known whether cell surface receptors (e.g. HER-2, EGFR) or gene expression profiles are altered by the exit of the tumor cells from the adjacent tumor and stroma cells and into the circulation, thus altering paracrine and possibly autocrine effects. Two small studies [14, 15] evaluating the presence of HER-2 in CTCs suggested that significant discordance may exist between the primary tumor and CTCs at the time of metastases, or during progression of metastatic disease. In addition, these studies demonstrate that it is possible to quantify receptors essential for rationally designed therapy using CTCs, and that this may be a more accurate measure of immunophenotype than the primary tumor. Technology is being developed to utilize the 4th channel of the Cell Search System to analyze surface markers.

1.2 Circulating endothelial cells (CECs)

Angiogenesis inhibition is a promising approach for new drug development in cancer therapy. Blocking VEGF has already been shown to have potent antivascular effects, as well as clinical activity demonstrated by an improvement in overall survival when combined with standard chemotherapy in colorectal cancer [16]. However, the antivascular effects of antiangiogenic (AA) therapy are not well understood and identifying clinically relevant intermediate markers for angiogenesis has been difficult. In addition, because AA compounds may primarily delay disease progression, standard objective response criteria (tumor shrinkage) may not be the best method of evaluating tumor effect. For these reasons, identification of biologic markers is necessary to help better define the effect of angiogenesis inhibition.

Data demonstrating a 5-fold increase in both resting and activated CECs in newly diagnosed breast cancer and lymphoma patients with a rapid fall following primary surgery [17] suggests that CECs may be an important non-invasive marker of tumor angiogenesis [18]. In addition, apoptosis of endothelial cells has been linked to tumor cell apoptosis in preclinical models. A single infusion of the VEGF inhibitor bevacizumab has been shown to decrease the number of CECs in patients with early stage rectal cancer; this was associated with direct antiangiogenic effects including decreased tumor perfusion, vascular volume, microvascular density and interstitial fluid pressure [19]. Recent studies in preclinical mouse models treated with a targeted VEGFR-2 antibody demonstrated a dose-dependent reduction in viable circulating endothelial progenitor cells that paralleled antitumor activity [20].

A recent phase II study evaluated serial CECs and CTCs in patients treated with bevacizumab and erlotinib for metastatic breast cancer [21]. CECs were measured using flow cytometry and defined as CD45-, CD34+, CD31+(Bright), thioflavin negative. Progenitor cells (CEP) were additionally defined as CD133+. In this exploratory analysis, the change in CEC from baseline to first subsequent clinic visit (week 3) predicted progression free survival at first tumor evaluation (9 weeks, p 0.014). In addition, CECs strongly correlated with progenitor cells as well as CTCs at week 0 and week 3. Preliminary data suggest that the change in CECs at week 3 predicts PFS in patients treated with letrozole and bevacizumab in the ongoing phase II trial [22].

Based on this and other pre-clinical data, CECs have been proposed as a possible surrogate marker of angiogenesis [23, 24]. We propose to use flow cytometry to analyze CECs in patients enrolled on this trial to explore the use of this non-invasive marker in predicting response to therapy. CECs will also be compared with CTCs. Surrogate markers of angiogenesis are critical to allow appropriate patient selection and a rational approach to

the use of antiangiogenic therapy given the recent positive data treating breast and other solid tumors with bevacizumab.

2.0 Objectives

2.1 To compare baseline and changes in serial levels of circulating endothelial cells and circulating tumor cells in patients treated with endocrine therapy alone or endocrine therapy plus bevacizumab, and to explore the relationship of these markers with response to study therapy.

3.0 Methods

3.1 Blood for CTC and CEC analysis will be drawn into two 10 mL CellSave tubes and one 5 mL EDTA lavender top tube shipped to the University of California San Francisco, Park Lab prior to the start of protocol therapy (baseline), then on the day of bevacizumab therapy weeks 4, 7 and 10 one CellSave tube and one EDTA should be drawn. Blood should be drawn *before* the bevacizumab infusion at each time point indicated.

Dr. Hope Rugo and the laboratory of Dr. John Park at the University of California, San Francisco has extensive expertise in circulating cell analysis by flow cytometry [25] and, more recently, the Cell Search System. They are performing cell analyses using these technologies for several multicenter trials and have recently presented data from a phase II trial [21] as well as preliminary data from the letrozole and bevacizumab phase II trial [22].

3.2 CTC analysis

CTC analysis will be performed using the Cell Search system [12]. This analysis will be performed on 7.5 mL of blood that has been drawn into a CellSave tube (tube will be provided to sites). Briefly, blood is diluted with buffer, enrichment is performed using the AutoPrep machine using antiEpCAM coated magnetic beads. Cells are then stained for CD45 and cytokeratin, and analyzed on an automated fluorescence microscope. Total number of cytokeratin positive, CD45 negative and DAPI positive (a nuclear stain) is reported per 7.5 mL of sample.

3.3 CEC analysis

CEC analysis is performed using cell cytometry. Using 5 mL of blood that has been drawn into a 5 mL EDTA lavender top tube, the specimen is stained with thioflavin (a nuclear stain), antiCD45, antiCD34, and antiCD31. CEDs are analyzed by flow cytometry, enumerated in TruCount tubes, and defined as CD45, CD34+ and CD31+; all cells must stain for thioflavin. Additional analyses are performed with antiCD146 and antiCD133 to evaluate progenitor cells.

4.0 References

1. Braun, S., et al., Comparative analysis of micrometastasis to the bone marrow and lymph nodes of node-negative breast cancer patients receiving no adjuvant therapy. J Clin Oncol, 2001. 19(5): p. 1468-75.
2. Braun, S., et al., Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. New England Journal of Medicine, 2000. 342(8): p. 525-33.
3. Braun, S., et al., Pooled analysis of prognostic impact of bone marrow micrometastasis: 10-year survivial of 4,199 breast cancer patients. Br Can Res Treat, 2003. 82: p. S8.
4. Bauernhofer, T., et al., Association of disease progression and poor overall survival with detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer. Oncology Reports, 2005. 13(2): p. 179-84.
5. Weigelt, B., et al., Marker genes for circulating tumour cells predict survival in metastasized breast cancer patients. British Journal of Cancer, 2003. 88(7): p. 1091-4.
6. Burchill, S.A., et al., Detection of epithelial cancer cells in peripheral blood by reverse transcriptase-polymerase chain reaction. British Journal of Cancer, 1995. 71(2): p. 278-81.
7. Terstappen, L.W., et al., Peripheral blood tumor cell load reflects the clinical activity of the disease in patients with carcinoma of the breast. International Journal of Oncology, 2000. 17(3): p. 573-8.
8. Burchill, S.A., et al., Circulating neuroblastoma cells detected by reverse transcriptase polymerase chain reaction for tyrosine hydroxylase mRNA are an independent poor prognostic indicator in stage 4 neuroblastoma in children over 1 year. J Clin Oncol, 2001. 19(6): p. 1795-801.
9. Tombal, B., et al., Prognostic value of circulating prostate cells in patients with a rising PSA after radical prostatectomy. Prostate, 2003. 56(3): p. 163-70.
10. Hayes, D., et al., Monitoring circulating tumor cell (CTC) levels to predict rapid progression in metastatic breast cancer (MBC): A prospective, multi-institutional trial. Proc Am Soc Clin Oncol., 2004.
11. Krishnan, K., et al., Evaluation of circulating tumor cell assays and clinical implications. Breast Cancer Res and Treat, 2003. 82(1): p. 99.
12. Cristofanilli, M., et al., Circulating tumor cells, disease progression, and survival in metastatic breast cancer. New England Journal of Medicine, 2004. 351(8): p. 781-91.
13. Slamon, D.J., et al., Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. New England Journal of Medicine, 2001. 344(11): p. 783-92.
14. Meng, S., et al., HER-2 gene amplification can be acquired as breast cancer progresses. Proceedings of the National Academy of Sciences of the United States of America, 2004. 101(25): p. 9393-8.
15. Hayes, D.F., et al., Monitoring expression of HER-2 on circulating epithelial cells in patients with advanced breast cancer. International Journal of Oncology, 2002. 21(5): p. 1111-7.
16. Hurwitz, H., et al., Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. New England Journal of Medicine, 2004. 350(23): p. 17. 2335-42.

17. Mancuso, P., et al., Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood, 2001. 97(11): p. 3658-61.

18. Bertolini, F., et al., Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. Cancer Res, 2003. 63(15): p. 4342-6.

19. Willett, C.G., et al., Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nature Medicine, 2004. 10(2): p. 145-7.

20. Shaked, Y., et al., Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; Implications for cellular surrogate marker analysis of antiangiogenesis. Cancer Cell, 2005. 7(1): p. 101-11.

21. Rugo, H.S., et al., Circulating endothelial cell (CEC) and tumor cell (CTC) analysis in patients (pts) receiving bevacizumab and erlotinib for metastatic breast cancer (MBC). Br Can Res Treat, 2004. 88: p. S142.

22. Traina, T., et al. *A phase II trial of letrozole in combination with bevacizumab, an anti*-22. *VEGF antibody, in patients with hormone receptor-positive metastatic breast cancer.* in 28th Annual San Antonio Breast Cancer Symposium 2005. San Antonio, Texas: 23.Breast Cancer Research and Treatment.

23. Mancuso, P., et al., Circulating endothelial cells as a novel marker of angiogenesis. 24. Advances in Experimental Medicine and Biology, 2003. 522: p. 83-97.

24. Schneider, M., M. Tjwa, and P. Carmeliet, A surrogate marker to monitor angiogenesis at last. Cancer Cell, 2005. 7(1): p. 3-4.

25. Park, J.W., et al., Comparative evaluation of micrometastasis (MM) in primary breast cancer as detected by immunocytochemistry (ICC), flow cytometry (FC), and reverse transcription-polymerase chain reaction (RT-PCR). Proc Am Soc Clin Oncol., 2002. 21.

PART B

Proteomic Analysis of Longitudinal Samples from Patients with Advanced Disease Undergoing Endocrine Therapy

1.0 Background

The investigators hypothesize that proteomic analysis of longitudinal samples from patients with advanced disease undergoing estrogen deprivation therapy will define new serum based biomarkers of disease activity.

The value of serum biomarkers in the treatment of breast cancer has been debated over the years but the rationale for a serum test for disease monitoring has been solidly established by the routine use of tumor markers in other malignancies such as testis cancer. The debate on the value of biomarkers therefore focuses more on the palliative intent of our therapies for advanced breast cancer rather then on the intrinsic potential of these proteins to assist in disease management. To date we have a limited repertoire of serum biomarkers in breast cancer, including CEA, CA15-3, CA27-29 and HER2 extra-cellular domain (ECD). Of these four assays HER2 ECD has the interesting property of an association with rapid disease progression despite endocrine therapy and a direct link to aggressive tumor biology through expression of a plasma membrane tyrosine kinase [1]. Serum biomarker discovery therefore has great potential to generate not only new tools to follow disease, but also assays on which to base therapeutic decisions and to predict resistance to standard therapies.

