A Phase II, Randomized, Open Label Trial of Pre-Operative (Neoadjuvant) Letrozole (Femara®) vs. Letrozole in Combination with Avastin® in Post-Menopausal Women with Newly Diagnosed Operable Breast Cancer (Breast Cancer Research Foundation - Johns Hopkins Consortium)

Study Protocol & Statistical Analysis Plan

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COMPREHENSIVE CANCER CENTER
UNIVERSITY OF ALABAMA AT BIRMINGHAM
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UAB 0648 and Affiliates: A Phase II, Randomized, Open Label Trial of Pre-Operative (Neoadjuvant) Letrozole (Femara®) vs. Letrozole in Combination with Avastin® in Post-Menopausal Women with Newly Diagnosed Operable Breast Cancer (Breast Cancer Research Foundation – Johns Hopkins Consortium)

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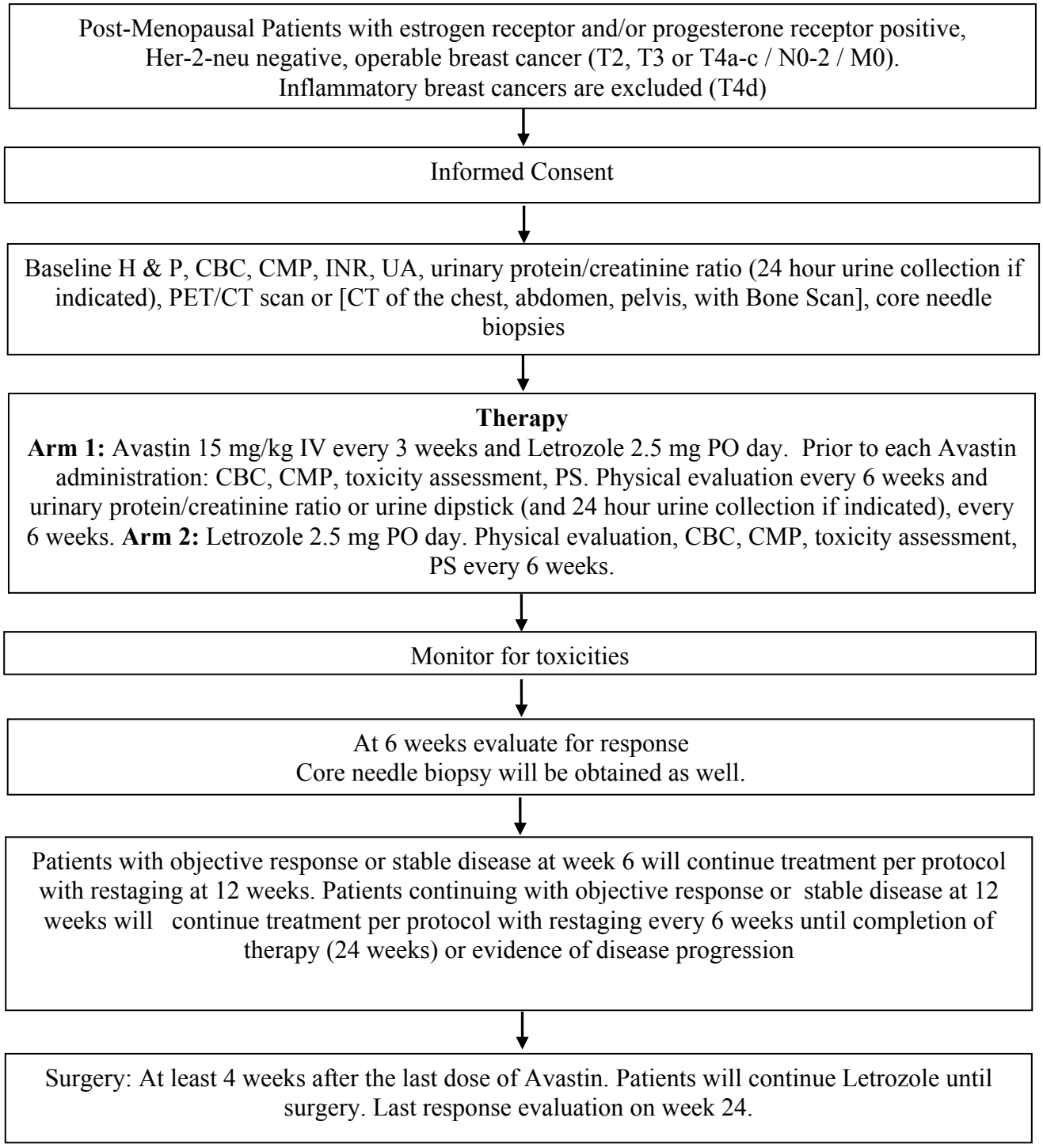