New techniques in proteomics are capable of high throughput screening of protein abundance with subsequent identification of proteins of interest through mass spectrometric (MS) techniques. To date the most common proteomics approach is based on two-dimensional gel electrophoresis (2DE). A standard 2DE assay is typically capable of resolving 10,000 proteins. This assay has some limitations for the large-scale analysis of complex protein mixtures (such as serum) because a number of potentially critical types of protein cannot be detected because their abundance is too low or they are too small or basic. Recent techniques in one-dimensional capillary separation techniques based on size, charge or hydrophobicity directly coupled to mass spectroscopy have been successfully applied to serum samples to define new cancer biomarkers [2]. These new techniques are available though the proteomics core at Washington University in St. Louis under the direction of Dr. Reid Townsend [3].

2.0 Objectives

2.1 To conduct proteomic analysis of longitudinal samples for patients with advanced-stage disease undergoing hormonal therapy to define new serum-based biomarkers related to disease activity.

3.0 Methods

10 mL of blood in a 10 mL SST (red/grey) tube and 8 mL of blood in a CPT tube will be obtained prior to the start of protocol therapy (baseline), then on the day of bevacizumab therapy weeks 4, 7 and 10. These samples will be shipped on a cold refrigerant pack over night to the Allinace PCO at Ohio State as described in Sections 6.2 and 6.2.1 of the protocol.

The samples will be analyzed for novel candidate biomarkers using unbiased proteomics methods, principally 2D gelelectrophoresis and high-resolution mass spectrometry coupled to nano-liquidchromatography. The quantity of plasma required for each analysis is ~ 0.5mL to measure ~ 2000 proteins. The serum samples will be used to compare/confirm proteins found in plasma and

will likely be used when higher throughput assays can be developed (e.g. antibody arrays and/or ELISA). The periperal whole blood samples are not banked, but will be aliquoted by the Alliance PCO and sent to Dr. Townsend's laboratory. The aliquoted amounts are then consumed in the performance of the proteomics analyses.

4.0 References

1. Lipton, A., et al., Serum HER-2/neu and response to the aromatase inhibitor letrozole versus tamoxifen. J Clin Oncol, 2003. 21(10): p. 1967-72.

2. Banks, R.E., et al., Proteomics: new perspectives, new biomedical opportunities. Lancet, 2000. 356(9243): p. 1749-56.

3. Adam, P.J., et al., Comprehensive proteomic analysis of breast cancer cell membranes reveals unique proteins with potential roles in clinical cancer. Journal of Biological Chemistry, 2003. 278(8): p. 6482-9.

PART C

Luminal Subtyping and Efficacy of Endocrine Therapy

1.0 Background

The course of ER+ advanced breast cancer is highly variable. Some patients have endocrine therapy sensitive disease and do relatively well even after relapse, responding to multiple lines of endocrine therapy. A second group of patients have ER+ endocrine therapy refractory tumors. The presence of these refractory patients in an endocrine therapy clinical trial population reduces the power to observe the beneficial interaction between bevacizumab in combination with AIs or tamoxifen.

One way to address this problem is to prospectively subtype the patient population into two broad prognostic groups based on transcriptional profiling studies, Luminal A (LumA) a relatively indolent subtype and Luminal B, a more aggressive and endocrine therapy refractory subtype (LumB) [1-3]. In early stage breast cancer approximately 50% of ER+ tumors are LumA and the rest are comprised of poor prognosis ER+ subtypes LumB and some misdiagnosed ER- Basal and ER- HER2+ subtypes. Since an ER+ metastatic population is likely to be enriched for the more aggressive subtypes we estimate that in an advanced disease trial, 50% of the relapses will be associated with tumors that were LumA at diagnosis and 50% LumB and misdiagnosed ER- subtypes We have therefore formulated the following hypothesis:

LumA tumors are more endocrine therapy sensitive than Lum B tumors or misdiagnosed ER- subtypes. LumA tumors therefore represent a population that is substantially enriched for tumors that respond to multiple lines of endocrine therapy in the advanced disease setting. The outcome of these patients is disproportionately improved by bevacizumab as Lum B tumors progress too rapidly for bevacizumab to exhibit a disease modulating effect in combination with endocrine interventions.

Methodological approaches to the identification of breast cancer subtypes. The initial approach to breast cancer sub-typing was transcriptional profiling using DNA micro-arrays. Alternative more clinically applicable approaches are under development, including qRT PCR and immunohistochemistry. The principle difference between these assays is the number of genes assayed, which has a significant impact on the complexity, cost and, potentially, the accuracy of the assay. There have been few attempts to compare the accuracy of these different assay approaches and to date no attempts in the context of large clinical trials that asked a critical therapeutic question. In this proposal we take advantage of data generated by co-investigators [4] that a small number of immunohistochemical markers can be used to identify the previously identified breast cancer subgroups [4, 5]. This approach is efficient and can be investigated in the context of the tissue micro-arrays (TMA) that are being developed in the context of cooperative group clinical trials. Analogous to transcriptional profiling data, investigators at the University of British Columbia and UNC Chapel Hill have shown that immuno-staining data using multiple biomarkers is superior to individual biomarkers when establishing prognosis [4, 5]. Further immuno-marker work by this group has established a simple approach to the identification of three of the five “intrinsic” subgroups of breast tumors identified by transcriptional profiling: an estrogen receptor positive (ER+) group (Luminal), and two ER- groups − one HER2 over-expressing and ER- and a second group that does not express HER2, but does express CK5/6 and/or HER1 (basal-type). This approach has now been successfully adopted by other groups to link biological subtypes to patient outcome and chemotherapy treatment [6]. Further sub-classification of the ER+ group into LumA and LumB is less well defined, and is currently under investigation. Almost all LumA and B tumors are ER and/or PgR positive but when HER2 is

amplified the tumor should always be considered a LumB tumor because this marks an ER+ tumor for a worse prognosis and at least some degree of endocrine therapy resistance. Our view of these immuno-marker studies is that they are certainly useful for rapid hypothesis generation. IHC data will lead to further diagnostic refinements using more sophisticated multi-gene qPCR assays that can be translated into clinical practice and a comparison between the approaches will help decide which should be recommended.

HER2 amplification is too infrequent in ER+ disease to explain most cases of breast cancer death despite endocrine treatment and so other markers must be developed to make the LumA (good prognosis) versus LumB (poor prognosis) distinction more accurate. In terms of immuno-markers, we will use a definition that includes information from three additional markers, HER1, BCL2 and GATA3. HER1 has been investigated extensively and it has been shown that strong HER1 immuno-staining in the setting of an ER+ tumor does portend a poor prognosis and lack of response to endocrine treatment [7]. However ER+ HER1+ tumors are uncommon − around 5% of ER+ disease [8] − and additional IHC biomarkers are required. Two further biomarkers have therefore been added to the experimental panel, BCL2 and GATA3. Based on microarray data, both biomarkers are expressed at high levels in LumA tumors and therefore they should be considered robust positive biomarkers for the LumA phenotype. Loss-of-function mutations have been described in GATA3, which together with functional data suggest an important role for GATA3 as a tumor suppressor in breast cancer [9]. High BCL2 expression is strongly associated with ER expression, good prognosis and possibly endocrine therapy sensitivity [10]. In addition BCL2 is a feature of the Oncotype DX assay [11]. TMA based immunoassays have been established for these proteins by Dr Nielsen. Preliminary data does support the proposition that the addition of these biomarkers adds significantly to the luminal classification.

In the analysis plan for the present study LumA will be defined as GATA3 and BCL2 positive and HER1 IHC negative as well as HER2 (amplification) negative. Further, recent data from transcriptional profiling studies indicates that within a subset of patients chacterized by relatively high ER expression the occurrence of metastasis is strongly predicted by a homogeneous gene expression pattern almost entirely consisting of cell cycle genes [12]. This finding suggests critical prognostic importance for proliferation genes in defining the boundary between LumA and LumB tumors. The analysis of Ki67, a measurement of proliferation, and the effect on LumA versus B classification will therefore also be assessed as a further promising biomaker that could add to the identification of endocrine therapy refractory patients.

2.0 Objectives

To identify biologic correlates that will predict progression –free survival (PFS) and response to therapy.

3.0 Methods

Biomarker analysis: Tissue blocks will be housed at the Alliance PCO and used for the construction of tissue microarrays. Sections from these arrays will be subjected to immunostaining (Table 1) and HER2 FISH in the laboratory of Dr. Nielsen. The stained slides will be digitally imaged, with primary image data archived and made available to all project investigators. Images from individual TMA cores will be assessed by Dr. Nielsen's laboratory using the following system for visual scoring. A score of 0 indicates invasive tumor present in the core and no staining seen (in some cases weak focal staining is also included in the 0 score), a score of 1 indicates invasive tumor present and weak staining intensity or less than 20% tumor cells stained, and a score of 2 indicates invasive tumor cells present with strong staining in > 20%. In addition, the scanned images will be subjected to quantitative digital image analysis using the Ariol system (Applied Imaging) located in Dr. Nielsen's lab. Following pathologist-

directed machine training, this system provides objective quantitations of the % of positive cancer cells and intensity of staining for nuclear and membranous biomarkers (applicable to all proposed biomarkers except ck5/6 and bcl2). Ki67 scoring will be based on the basis of a percentage of infiltrating cells detected by image analysis that are positive for this nuclear stain, to generate a more accurate proliferative index than is possible by visual assessment. HER2 will be assessed by fluorescent in situ hybridization to assign cases as amplified or non-amplified according to standard laboratory diagnostic methodology.

Table 1

Marker	Source	Dilution	Pretreatment	IHC Score	Stain
ER	DAKO	1:100	Steam 20 mins EDTA	0, 1, 2	Nuclear
PgR	DAKO	1:100	Steam 20 mins EDTA	0, 1, 2	Nuclear
HER1	Pharm DX (DAKO)	Ready to use	Proteinase K for 5 mins	0, 1, 2	Plasma membrane
CK5/6	Zymed Laboratories	1:100	Heat, CC1 mild (EDTA, 20 min)	0, 1, 2	Cytoplasmic
GATA3	Santa Cruz Biotechnology	1:50	Steam in Citrate buffer pH6) for 30 mins	0, 1, 2	Nuclear
BCL2	DAKO	1:20	Heat, CC1 mild (EDTA for 20 mins)	0, 1, 2	cytoplasmic
Ki67	Novocastra	1:100	Pressure Cooker in MV, 2 mins	% positive by image analysis	Nuclear

Subtyping analysis:. The IHC score will be "dichotomized" for the purposes of correlational and multivariate analysis [5]. The transformation of the three point score (see Table 1) into positive versus negative will be based on previously established cut offs, in most cases 0 *versus* 1 or 2.

LumA ER+ and/or PgR+; HER1- and HER2 Not amplified and GATA3+ and BCL2+

LumB ER+ and/or PgR+; HER1+ OR HER2 amplified OR GATA3 – OR BCL2 –

ER-, HER2+: ER- and PgR- and HER2 Amplified

Basal: ER-, PgR-, HER1+ and/or CK5/6+

Unclassified: any tumor not falling into one of these patterns.

Ki67 analysis: Ki67 defines a proliferation index which is best assessed as a continuous variable. Visual scoring shows poor reproducibility and for this reason quantitative image analysis will be employed. To determine the best cutpoint of Ki67 that best predicts PFS, we will consider two different methods. We will use regression tree analysis on the entire dataset, and generate bootstrap samples to examine the amount of variance in this cutpoint. Second, we will randomly select 50% of the patients in which to do the regression tree analysis, and try to validate this cutpoint in the remaining 50% of the cases. The resulting dichotomized Ki67 will be crosstabulated with breast cancer subtype, with the expectation that Ki67 redefines as poor prognosis a proportion of LumA tumors and places them in the LumB category. The effect is to improve the predictive value of the test in placing patients into a group that benefit from

endocrine therapy and the addition of bevacizumab (LumA) and a group that do not (LumB). These associations will be tested with the chi-square test.

Finally, in order to explore the degree to which the best Ki67 cutpoint depends on subtype, we may also try to define cutpoints within each subtype separately.

4.0 References

1. Perou, C.M., et al., Molecular portraits of human breast tumours. Nature, 2000. 406(6797): p. 747-52.

2. Sorlie, T., et al., Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences of the United States of America, 2001. 98(19): p. 10869-74.

3. Sorlie, T., et al., Repeated observation of breast tumor subtypes in independent gene expression data sets. Proceedings of the National Academy of Sciences of the United States of America, 2003. 100(14): p. 8418-23.

4. Nielsen, T.O., et al., Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clinical Cancer Research, 2004. 10(16): p. 5367-74.

5. Makretsov, N.A., et al., Hierarchical clustering analysis of tissue microarray immunostaining data identifies prognostically significant groups of breast carcinoma. Clinical Cancer Research, 2004. 10(18 Pt 1): p. 6143-51.

6. Rodriguez-Pinilla, S.M., et al., Prognostic significance of basal-like phenotype and fascin expression in node-negative invasive breast carcinomas. Clin Cancer Res, 2006. 12(5): p. 1533-9.

7. Arpino, G., et al., HER-2 amplification, HER-1 expression, and tamoxifen response in estrogen receptor-positive metastatic breast cancer: a southwest oncology group study. Clinical Cancer Research, 2004. 10(17): p. 5670-6.

8. Ellis, M., et al., Letrozole is more effective neoadjuvant endocrine therapy than Tamoxifen for ERB-1 and/or Erb-2 positive, estrogen receptor-positive primary breast cancer: Evidence from a Phase III randomized trial. J Clin Oncol, 2001. 19: p. 3808-3816.

9. Usary, J., et al., Mutation of GATA3 in human breast tumors. Oncogene, 2004. 23(46): p. 7669-78.

10. Callagy, G.M., et al., Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. Clin Cancer Res, 2006. 12(8): p. 2468-75.

11. Paik, S., et al., A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. New England Journal of Medicine, 2004. 351(27): p. 2817-26.

12. Dai, H., et al., A cell proliferation signature is a marker of extremely poor outcome in a subpopulation of breast cancer patients. Cancer Res, 2005. 65(10): p. 4059-66.

PART D

The Impact of PIK3CA Mutation on the Efficacy of Bevacizumab

1.0 BACKGROUND

PIK3CA encodes p110, the predominant isoform of the catalytic subunit of Class 1A phosphatidylinositol 3-kinase (PI3K), a lipid phosphokinase. The family of PI3Ks provides signaling for diverse cellular functions including proliferation, metabolism, migration, polarity, energy expenditure, translation, apoptosis avoidance and angiogenesis (for review [1,2]). Over the past several years it has been shown that disruption of this normally tightly regulated pathway by gene loss (PTEN), mutation (PIK3CA or less commonly PIK3R1) or amplification (PIK3CA) is one of the most common alterations occurring in human cancers. PIK3CA mutations result in constitutive activation of p110α which in turn increases lipid kinase activity resulting in an increase in activated Akt [3-5]. Cultured cells that express the PIK3CA mutations commonly identified in human tumors do not undergo growth arrest when starved of growth factors, demonstrate increased angiogenesis, acquire features of cellular transformation, and are resistant to cell death [3-9]. In breast cancer cells ligand-independent proliferation by activated-Akt results in anti-estrogen insensitivity [10]. Activated Akt has been shown to correlate with decreased overall survival in tamoxifen treated patients [11]. In addition, deregulation of the PI3K/Akt pathway through either loss of PTEN or through increased PIK3CA activity has been shown to enhance angiogenesis and promote tumor invasiveness in animal models [6, 9, 12-14]. In human ovary cancer PIK3CA is commonly amplified [15], and over-expression of p110(correlates with increased VEGF expression and increased microvascular density [16]. A correlation between high PIK3CA expression and increased proliferation and decreased apoptosis was also identified.

Mutation of PIK3CA is noted in 8-40% of human breast cancers, and in larger studies the average incidence is 26 - 35% [15, 17-21]. The majority of mutations in breast cancer occur at two hotspots; at exon 9, which encodes the helical domain, the mutations involve codons 542 or 545 and at exon 20, which encodes the kinase domain, the mutation involves codon 1047 [18-20]. These three missense mutations comprise > 85% PIK3CA mutations, however many reports often limited the analysis for mutation to the exon 9 and exon 20 hotspot locations. In several larger studies where the entire coding region and intron-exon boundaries were analyzed, missense mutations in other regions of the protein occurred in up to 18% of breast cancers [19, 20]. A recent study confirmed the finding that 19% of PIK3CA mutations in breast cancer occur at non-hotspot codons and many of these rare mutations (0.6-4.2% in breast cancer) confer a gain of function as measured by lipid kinase activity, cellular transformation, and constitutive activation of Akt [3]. In addition, a range of oncogenic potency was ascribed to the different missense mutations. Whether different PIK3CA mutations confer differences in clinical phenotype is not known, but given that these low incidence mutations collectively comprise up to 19% of somatic PIK3CA mutations in breast cancer, further analysis should be considered. There has been discrepancy as to the prognostic implication of a PIK3CA mutation, where studies have shown no correlation to relapse or survival [20], decreased overall survival [18] and an improved RFS [19]. Further clarification regarding the prognostic impact of a PIK3CA mutation may become evident through mutation site stratification.

The majority of retrospective studies assessing the incidence of PIK3CA mutation in breast cancer report a positive correlation between PIK3CA mutation and hormone receptor positive (HR+) [18-20]. In a retrospective analysis of the incidence of PIK3CA mutation status in 98 invasive breast cancers performed at MSKCC a positive correlation with nuclear steroid receptor positive breast cancers and mutated PIK3CA was identified, whereby 85% of PIK3CA mutated breast cancers were ER, PR or androgen receptor (AR) positive [22]. The significant association

of AR positivity and PIK3CA mutation was particularly striking in a subset of ER negative, AR positive tumors, whereby 80% were PIK3CA mutated (p=0.013). In addition, the exon 20 hotspot mutation located in the kinase domain of PIK3CA was over-represented in the ER negative, AR positive tumors as compared to the exon 9 PIK3CA mutations (unpublished data). Historically, the majority of ER positive tumors are also AR positive; however it is currently not known whether the significant association of AR positivity and PIK3CA mutation extends to ER/PR positive breast cancers.

It is hypothesized that in HR+ invasive breast cancer somatic PIK3CA mutation imparts a multi-faceted growth advantage including resistance to anti-estrogen therapy, stimulation of angiogenesis and increased invasiveness. Inhibition of multiple targets may be needed therapeutically to uncouple these growth advantages and counteract resistance to anti-estrogen therapy. There has been no prospective analysis on the clinical and biologic impact of a PIK3CA mutation. We are proposing an assessment of PIK3CA mutation status to assess the clinical correlates on the treatment of HR+ recurrent breast cancer, particularly as it relates to combined anti-estrogen and anti-angiogenesis therapy. Inhibitors of PI3K and mTOR pathways can reverse many of the effects of the mutant proteins, as demonstrated by decreased pAkt and p70S6K, decreased transformation and diminished angiogenesis [5, 7, 8, 12, 13]. Although we anticipate that bevacizumab therapy will reverse the enhanced angiogenesis induced by a PIK3CA mutation and result in a longer PFS, other pAkt tumor promoting effects will not be affected and therefore we expect the benefit of combined therapy to be modest.

It is anticipated that correlative science studies will improve clinical benefit by biologically defining clinical populations that will benefit from multi-targeted therapy. Clinical trials assessing PI3K inhibitors either alone or in combination with anti-estrogen and anti-angiogenesis therapy will be a near future consideration given the common finding of PIK3CA mutation in HR+ breast cancer. In addition to determining the clinical relevance of a PIK3CA mutation, we plan to explore biologic correlates that the mutation imparts. Predictors of response to angiogenesis inhibitors have not been fully identified in treated populations [23]. Recently, a correlation was observed with an increase in circulating endothelial and progenitor cells observed in patients treated with a pan-VEGFR inhibitor following tumor progression and treatment interruption [24]. It is expected that a somatic PIK3CA mutation in breast cancer will correlate with markers of increased angiogenesis as was shown with PIK3CA over-expression in ovary cancer. However, tumor angiogenesis through deregulated HIF-1(α and over-expression of VEGF and other vascular mediators occurs through a variety of oncogenes and tumor suppressors [25]. We will correlate PIK3CA mutation status with VEGF expression as measured by quantitative real time RT-PCR and VEGF immunostaining, microvessel density as measured by CD31 immunostaining, and quantitation of circulating CECs and CTCs (interaction with Appendix I, Part A, performed at UCSF, CA). Changes in cell polarity, along with increased migration and invasiveness as can be identified with increased Akt activation may be observed in cells expressing mutated PIK3CA.

2.0 OBJECTIVES

2.1 To determine the clinical correlates of a PIK3CA mutation in HR+ recurrent breast cancer with regard to time to progression with combined anti-estrogen and bevacizumab therapy as compared to anti-estrogen therapy alone.

2.2 To examine biologic correlates associated with PIK3CA mutations. These include activation of angiogenesis assessed by tumor VEGF expression measured by IHA and quantitative real time RT-PCR and IHA of CD31 for tumor microvascular density, enhanced migration through quantitation of circulating tumor and endothelial cells (CTCs and CECs) (Interaction with Appendix I, Part A, performed at UCSF, CA), correlation with AR expression and assessment of a differential effect dependent on helical versus kinase versus non-hotspot PIK3CA mutation site.