1.0 BACKGROUND

1.1 Hormonal Therapy for Breast Cancer

Breast Cancer is the most common cancer in the United States and the second most deadly cancer in woman with an estimated 212,930 invasive breast cancer diagnoses and 40,870 breast cancer deaths in 2005; although the incidence has increased for several decades, the mortality rate has decreased **(1)**. The introduction of effective therapies, including chemotherapy, hormonal therapies and targeted therapies, has been a major factor contributing to improved outcomes **(2)**.

The link between the endocrine system and breast cancer was first recognized more than 100 years ago with the use of bilateral oophorectomy in patients with breast cancer **(3).** It is now established that estrogen stimulates the growth of some breast tumors; thus, hormone-sensitive breast cancer can be effectively treated with agents that reduce estrogen stimulation of the estrogen receptor. Since the late 1970s, numerous clinical trials have shown that the selective estrogen receptor (SERM) antagonist tamoxifen is as effective for the initial treatment of metastatic breast cancer as other hormonal therapies or chemotherapy but with fewer secondary effects in women with hormone receptor positive tumors **(4,5)**. As a result, tamoxifen became the gold standard for first-line treatment for hormone-sensitive metastatic breast cancer. In contrast to tamoxifen, which inhibits the activity of estrogen by competitively binding to the estrogen receptor, aromatase inhibitors (AIs) block the conversion of androgens to estrogens and reduce estrogen levels in tissue and plasma. Third-generation AIs include the nonsteroidal inhibitors letrozole and anastrozole and the steroidal inhibitor exemestane. With daily oral administration, anastrozole and exemestane inhibit aromatase activity in vivo by 97 to 98 percent and letrozole inhibits aromatase by more than 99 percent. Over the last years, the aromatase inhibitors (AIs) have been developed and evaluated in clinical trials. As first-line treatment for metastatic breast cancer, third-generation AIs are equivalent or superior to tamoxifen. Women with metastatic breast cancer who were given letrozole as first-line treatment had a significantly higher response rate, a significantly longer time to progression, and a significant improvement in one and two-year survival rates, as compared with women given tamoxifen. Thus, these agents have been demonstrated to be more effective than tamoxifen in the metastatic setting with less secondary effects and they are now considered the first line therapy in postmenopausal patients with hormone receptor positive metastatic breast cancer.

- Adjuvant Hormonal therapy for early breast cancer in postmenopausal women

The proven efficacy of hormonal therapy in metastatic disease also made it the ideal test agent for adjuvant hormonal therapy in early stage operable breast cancer **(6)**. In the 1980's the use of tamoxifen expanded as adjuvant therapy for early-stage hormone receptor positive breast cancer reducing reoccurrences by 47% and the risk of death by 26% among patients with hormone-receptor-positive breast cancer.

Historically, breast cancer was thought to be a local disease with predictable progression through the mammary ducts, lymphatics, and draining lymph nodes before more widespread dissemination. A more recent divergent view of breast cancer is the recognition of the potential early micrometastases of cancer cells from the primary tumor; in this view, breast cancer is seen