3.0 METHODS

The Beene Translational Oncology Core Facility, under the direction of Adriana Heguy, PhD., within the Human Oncology and Pathogenesis Program (HOPP) at MSKCC has been established to provide state of the art genome-scale molecular profiling technologies. The Core will perform semi-automated high throughput nucleic acid (DNA and RNA) extraction from the CALGB Pathology core provided FFPE sections (2-3 5_m), whole genome amplification (WGA) of DNA extracted from these samples and fully automated high throughput PCR in 384 well plates will provide templates for PIK3CA exon mutation analysis. The PCR amplified fragments generated in the Core facilities will be sent for high volume sequencing of PIK3CA exons 1, 2, 3, 5, 7, 9, 12, 13, 18 and 20 with primers previously described [20] to outside contractors (Agencourt), resulting in lower operational costs due to high scale. The Core will also provide data analysis from the resulting sequences. Quantitative real-time RT-PCR for VEGF will be performed with previously described primers and probe [16] using the Sequenom platform located within the Molecular Pathology R&D Core. Sections of the tissue microarray constructed by Part C Investigators will be immunostained and scored for VEGF, CD31 and AR within the Pathology Core of HOPP at MSKCC. Statistical analysis will be performed as described in 14.7.4.

4.0 REFERENCES

1. Bader, A.G., et al., *Oncogenic PI3K deregulates transcription and translation.* Nat Rev Cancer, 2005. **5**(12): p. 921-9.
2. Engelman, J.A., J. Luo, and L.C. Cantley, *The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism.* Nat Rev Genet, 2006. **7**(8): p. 606-19.
3. Gymnopoulos, M., M.A. Elsliger, and P.K. Vogt, *Rare cancer-specific mutations in PIK3CA show gain of function.* Proc Natl Acad Sci U S A, 2007. **104**(13): p. 5569-74.
4. Ikenoue, T., et al., *Functional analysis of PIK3CA gene mutations in human colorectal cancer.* Cancer Res, 2005. **65**(11): p. 4562-7.
5. Kang, S., A.G. Bader, and P.K. Vogt, *Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic.* Proc Natl Acad Sci U S A, 2005. **102**(3): p. 802-7.
6. Bader, A.G., S. Kang, and P.K. Vogt, *Cancer-specific mutations in PIK3CA are oncogenic in vivo.* Proc Natl Acad Sci U S A, 2006. **103**(5): p. 1475-9.
7. Isakoff, S.J., et al., *Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells.* Cancer Res, 2005. **65**(23): p. 10992-1000.
8. Samuels, Y., et al., *Mutant PIK3CA promotes cell growth and invasion of human cancer cells.* Cancer Cell, 2005. **7**(6): p. 561-73.
9. Zhao, J.J., et al., *The oncogenic properties of mutant p110alpha and p110beta phosphatidylinositol 3-kinases in human mammary epithelial cells.* Proc Natl Acad Sci U S A, 2005. **102**(51): p. 18443-8.
10. Stoica, G.E., et al., *Effect of estradiol on estrogen receptor-alpha gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway.* Oncogene, 2003. **22**(39): p. 7998-8011.
11. Kirkegaard, T., et al., *AKT activation predicts outcome in breast cancer patients treated with tamoxifen.* J Pathol, 2005. **207**(2): p. 139-46.
12. Hu, L., J. Hofmann, and R.B. Jaffe, *Phosphatidylinositol 3-kinase mediates angiogenesis and vascular permeability associated with ovarian carcinoma.* Clin Cancer Res, 2005. **11**(22): p. 8208-12.
13. Su, J.D., et al., *PTEN and phosphatidylinositol 3'-kinase inhibitors up-regulate p53 and block tumor-induced angiogenesis: evidence for an effect on the tumor and endothelial compartment.* Cancer Res, 2003. **63**(13): p. 3585-92.
14. Wen, S., et al., *PTEN controls tumor-induced angiogenesis.* Proc Natl Acad Sci U S A, 2001. **98**(8): p. 4622-7.
15. Campbell, I.G., et al., *Mutation of the PIK3CA gene in ovarian and breast cancer.* Cancer Res, 2004. **64**(21): p. 7678-81.
16. Zhang, L., et al., *The oncogene phosphatidylinositol 3'-kinase catalytic subunit alpha promotes angiogenesis via vascular endothelial growth factor in ovarian carcinoma.* Cancer Res, 2003. **63**(14): p. 4225-31.
17. Buttitta, F., et al., *PIK3CA mutation and histological type in breast carcinoma: high frequency of mutations in lobular carcinoma.* J Pathol, 2006. **208**(3): p. 350-5.

18. Li, S.Y., et al., *PIK3CA mutations in breast cancer are associated with poor outcome.* Breast Cancer Res Treat, 2006. **96**(1): p. 91-5.

19. Maruyama, N., et al., *Clinicopathologic analysis of breast cancers with PIK3CA mutations in Japanese women.* Clin Cancer Res, 2007. **13**(2 Pt 1): p. 408-14.

20. Saal, L.H., et al., *PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma.* Cancer Res, 2005. **65**(7): p. 2554-9.

21. Samuels, Y., et al., *High frequency of mutations of the PIK3CA gene in human cancers.* Science, 2004. **304**(5670): p. 554.

22. Doane, A.S., et al., . *PIK3CA mutation is frequent in class A estrogen receptor-negative breast cancer and contributes to the distinct molecular profile*. in *SABC*. 2005:. San Antonio, TX.

23. Jain, R.K., et al., *Lessons from phase III clinical trials on anti-VEGF therapy for cancer.* Nat Clin Pract Oncol, 2006. **3**(1): p. 24-40.

24. Batchelor, T.T., et al., *AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients.* Cancer Cell, 2007. **11**(1): p. 83-95.

25. Ferrara, N. and R.S. Kerbel, *Angiogenesis as a therapeutic target.* Nature, 2005. **438**(7070): p. 967-74.

APPENDIX II

PHARMACOGENOMIC STUDIES FOR CALGB 40503

Pharmacogenomic Investigator:

Federico Innocenti, M.D. University of Chicago

Pharmacogenomic Studies

1.0 Background

The investigators hypothosize that the presence of common germ line polymorphisms in patients will be associated with differences in PFS among patients and serve as predictive markers of response.

There is increasing evidence which suggests that germ line polymorphisms related to anticancer therapeutics, metabolism, transport, and resistance correlate with drug response; furthermore, germ line polymorphisms related to therapeutic targets and/or therapeutic pathways might also help predict therapeutic outcomes [1-4].

1.1 Candidate gene approach

Assays for genetic variants will be performed for the *VEGF* (bevacizumab), *CYP2D6* (tamoxifen) and *CYP1A9* (aromatase) genes. These candidate genes have been chosen based on their potential influence on activation, degradation, transport disposition or cytotoxicity of study drugs.

Bevacizumab is a recombinant humanized monoclonal antibody to VEGF that blocks angiogenesis via neutralization of VEGF, and it has been shown to recognize and neutralize all VEGF isoforms. The regulatory region of *VEGF* contains many transcription factor binding sites and its transcriptional as well as translational regulation appears to be quite complex. Over the past few years several *VEGF* variants have been identified in the *VEGF* promoter and UTRs, and some of the variants have been associated with altered VEGF levels [5, 6]. In a few studies, *VEGF* polymorphisms have also been linked to altered disease risk and have been proposed as prognostic markers in breast cancer patients [6-8]. Given the fact that bevacizumab directly neutralizes VEGF, it is quite likely that *VEGF* variants which are associated with higher VEGF levels could influence the drug response to such a therapy. One such common variant (936C>T) in the 3'UTR of the *VEGF* gene has been associated with VEGF plasma levels such that the individuals with CC genotype had significantly higher VEGF levels than individuals with CT or TT genotypes, and about 71% of a Caucasian population studied carried the CC genotype [6]. We hypothesize that patients with significantly higher expression of VEGF might most likely benefit from anti-VEGF therapy. Furthermore, evaluating the role of other *VEGF* variants might also clarify any underlying influence that heritable differences in altered VEGF levels might have on breast cancer biology or treatment.

We will also genotype for variants in the *CYP2D6* and *CYP1A9* genes. Recent data are suggesting that the conversion of tamoxifen into endoxifen is an important step in the inhibitory properties of tamoxifen [9]. The formation of endoxifen is mediated by the CYP2D6 enzyme. CYP2D6 is highly polymorphic in the population, and it has been demonstrated that patients carrying nonfunctional CYP2D6 alleles (*4, *6, or *8) had lower endoxifen plasma concentrations than patients with functional alleles [10]. Preliminary data suggest that 5-year disease-free survival of patients who received tamoxifen in a previous trial was only 46% in patients homozygous for nonfunctional CYP2D6 variants compared to 83% in patients without them [11]. Moreover, a functional repeat polymorphism (TTTA in intron 4) of the *CYP1A9* gene has been associated with changes in aromatase activity, circulating estrogen levels, and breast cancer risk [12, 13]. Hence, genotyping of patients for common functional CYP variants is proposed in order to assess their effect on study outcomes.

1.2 Genome wide approach

In addition the investigation of the candidate gene variation and its association with treatment outcomes, DNA of patients extracted form peripheral blood will have used to scan their entire genome. Using genetic information collected from selected candidate genes has the disadvantage of relying on existing data regarding the role of those genes in the pharmacology of tamoxifen, aromatase inhibitors and bevacizumab. Novel high-density single-nucleotide polymorhphisms (SNP) platforms are now available to survey the pattern of variation of the entire genome of an individual, allowing the identification of genes that have not previously related to the pharmacology of the drugs of interest or to a certain biological pathway. This comprehensive genome-wide approach has the potential to lead to new discoveries of genes of clinical importance in pharmacogenomics.

Currently, platforms with hundreds of thousands of SNPs have been extensively used in case-control studies of cancer risk in germ line DNA of subjects [14]. These platforms do not only provide information of the SNP pattern of a individual, but also on the quantitative pattern on copy number variation (including loss of heterozygosity, LOH). Recent genome-wide association studies (GWAS) have also discovered novel genes of treatment outcome to chemotherapy, including our GWAS on CALGB 80303 [15, 16].

The present study is relatively large and randomized, and a genome-wide investigation is in the perfect position to indentify new genes (additional to the candidate genes) that are associated with the treatment outcomes. Ultimately, subsets of patients with better response and/or less risk of toxicity can be indentified based upon their genetic makeup.

2.0 Objectives

2.1 To conduct pharmacogenomic assessment of candidate variants in the VEGF, CYP2D6 and CYP19 genes and evaluate their association with PFS and other study outcomes.

2.2 To identify SNPs associated with progression free survival in the genome-wide approach (GWAS).

3.0 Methods

One blood sample will be obtained from all patients enrolled in the study for pharmacogenetic evaluation. Investigation of the relevant polymorphisms will take place in germ line DNA extracted from peripheral whole blood (10 ml) collected in an EDTA (lavender) Vacutainer tube. Blood will be sent to the Alliance Pathology Coordinating Office (PCO) (see Sections 6.2 and 6.2.2) for DNA extraction. DNA quality will be assessed by UV spectrophotometry and by agarose gel electrophoresis. All DNA samples will be stored at the PCO until they are distributed to the appropriate laboratory for analysis. Phenotypic data will be extracted from the CALGB database by the CALGB statistical group.

3.1 Candidate gene approach

For genotyping variants in the VEGF, CYP2D6, and CYP1A9 genes, a standard procedure will be used for primer design for all genotype assays developed. All primers will be designed for the gene and/or variant of interest using the Oligo Primer Analysis Software (Molecular Biology Insights, http://www.oligo.net). The specificity and optimization of all primers will be determined using the BLAST algorithm from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the BLAT algorithm [17]. All primer sequences will also be carefully checked to ensure they do not encompass SNP locations. Accurate quantification of all DNA samples to be genotyped will be performed by use of a picogreen-based assay followed by normalization of DNA concentration in 96

or 384 well plates. Since many genotyping assays are now based on clustering (e.g. TaqMan and Invader), the normalization of DNA concentration provides more definitive clustering and reduces the number of repeats necessary on a plate-wide and individual genotype basis. For all genotype assays performed, appropriate controls for each genotype will also be included to ensure the assay is performing optimally.

A number of different methods of SNP genotyping will be used. For optimal efficiency, the primary method of genotyping to be used for this study will be single base extension (SBE) with separation of extension products by denaturing high performance liquid chromatography (DHPLC). This method has proven more robust and reliable over other genotyping methods, and for some SNPs was the only genotyping method that was successful. SBE-DHPLC is more cost-effective compared to some of the other methods of genotyping, as the extension primers used are not fluorescently labeled. In addition, as all four ddNTPs are included in the SBE reaction and, in the rare cases of tri-allelic or even tetra-allelic SNPs, all different alleles will be detected by this method. This method is suitable for medium-scale genotyping projects (up to ~ 500 samples) and for the sample size proposed in this project is an ideal method for genotyping.

The principle of SBE-DHPLC is as follows: DNA sequence fragments that differ at a single base pair position can be distinguished on the DHPLC due to the differing hydrophobicities of different base pairs that can cause a change in their elution profile [18]. This characteristic is taken advantage of in the SBE-DHPLC genotyping methodology [19]. SBE-DHPLC is performed by an initial amplification by PCR of the DNA fragment that contains the SNP to be genotyped followed by an extension reaction using an oligonucleotide that acts as an extension primer. The SBE primer is annealed downstream or upstream immediately adjacent to the SNP to be genotyped in the 5' to 3' direction. Thermosequenase extends the 3' end of the extension primer with the appropriate ddNTP. The primer extends one base only because the ddNTP terminates further extension. Extended products are separated on the DHPLC based on the hydrophobicity of the last base, so although the lengths of the extended products are the same for different alleles, the hydrophobicity of the extended products of each allele will be different. For each SNP to be genotyped, appropriate controls representative of the different genotypes will be used. Duplex SBE reactions will be also performed, where two SNPs are genotyped simultaneously and run together on the DHPLC. This allows for increased throughput and also decreases the cost of genotyping.

Other methodologies might be also taken into consideration, such as SBE with fluorescent polarization [20], SBE with detection by capillary electrophoresis (SnaPShot) (Applied Biosystems, Inc.) and the Invader method (Third Wave Technologies, Inc.).

Genotyping of indel gene variants will be performed by PCR and sizing by capillary electrophoresis, followed by analysis using the Genemapper genotyping software (Applied Biosystems, Inc.). Appropriate controls that represent the different genotypes will be used.

For all the variants tested, the presence of deviation for the Hardy-Weinberg equilibrium will be calculated by using Arlequin software 2.0.

3.2 Genome-wide approach

Aliquots of DNA will be sent to the Riken Institute for the whole-genome analysis. For the whole-genome analysis, the genotyping will be performed at the laboratory of Dr. Yusuke Nakamura and Dr. Hitoshi Zembutsu at the Riken SNP Research Center and the University of Tokyo Human Genome Center, Japan. In the Nakamura/Zembutsu laboratory, the current plan is that each DNA sample will be analyzed by two platforms. The first platform is the Illumina HumanHap550 Genotyping BeadChip for genome-wide screening and the analysis will be performed according to the recommended Illumina protocol. Each Illumina

HumanHap550 chip requires 750 ng of DNA and additional sample will be used for repeat assays where necessary.

Illumina's HumanHap550 Genotyping BeadChip enables whole-genome genotyping of over 555,000 single nucleotide polymorphism (SNP) loci efficiently and accurately on a single BeadChip. The HumanHap550 BeadChip is powered by the Infinium™ II assay, which uses a single-tube, whole-genome amplification method that does not require PCR and enables intelligent SNP selection using tagSNPs. TagSNPs are loci that can serve as proxies for many other SNPs. The use of tagSNPs greatly improves the power of association studies, as the same information and power from a larger number of SNPs can be gathered by genotyping only a subset of loci. TagSNPs on the HumanHap550 BeadChip were selected from the recently completed International HapMap Project.

The second platform that will be used by the RIKEN investigators is the combination of Invader assays with multiplex-PCR for target SNP genotyping. More than 7,000 variants in 267 possible drug-related genes can be genotyped using re-established assays developed in Dr. Nakamura's lab [21]. The number of variants to be genotyped with the Invader assays might be less than 7,000 due to redundancy with the coverage in the Illumina platform.

4.0 References

1. DeMichele, A., et al., Drug-metabolizing enzyme polymorphisms predict clinical outcome in a node-positive breast cancer cohort. J Clin Oncol, 2005. 23(24): p. 5552-9.
2. Stanulla, M., et al., Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. JAMA, 2005. 293(12): p. 1485-9.
3. Villafranca, E., et al., Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. J Clin Oncol, 2001. 19(6): p. 1779-86.
4. Dall'Ozzo, S., et al., Rituximab-dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship. Cancer Res, 2004. 64(13): p. 4664-9.
5. Renner, W., et al., A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. Journal of Vascular Research, 2000. 37(6): p. 443-8.
6. Krippl, P., et al., A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. International Journal of Cancer, 2003. 106(4): p. 468-71.
7. Lee, S.J., et al., Vascular endothelial growth factor gene polymorphisms and risk of primary lung cancer. Cancer Epidemiology, Biomarkers and Prevention, 2005. 14(3): p. 571-5.
8. Lu, H., et al., Association of genetic polymorphisms in the VEGF gene with breast cancer survival. Cancer Res, 2005. 65(12): p. 5015-9.
9. Jin, Y., et al., CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst, 2005. 97(1): p. 30-9. Jin, Y., et al., CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst, 2005. 97(1): p. 30-9.
10. Stearns, V., et al., Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst, 2003. 95(23): p. 1758-64.
11. Garber, K., Tamoxifen pharmacogenetics moves closer to reality. J Natl Cancer Inst, 2005. 97(6): p. 412-3.
12. Gennari, L., et al., A polymorphic CYP19 TTTA repeat influences aromatase activity and estrogen levels in elderly men: effects on bone metabolism. Journal of Clinical Endocrinology and Metabolism, 2004. 89(6): p. 2803-10.
13. Kristensen, V.N., et al., Genetic variants of CYP19 (aromatase) and breast cancer risk. Oncogene, 2000. 19(10): p. 1329-33.
14. Easton, D.F., Eeles, R.A, Genome-wide associations in cancer. Hum Mol Gent. 2008. 17(R2): p. R109-15.
15. Yang, J.J., et al., Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. Jama, 2009. 301(4): p. 393-403.

16. Innocenti, F., et al., Heritable interleukin-17F(IL17F) gene variation is associated with overall survival (OS) in pancreatic cancer patients (pts): results from genome-wide association study (GWAS) in CALGB 80303. Proc ASCO 2009

17. Kent, W.J., et al., The human genome browser at UCSC. Genome Res, 2002. 12(6): p. 996-1006.

18. Oefner, P.J., Allelic discrimination by denaturing high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl, 2000. 739(2): p. 345-55.

19. Devaney, J.M., et al., Genotyping of two mutations in the HFE gene using single-base extension and high-performance liquid chromatography. Anal Chem, 2001. 73(3): p. 620-4.

20. Chen, X., L. Levine, and P.Y. Kwok, Fluorescence polarization in homogeneous nucleic acid analysis. Genome Res, 1999. 9(5): p. 492-8.

21. Iida, A., et al., Japanese single nucleotide polymorphism database for 267 possible drug-related genes. Cancer Sci, 2006. 97(1): p. 16-24.

APPENDIX III

Procedure for Calculating a urine protein/creatinine (upc) ratio

1) Obtain at least 4mL of random urine sample (does not have to be a 24 hour urine).

2) Determine protein concentration (mg/dL).

3) Determine creatinine concentration (mg/dL).

4) Divide #2 by #3 above:
   Urine protein/creatinine ratio = protein concentration (mg/dL) / creatinine concentration (mg/dL).

The UPC ratio directly correlates with the amount of protein excreted in the urine per 24 hrs (i.e. a UPC of 1 should be equivalent to 1g protein in a 24 hr urine collection). The UPC ratio can be used in place of a 24 hr urine collection.

Protein and creatinine concentrations should be available on standard reports of urinalysis, not dipsticks. If protein and creatinine concentrations are not routinely reported at an Institution, their measurements and reports may need to be requested.

APPENDIX IV

New York Heart Association classification for congestive heart failure

Class	Definition
I	No limitation: Ordinary physical activity does not cause undue fatigue, dyspnea, or palpitation.
II	Slight limitation of physical activity: Such patients are comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea, or angina.
III	Marked limitation of physical activity: Although patients are comfortable at rest, less than ordinary physical activity will lead to symptoms.
IV	Inability to carry on physical activity without discomfort: Symptoms of congestive heart failure are present even with rest. With any physical activity, increased discomfort is experienced.

APPENDIX V

UNDERSTANDING PATIENT CHARACTERISTICS ASSOCIATED WITH RISK OF TOXICITY TO BEVACIZUMAB AND HORMONAL THERAPY AND UNDERSTANDING LONGITUDINAL CHANGES IN PHYSICAL STATE, COMORBID MEDICAL CONDITIONS AND PSYCHOLOGICAL STATE WHILE ON TREATMENT

1.0 Background

Among patients of the same chronological age, there is wide heterogeneity in physical and psychological functioning. A brief, comprehensive measure is needed that can help characterize the "functional age" of a patient, in order to optimize treatment decisions and stratify outcomes based on factors other than chronological age. Such a measure has been developed in the CALGB Cancer in the Elderly Committee [1] and includes an evaluation of the following domains: functional status, comorbid medical conditions, cognition, nutritional status, psychological status, and social support, each of which is an independent predictor of morbidity and mortality. This correlative study offers the opportunity to identify which of these domains predicts the risk of grade 3, 4, or 5 toxicity with bevacizumab and endocrine therapy and to establish longitudinal changes in these domains while on therapy.

1.1 Domains of assessment

The essential components of the assessment will be reviewed below.

1.1.1 Functional status

Functional status is traditionally assessed in oncology clinical trials by rating a patient's Karnofsky Performance Status (KPS) or Eastern Cooperative Oncology Group (ECOG) Performance Status. These brief assessments predict treatment morbidity and mortality regardless of age; however, they do not provide details regarding the impact of functional decline on everyday activities required to maintain independence at home or in the community. In this study, in addition to rating the patient's KPS we will evaluate the patient's ability to complete activities of daily living (ADLs) and instrumental activities of daily living (IADLs). ADLs are basic self-care skills needed to maintain independence at home; IADLS are skills needed to maintain independence at home and in the community. These scales provide information about physical functioning above and beyond that provided by the Karnofsky or ECOG performance status. In a study of patients with cancer with a good ECOG performance status (defined as < 2), 37.7% had limitations in IADLs and 9.3% had limitations in ADLs [2]. Functional limitations in ADLs and IADLs predict morbidity and mortality [3, 4]. In a study of 566 patients age 70 and older with non–small cell lung cancer, those who did not require assistance in performing instrumental activities of daily living had a better overall survival than patients who did require assistance (P=.04) [5].

1.1.2 Comorbid Medical Conditions

Comorbid medical conditions are concurrent medical problems that are a competing source of morbidity or mortality. The number of comorbid medical conditions increases as one ages and adversely impacts on projected life expectancy [6]. A thorough understanding of comorbid medical conditions is important in order to: 1) determine whether another competing cause of mortality will limit an individual's life expectancy more than the cancer and 2) consider the impact of these co-existing medical problems

on the patient's ability to tolerate treatment. In a study by Extermann and colleagues of 203 patients with cancer (median age 75; range 63-91), there was low correlation between comorbidity and functional status [7]. Therefore, each is an important domain to assess.

1.1.3 Nutrition

Poor nutritional status, defined as a body mass index < 22kg/m^2, is associated with increased dependence in activities of daily living (odds ratio 1.21; 95% CI 1.01-1.45) and decreased one year survival [RR 0.85 (95% CI 0.74-0.97)] [8]. In addition, unintentional weight loss is associated with lower chemotherapy response rates and decreased performance status [9]. Weight loss of 5% or greater is associated with an increased risk of mortality (Hazard ratio = 1.67, 95% CI = 1.29-2.15) [10].

1.1.4 Cognition

The presence of dementia is an independent prognostic indicator of survival [11, 12]. A baseline assessment of cognition is important in order to rule out subtle findings of metastatic disease and to determine if a patient needs additional assistance to participate in a complex treatment plan. A caregiver can be essential in maintaining safety by ensuring adherence to the treatment plan.

1.1.5 Psychological State and Social Support

Social isolation is an independent predictor for mortality [13]. Studies demonstrate that, in general, older patients with cancer experience similar or less psychological distress than younger patients [14, 15]. However, older individuals who are most vulnerable to psychological distress are usually socially isolated [16]. Depression in the geriatric population is associated with functional decline and increased need for informal care giving [17, 18]. From a practical standpoint, because patients with cancer are more likely to require functional assistance, an assessment of social support is essential to determine healthcare needs and to plan for the resources required during cancer therapy.

1.1.6 Medication Review

Age-related changes in physiology may affect a drug's pharmacokinetics and pharmacodynamics, placing the patients at increased risk for adverse drug events [19, 20]. During cancer therapy, several medications in addition to the cancer therapy are often prescribed such as antiemetics or other supportive-care medications. In addition to these medications for the cancer therapy, individuals are often already taking several prescribed medications as well as over-the-counter drugs, leading to a risk for drug interactions [19, 21, 22].

2.0 OBJECTIVES

2.1 Primary objective

To identify factors other than chronological age that predict the risk of grade 3, 4, or 5 toxicity in patients receiving endocrine therapy with bevacizumab. The factors to be studied include:

a) OARS MFAQ (IADL)

b) MOS Physical Functioning

c) Karnofsky Performance Status Rated Healthcare Professional

d) Timed "Up and Go"

e) OARS Physical Health Section

2.2 Secondary objectives

2.2.1 To perform an exploratory analysis of whether other factors included in patient assessments (either individually or in combination) predict the risk of grade 3, 4, or 5 toxicity in patients receiving endocrine therapy with bevacizumab.

2.2.2 To compare the associations of baseline factors to grade 3, 4, or 5 toxicity in patients receiving endocrine therapy with and without bevacizumab.

2.2.3 To explore whether longitudinal changes in factors are in association with the occurrence of grade 3, 4, or 5 toxicities in patients receiving endocrine therapy with bevacizumab.

3.0 METHODS

3.1 Institutional nurse and CRA training

Nurses and/or CRA who will administer the assessments must contact Dr. Hurria (626-256-4673) to review the assessment via telephone conference, prior to performing the first patient assessment.

3.2 The instrument used to measure these domains is the CALGB 40503 Functional Age Assessment Measure Health (Care Professional) C-1776 and the CALGB 40503 Functional Age Assessment Measure (Patient Questionnaire) C-1777, developed by the Cancer in the Elderly Committee. In this protocol we will apply the assessment to patients of all ages. The components of that instrument are:

Background Information

Functional Status:

a) OARS MFAQ (IADL) [23, 24]
b) MOS Physical Functioning [25]
c) Karnofsky Performance Status Rated Healthcare Professional [26]
d) Karnofsky Performance Status Rated by Patient [27]
e) Timed “Up and Go” [28]
f) Number of falls in last 6 months

Comorbidity:

OARS Physical Health Section [23]

Medication Review:

Patient reports number and names of medications, herbs, or vitamins

Cognition:

Blessed Orientation-Memory-Concentration Test* [29]

Psychological Status:

Mental Health Inventory [30]

Nutritional Status:

a) % Unintentional Weight Loss in last 6 months
b) Body Mass Index

Social Functioning and Social Support:

a) MOS Social Activity Limitation Scale [25]
b) Medical Outcomes Study (MOS) Social Support Survey Subscale [25, 31]

*If a patient has a Blessed Orientation-Memory-Concentration score ≥ 11 (suggesting gross cognitive impairment), the treating physician should be notified so that further evaluation or intervention can be performed as deemed medically appropriate by the treating physician. Patients must be able to understand and follow directions in English due to the lack of availability of the instruments in other languages.

3.3 Assessment Procedures

Patients will undergo a full informed consent process and patients who agree to participate in this study will undergo the assessment. This consists of a two questionnaires which will be completed: 1) prior to initiation of protocol therapy; 2) restaging time points 1 (after 3 cycles) and 2 (after 6 cycles) and every other restaging thereafter (ie, restaging time point #4, #6, #8, etc…); and 3) and on the last day of treatment or up to 1 month later, but prior to the start of a new treatment. The Blessed Orientation-Memory-Concentration test only needs to be completed prior to initiation of protocol therapy and end of treatment.
The reason and time points for missing data will be captured.

3.3.1 Form C-1776 Healthcare Professional Questionnaire

Three items will be administered by a member of the enrolling institutions healthcare team (physician, RN, or CRA). This individual will also calculate the patient's body mass index and % weight loss in the last 6 months. These items are described below:

1) The Blessed Orientation-Memory-Concentration test (only needs to be completed prior to initiation of protocol therapy and end of treatment) provides an objective measure of cognition [29]. If patients score above a certain score (≥ 11), signifying possible cognitive impairment, then the remainder of the self-administered assessment would be considered unreliable and the self-reported data will not be used in the final data analysis. If a patient scores ≥ 11 on the Blessed O-M-C test, the patient's treating physician will be informed so that further evaluation or intervention can be performed as deemed medically appropriate by the treating physician.
2) The Timed Up and Go is a timed performance-based measure of functional status [28]. Patients rise from a seated chair, walk 10 feet, turn, and return to the chair and sit down. This provides an objective assessment of the patient's functional status in comparison to a subjective assessment provided through the patient-rated functional status questionnaires.
3) The Karnofsky Performance Status is completed by a trained member of the enrolling institution's healthcare team [26].

3.3.2 Form C-1777 Patient Questionnaire

This questionnaire is self-administered and completed by the patient. Patients who cannot complete the assessment on their own will receive assistance by a member of the enrolling institution's healthcare team. The reason why a patient requires assistance will be noted on the form.

3.4 Description of Measures

3.4.1 Functional Status

a) Instrumental Activities of Daily Living (IADL):

[Subscale of the Multidimensional Functional Assessment Questionnaire (MFAQ)]: Older American Resources and Services (OARS) [23]

The OARS MFAQ was developed to provide a profile of the level of functioning and need for services of older persons who live at home but may have some degree of

impairment. The MFAQ has been tested in over 6,000 older community residents. The IADL subscale consists of 7 questions rated on a 3-point Likert scale measuring degree to which an activity can be performed independently. Five week test-retest correlation is 0.71 for the IADL subscale.23

b) Activities of Daily Living:

[Subscale of Medical Outcomes Study (MOS) Physical Health] [25]

The MOS Physical Health Scale measures a broad range of physical functioning, with questions ranging from "Can you bathe and dress yourself?" to "Can you perform vigorous activities, such as running or lifting heavy objects?" Items are rated on a 3-point Likert scale measuring independence in performing the activity. Internal consistency of the physical function score is 0.92 [25].

c) Karnofsky Performance Rating Scale (Healthcare Professional Rating)

The Karnofsky performance status is a general measure of patient independence in carrying out normal activities and self-care needs. The scale, developed in 1948, has been widely used in the evaluation of cancer patients. Patients are given a score on a numerical scale of 0-100 as a global indicator of functional status. There is a moderate degree of inter-rater reliability between nurse and social worker KPS ratings with a pearson correlation of 0.69 ($p<0.001$). In terms of validity, KPS most strongly correlates with variables related to physical functioning [difficulty with stair, difficulty with balance; pearson correlation 0.63 ($p<0.001$) and 0.61 ($p<0.001$) respectively] [26, 32]

d) Karnofsky Self-Reported Performance Rating Scale

A self-reporting version of the Karnofsky performance scale was developed to assess the patients' perception of their own performance status. With this scale, patients rate their own functional status and choose from a range of functioning from "able to carry out normal activities requiring no assistance" to "severely disabled, requiring continuous nursing care." Among patients with cancer participating in clinical trials, the patient rated KPS was significantly related to survival ($p < 0.05$) and provided information independent from that obtained by the physician scored performance status [27].

e) Timed Up and Go

The "Timed Up & Go" is a performance test of physical mobility. The test measures how many seconds it takes for an individual to stand up from a standard arm-chair (approximate seat height of 46 cm), walk a distance of 3 meters (10 feet), turn, walk back to the chair, and sit down again. The test was originally reported by Mattias and colleagues, and subsequently modified by Podsiadlo and colleagues to be a timed test. In a population of frail, community dwelling older adults, there was good inter-rater and intra-rater reliability (intraclass correlation coefficient 0.99 for both). The timed "Up and Go" score correlated to the scores on the Berg Balance Scale ($r = -0.72$), gait speed ($r = -0.55$) and Barthel Index of ADL ($r = -0.51$) [28]. Guralnik and colleagues reported gait speed as an important predictor of disability [33].

f) Number of Falls in Last 6 Months

Older patients are at risk for falls because of limited mobility, gait, and balance impairments [34, 35]. Falls may place patients with cancer at greater than average risk for injury because bony metastases place them at risk for a pathologic fracture. Patients receiving anti-angiogenic agents may be at greater risk of hemorrhage. Patients will be asked to report their number of falls in the last 6 months.

3.4.2 Comorbidity

Physical Health Section

[Subscale of The Older American Resources and Services Questionnaire (OARS)]:

The OARS Physical Health Section is a comorbidity scale that contains a list of current illnesses and conditions an individual might have, and the degree to which they impair daily activities, rated on a 3-point scale of "not at all" to "a great deal." A list of current medications is also recorded. Test-retest reliability for the Physical Health subscale over five weeks was .66. In terms of validity, the Physical Health subscale correlated significantly with health professional ratings (Kendall's tau coefficients = 0.75) [23]. A modified shorted version of the OARS will be used in this protocol.

3.4.3 Cognition

Blessed Orientation-Memory-Concentration Test

The BOMC consists of 6 questions designed to screen for gross cognitive impairment. A score > 11 signifies cognitive impairment. The test-retest reliability is high (Spearman Rank Correlation 0.96; $p < 0.001$) [29]. The BOMC has excellent validity as a screening instrument, correlates highly with clinicians' ratings of dementia severity (r=0.89), predicts results from a longer (26 item) mental status questionnaire, and discriminates between patients with mild, moderate, and severe cognitive deficits [36].

3.4.4 Nutritional Status

a) Percentage of Unintentional Weight Loss in Last 6 Months

The prognostic effect of unintentional weight loss in patients with cancer was evaluated in a study of 3,047 patients enrolled in Ester Cooperative Oncology Group chemotherapy trials [9]. Weight loss during the 6 months prior to chemotherapy was associated with poorer survival (statistically significant in 9 out of 12 tumor types). In addition, weight loss was associated with lower chemotherapy response rates (significant only in patients with breast cancer). Decreasing weight correlated with decreased performance status in all tumor types except pancreatic and gastric cancer [9].

The following is the calculation for the percentage of unintentional weight loss: Percentage of unintentional weight loss = (weight loss in last 6 months/body weight 6 months ago) x 100.

b) Body Mass Index (BMI)

In a prospective cohort study of 214 older community-dwelling adults, a low body mass index, defined as a body mass index ‹ 22 kg/ m2 was associated with dependency in activities of daily living (odds ratio 1.21; 95% CI 1.01-1.45). After adjusting for potential confounding factors including age, gender, mental status, comorbidity and functional dependency, body mass index ‹ 22 kg/m^2 was associated with decreased one year survival [RR 0.85 (95% CI 0.74-0.97)] [8].

Body mass index is calculated by the following equation: Body mass index = $weight/(height)^2$.

3.4.5 Psychological Status

a) Rand Mental Health Inventory-17 [30]

The Mental Health Inventory (MHI) (Revised General Well-Being Scale) is based upon the General Well-Being Scale, developed by Dupuy for the National Health Interview

Survey, and was included in a battery of health measures in the Rand Health Insurance Study [37]. The full length MHI consists of 38 items grouped into the following five subscale and three global scores: anxiety, depression, general positive affect, emotional ties, and loss of behavioral emotional control, Psychological Distress (negative affect), Psychological Well-Being (positive affect), and the MHI total score. The MHI has community norms, based upon 5,000 respondents from six communities [37]. In order to reduce respondent burden, a 17 item version of the MHI will be used, which will yield three global scores of Psychological Distress, Psychological Well-Being and MHI total score, as in the original 38 item MHI [30]. The MHI-18 had been developed and tested, and detected differences between medical and psychiatric patients to a similar degree [38]. One item of the MHI-18 has been removed by its developers because of its questionable content as a positive affect item, reducing the scale to the MHI-17. The MHI-17 has an excellent internal consistency (alpha coefficient=.96) [30].

3.4.6 Social Functioning and Social Support

a) Medical Outcomes Study (MOS) Social Activity Limitations Measure

The impact of cancer on patients' social functioning will be assessed by the Social Activity Limitations scale from the Medical Outcome Study (MOS) [25]. As with all MOS measures, the Social Activity Limitations scale was developed from a national sample of medically ill patients being treated in outpatient facilities. The 4-item scale includes the extent to which physical or emotional problems have interfered with their social activities. All items are rated on a 5-point Likert scale, with response categories varying with each item. Internal consistency was good (alpha coefficient = .77). The scale correlates significantly with a range of measures: role limitations due to physical (r = .52) and emotional (r = .49) health, psychological distress (r = .64) and pain 9r = .55). The mean of the total score is transformed to a scale of 0-100, with a higher number indicating greater support.

b) Medical Outcomes Study (MOS) Social Support Survey Emotional/ Information and Tangible Subscales

This is a 20-item measure of perceived availability of social support, with four subscales: emotional/informational, tangible, affectionate, and positive social interactions. The scale was developed as part of the Medical Outcome Study, tested on 2,987 patients and designed to assess quality of life of patients across medical conditions. All but one item is rated on a five-point Likert scale from 'None of the Time' to 'All of the Time.' Internal consistency of the subscales and total score are excellent (alpha coefficient $\geq$ 0.91). Convergent validity was demonstrated by significant correlations of social support total score with measures of mental health (r = .45; p = ‹ .01). In order to reduce respondent burden, we will use only the Tangible and Emotional/ Information Subscales, two of the most important subscales for this patient population [31].

4.0 References

1. Hurria A, Gupta S, Zauderer M, et al. Developing a cancer-specific geriatric assessment: a feasibility study. Cancer. Nov 1 2005;104(9):1998-2005.
2. Repetto L, Fratino L, Audisio RA, et al. Comprehensive geriatric assessment adds information to Eastern Cooperative Oncology Group performance status in elderly cancer patients: an Italian Group for Geriatric Oncology Study. J Clin Oncol. Jan 15 2002;20(2):494-502.
3. Narain P, Rubenstein LZ, Wieland GD, et al. Predictors of immediate and 6-month outcomes in hospitalized elderly patients. The importance of functional status. J Am Geriatr Soc. Sep 1988;36(9):775-783.
4. Reuben DB, Rubenstein LV, Hirsch SH, Hays RD. Value of functional status as a predictor of mortality: results of a prospective study. Am J Med. Dec 1992;93(6):663-669.
5. Maione P, Perrone F, Gallo C, et al. Pretreatment quality of life and functional status assessment significantly predict survival of elderly patients with advanced non-small-cell lung cancer receiving chemotherapy: a prognostic analysis of the multicenter Italian lung cancer in the elderly study. J Clin Oncol. Oct 1 2005;23(28):6865-6872.
6. Yancik R, Wesley MN, Ries LA, Havlik RJ, Edwards BK, Yates JW. Effect of age and comorbidity in postmenopausal breast cancer patients aged 55 years and older. Jama. Feb 21 2001;285(7):885-892.
7. Extermann M, Overcash J, Lyman GH, Parr J, Balducci L. Comorbidity and functional status are independent in older cancer patients. J Clin Oncol. Apr 1998;16(4):1582-1587.
8. Landi F, Onder G, Gambassi G, Pedone C, Carbonin P, Bernabei R. Body mass index and mortality among hospitalized patients. Arch Intern Med. Sep 25 2000;160(17):2641-2644.
9. Dewys WD, Begg C, Lavin PT, et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group. Am J Med. Oct 1980;69(4):491-497.
10. Newman AB, Yanez D, Harris T, Duxbury A, Enright PL, Fried LP. Weight change in old age and its association with mortality. J Am Geriatr Soc. Oct 2001;49(10):1309-1318.
11. Eagles JM, Beattie JA, Restall DB, Rawlinson F, Hagen S, Ashcroft GW. Relation between cognitive impairment and early death in the elderly. Bmj. Jan 27 1990;300(6719):239-240.
12. Wolfson C, Wolfson DB, Asgharian M, et al. A reevaluation of the duration of survival after the onset of dementia. N Engl J Med. Apr 12 2001;344(15):1111-1116.
13. Seeman TE, Berkman LF, Kohout F, Lacroix A, Glynn R, Blazer D. Intercommunity variations in the association between social ties and mortality in the elderly. A comparative analysis of three communities. Ann Epidemiol. Jul 1993;3(4):325-335.
14. Kua J. The prevalence of psychological and psychiatric sequelae of cancer in the elderly - how much do we know? Ann Acad Med Singapore. Apr 2005;34(3):250-256.
15. Vinokur AD, Threatt BA, Vinokur-Kaplan D, Satariano WA. The process of recovery from breast cancer for younger and older patients. Changes during the first year. Cancer. Mar 1 1990;65(5):1242-1254.

16. Kornblith AB, Herndon JE, 2nd, Weiss RB, et al. Long-term adjustment of survivors of early-stage breast carcinoma, 20 years after adjuvant chemotherapy. Cancer. Aug 15 2003;98(4):679-689.

17. Penninx BW, Guralnik JM, Ferrucci L, Simonsick EM, Deeg DJ, Wallace RB. Depressive symptoms and physical decline in community-dwelling older persons. Jama. Jun 3 1998;279(21):1720-1726.

18. Langa KM, Valenstein MA, Fendrick AM, Kabeto MU, Vijan S. Extent and cost of informal caregiving for older Americans with symptoms of depression. Am J Psychiatry. May 2004;161(5):857-863.

19. Delafuente JC. Understanding and preventing drug interactions in elderly patients. Crit Rev Oncol Hematol. Nov 2003;48(2):133-143.

20. Vestal RE. Aging and pharmacology. Cancer. Oct 1 1997;80(7):1302-1310.

21. Gandhi TK, Weingart SN, Borus J, et al. Adverse drug events in ambulatory care. N Engl J Med. Apr 17 2003;348(16):1556-1564.

22. Hanlon JT, Pieper CF, Hajjar ER, et al. Incidence and predictors of all and preventable adverse drug reactions in frail elderly persons after hospital stay. J Gerontol A Biol Sci Med Sci. May 2006;61(5):511-515.

23. Fillenbaum GG, Smyer MA. The development, validity, and reliability of the OARS multidimensional functional assessment questionnaire. J Gerontol. Jul 1981;36(4):428-434.

24. Fillenbaum GG. Screening the elderly. A brief instrumental activities of daily living measure. J Am Geriatr Soc. Oct 1985;33(10):698-706.

25. Stewart AL, & Ware, J.E. Jr. Measuring functioning and well-being: The Medical Outcomes Study approach. . Durham, NC: Duke University Press; 1992.

26. Yates JW, Chalmer B, McKegney FP. Evaluation of patients with advanced cancer using the Karnofsky performance status. Cancer. Apr 15 1980;45(8):2220-2224.

27. Loprinzi CL, Laurie JA, Wieand HS, et al. Prospective evaluation of prognostic variables from patient-completed questionnaires. North Central Cancer Treatment Group. J Clin Oncol. Mar 1994;12(3):601-607.

28. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc. Feb 1991;39(2):142-148.

29. Kawas C, Karagiozis H, Resau L, Corrada M, Brookmeyer R. Reliability of the Blessed Telephone Information-Memory-Concentration Test. J Geriatr Psychiatry Neurol. Oct 1995;8(4):238-242.

30. Stewart AL K, CJ,. . Measuring functioning and well-being; the Medical Outcomes Study approach. 1992 ed. Durham, North Carolina; Duke University Press; 1992.

31. Sherbourne CD, Stewart AL. The MOS social support survey. Soc Sci Med. 1991;32(6):705-714.

32. Karnofsky D, Burchenal J. The clinical evaluation of chemotherapeutic agents in cancer. In: Macleod CM, ed. Evaluation of chemotherapeutic agents. New York: Columbia University Press; 1948:191-205.

33. Guralnik JM, Ferrucci L, Pieper CF, et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone

compared with the short physical performance battery. J Gerontol A Biol Sci Med Sci. Apr 2000;55(4):M221-231.

34. Tinetti ME. Clinical practice. Preventing falls in elderly persons. N Engl J Med. Jan 2 2003;348(1):42-49.

35. Tinetti ME, Williams CS. The effect of falls and fall injuries on functioning in community-dwelling older persons. J Gerontol A Biol Sci Med Sci. Mar 1998;53(2):M112-119.

36. Katzman R, Brown T, Fuld P, Peck A, Schechter R, Schimmel H. Validation of a short Orientation-Memory-Concentration Test of cognitive impairment. Am J Psychiatry. Jun 1983;140(6):734-739.

37. Veit CT, J.E. Ware J. The structure of psychological distress and well-being in general populations. Journal of Consulting and Clinical Psychology. 1983;51:730-742.

38. McHorney CA, J.E. Ware J, Rogers W, Raczek AE, Lu JFR. The validity and relative precision of MOS Short and Long-Form Health Status Scales and Dartmouth COOP charts. Medical Care. 1992;30(5, (suppl)):MS 253-256.

APPENDIX VI

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

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

For patient enrollments:
CTSU Patient Registration
Voicemail: 1-888-691-8039
Fax: 1-888-691-8039
Hours: 9:00 AM – 5:30 PM Eastern Time, Monday – Friday (excluding holidays)

[Registrations received after 5:00 PM ET will be handled the next business day. For CTSU patient enrollments that must be completed within approximately one hour, or other extenuating circumstances, call 301-704-2376 between 9:00 am and 5:30 pm.]

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

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

Sites should submit Teleforms via Fax or Mail. See section 6.1 Data Submission Section for details on forms submission.

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

For patient eligibility or treatment related questions: Contact the CALGB 40503 Study Chair.

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

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

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

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

REGISTRATION/RANDOMIZATION

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

Each CTSU investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office

before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site at http://members.ctsu.org

All forms and documents associated with this study can be downloaded from the CALGB-40503 Web page on the CTSU registered member Web site (https://members.ctsu.org). Patients can be registered only after pre-treatment evaluation is complete, all eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS.

Requirements for CALGB 40503 site registration:

- CTSU IRB Certification
- IRB/Regulatory Approval Transmittal Sheet

Prestudy requirements for patient enrollment on CALGB 40503

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

CTSU Procedures for Patient Enrollment

1. Contact the CTSU Patient Registration Office by calling 1-888-462-3009 between 9:00 am and 5:30 pm Eastern Time, Monday-Friday. Leave a voicemail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs, e.g. within one hour, call the registrar cell phone at 1-301-704-2376.
2. Complete the following forms:
   - CTSU Patient Enrollment Transmittal Form
   - CALGB 40503 Eligibility Checklist
   - CALGB Registration Worksheet (sites should indicate patient participation on companion studies CALGB 150605 and CALGB 60605).
3. Fax these forms to the CTSU Patient Registrar at 1-888-691-8039 between the hours of 9:00 a.m. and 5:50 p.m., Mon-Fri, Eastern Time (excluding holidays). This is limited to the operating hours of the CALGB Registration Office. The CTSU registrar will check the investigator and site information to ensure that all regulatory requirements have been met. The registrar will also check that forms are complete and follow-up with the site to resolve any discrepancies.
4. Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will contact the CALGB, within the confines of CALGB's registration hours. The CTSU registrar will access the CALGB's on-line registration system, to obtain assignment of a unique patient ID (to be used on all future forms and correspondence). Since this is a randomized study, a treatment arm will not be assigned. The CTSU registrar will confirm registration by fax.
   - Protocol treatment should begin within 14 days of registration/randomization.

- Registration to the correlative sciences companion studies (CALGB 150605 and CALGB 60605) for those patients who have agreed to participate should be performed at the same time as registration to the treatment study.

Procedures for late enrollment on CALGB 60605 (pharmacogenomic studies):

- Submit CTSU Patient Enrollment Transmittal Form (with note indicating delayed registration to CALGB 60605).
- Submit revised CALGB 40503 registration worksheet (indicating patient consent for CALGB 60605).

Note: Although it is preferable that patients are registered to CALGB 60605 at the same time they are registered for CALGB 40503, registration to CALGB 60605 may occur up to 60 days following registration to the treatment trial. However, registration to CALGB 150605 must be done at the time the patient is registered to the treatment trial 40503.

Data Submission and Reconciliation

1. All case report forms (CRFs) associated with this study must be downloaded from the CALGB-40503 Web page located on the CTSU registered member Web site (https://members.ctsu.org). Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.
2. Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and transmittals directly to the CALGB Statistical Center, [see contact table and section 6.1 of protocol] unless an alternate location is specified in the protocol. Do not send study data to the CTSU. A completed CTSU-CALGB coversheet should accompany all data submissions.
3. The CALGB Statistical Center will send (generally via email but may be sent via postal mail or fax) query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the CALGB Statistical Center (via postal mail) and do not copy the CTSU Data Operations. Each site should have a designated CTSU Administrator and Data Administrator and **must keep their CTEP AMS account contact information current**. This will ensure timely communication between the clinical site and the CALGB Statistical Center.

Special Materials or Substudies

There are two substudies within CALGB 40503. These correlative science and pharmacogenomic studies must be offered to all patients enrolled on CALGB 40503, although patients may opt to not participate.

1. Specimen collection for correlatives (Protocol section 6.0, Appendices I and II)
   - The substudies included within CALGB 40503 are: Correlative Science Studies, CALGB 150605 (Appendix I) and Pharmacogenomic Studies, CALGB 60605 (Appendix II)
   - Collect, prepare, and submit specimens as outlined in the protocol
   - Do not send specimens, supporting clinical reports, or transmittals to the CTSU
2. All patients who understand and are able to follow directions in English (as the assessment instruments are only available in English), and have agreed to participate, will take part in the functional age assessments. **Please see Appendix V, Section 3.0 for instruction regarding assessment administration. Nurses and/or CRA who will administer the assessments must contact Dr. Hurria (626-256-4673) to review the assessment measures via telephone conference, prior to performing the first patient assessment.** See Section 6.1 for the submission instructions for the CALGB: 40503 Functional Age Assessment Measure (Healthcare

Professional Questionnaire) (C-1776) and the CALGB 40503: Functional Age Assessment Measure (Patient Questionnaire) (C-1777).

Serious Adverse Event (AE) Reporting (Section 15.0)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting beginning October 1, 2010. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov). CTSU investigators should assess adverse events according to the instructions and tables in section 15.0 of the protocol. All reporting should be conducted within the time frames specified in section 15.0 of the protocol.

1. CTSU sites must comply with the expectations of their local Institutional Review Board (IRB) regarding documentation and submission of adverse events. Local IRBs must be informed of all reportable serious adverse reactions.
2. CTSU sites will assess and report adverse events according to the guidelines and timelines specified in the protocol. You may navigate to the CTEP Adverse Event Expedited Report System (AdEERS) from either the Adverse Events tab of the CTSU member homepage (https://members.ctsu.org) or by selecting Adverse Event Reporting Forms from the document center drop down list on the protocol number Web page.
3. Do not send adverse event reports to the CTSU.
4. **Secondary AML/MDS/ALL reporting:** Report occurrence of secondary AML, MDS, or ALL via the NCI/CTEP AML-MDS Report Form in lieu of AdEERS. Submit the completed form and supporting documentation as outlined in the protocol.
5. **Reporting other secondary malignancies:** Secondary malignancies must be reported on the C-1555, CALGB-40503 Follow-up Form and submitted to the CTSU Data Operations Office for forwarding to CALGB.

DRUG PROCUREMENT (Section 10.0)

Investigational agents: Bevacizumab (NSC 704865) will be provided free of charge by Genentech and distributed by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI).

1. Information on drug formulation, procurement, storage and accountability, administration, and potential toxicities are outlined in section 10.0 of the protocol.
2. For drug reordering you may navigate to the drug forms by selecting Pharmacy Forms from the document center drop down list on the CALGB-40503 Web page.

Supplied commercial agent: Letrozole tablets will be provided for this trial, free of charge, by Novartis. Letrozole should be ordered using the 40503 Drug Shipment Request, which is available on the CTSU web site, on the 40503 study page under Supplemental materials. Letrozole orders must be accompanied by the study specific Form FDA 1572, also available on the 40503 study page. Please allow 4-5 business days for drug delivery.

REGULATORY AND MONITORING

Study Audit

To assure compliance with Federal regulatory requirements [CFR 21 parts 50, 54, 56, 312, 314 and HHS 45 CFR 46] and National Cancer Institute (NCI)/Cancer Therapy Evaluation Program (CTEP) Clinical Trials Monitoring Branch (CTMB) guidelines for the conduct of clinical trials and study data

validity, all protocols approved by NCI/CTEP that have patient enrollment through the CTSU are subject to audit.

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

For patients enrolled through the CTSU, you may request the accrual be credited to any Group for which you have an affiliation provided that Group has an active clinical trials program for the primary disease type being addressed by the protocol. Per capita reimbursement will be issued by the credited Group provided they have endorsed the trial, or by the CTSU if the Group has not endorsed the trial.

Details on audit evaluation components, site selection, patient case selection, materials to be reviewed, site preparation, on-site procedures for review and assessment, and results reporting and follow-up are available for download from the CTSU Operations Manual located on the CTSU Member Web site.

Health Insurance Portability and Accountability Act of 1996 (HIPAA)

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

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

Clinical Data Update System (CDS) Monitoring

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