as a systemic disease very early in its development. This later model of breast cancer is increasingly felt to be the correct one based on consistent data which demonstrate a reduction in the risk of recurrence of breast cancer in women who received adjuvant therapy. An overview of 55 trials of adjuvant tamoxifen for 1, 2, or 5 years versus no therapy for patients with early breast cancer showed that the treatment produced highly significant benefits in terms of both recurrence and survival; reductions in recurrence were 18%, 25%, and 41%, and reductions in death rate were 10%, 15%, and 22% for 1, 2, and 5 years of tamoxifen treatment, respectively **(7)**. These benefits are durable as well after discontinuation of tamoxifen with a 47 % reduction in recurrence and 27% improvement in overall survival after 10 years of follow up. These benefits were found to occur almost exclusively in the hormone receptor positive population. The optimal duration of tamoxifen is unresolved, however 5 years of adjuvant therapy has demonstrated superior recurrence rates, and overall survival compared to shorter durations of therapy. Three additional trials, including the NSABP-14, studying extending the duration of tamoxifen beyond 5 years have not showed any benefit and rather an increase in endometrial carcinoma and a decrease in the disease-specific survival. Two definitive trials, ATLAS and aTTom, comparing the effect of 5 years of treatment with tamoxifen vs. the effect of more than 5 years are currently ongoing. Although the results have not been conclusive, it appears that 5 years of tamoxifen is the optimal duration in terms of patient benefit **(8-12)**. In addition, the results of the 1998 Oxford Early Breast Cancer Trialist's Collaborative Group showed that the significant improvements in the risk of recurrence and overall survival, with combination chemotherapy and tamoxifen in patients with estrogen receptor positive disease were irrespective of age or menopausal status **(7)**.

Based on the anti-tumor activity of the third-generation AIs (letrozole, anastrozole, exemestane) in the metastatic disease setting **(13-21)**, these medications are currently being assessed in the adjuvant setting. Multiple trials are being conducted at this time, and the data from five randomized trials is now available. The first trial, ATAC (Arimidex, Tamoxifen, Alone or in Combination) randomized 9,366 postmenopausal women with invasive operable breast cancer who completed primary therapy to one of the three arms: tamoxifen alone, anastrozole alone or the combination of both. After the first evaluation, the arm using the combination was discontinued due to lack of superiority over tamoxifen alone. At the updated efficacy analysis after 68 months of follow up , DFS remained significantly longer in the anastrozole arm than in the tamoxifen arm with a HR 0.87; P = .01 with an absolute difference of 3.8%, demonstrating a continued benefit after completion of 5 years of therapy. Time to progression was significantly longer for those patients receiving anastrozole compared with the patients receiving tamoxifen (HR = 0.79; P = .0005), time to distant metastases (HR = .86; P = .04) and a larger benefit was seen in the hormone receptor positive population. There was also a 42% decrease in contralateral breast cancer (P = .01). Tolerance was better for anastrozole **(22-24)**. Thus, anastrozole was the first AI approved by the FDA for the adjuvant treatment of early breast cancer in postmenopausal women with hormone receptor positive disease. A second adjuvant trial, IES (Intergroup Exemestane Study) randomized 4,742 patients after 2-3 years of adjuvant tamoxifen to continued tamoxifen versus exemestane for a total of 5 years. After 30.6 months of follow up, exemestane demonstrated an improved DFS with exemestane (HR 0.68; P < .001), and an absolute difference of 4.7%. Long term data including survival and toxicity data have not been presented. Several other supportive studies with the use of letrozole in the BIG FEMTA and BIG 1-98 trials have confirmed similar benefits demonstrating a class effect of the AI.

Additional data has also been published using five years of letrozole following five years of adjuvant tamoxifen, the MA-17 trial, in postmenopausal patients with hormone receptor positive tumors (randomized study between placebo and letrozole). At a median follow-up of 2.4 years, the estimated 4-year DFS rate was 93% in the letrozole group compared with 87% in the placebo group **(25)**.

While both surgery and radiation therapy treatments affect the local disease, adjuvant therapy is employed to reduce the risk of systemic disease. Thus, the current management of breast cancer attempts to eradicate breast cancer both at the primary tumor site and by affecting distant metastases. The timing of these therapies is undergoing re-examination. Observation of effects both on the primary tumor and on systemic metastases has suggested that systemic therapy might provide greater efficacy if employed earlier in the treatment of breast cancer (neoadjuvant therapy).

- **Neoadjuvant therapy for breast cancer**

Preoperative chemotherapy has been employed as part of multimodality therapy for patients with locally advanced breast cancer (LABC) or inflammatory breast cancer (IBC). These patients fared poorly with single-modality therapy alone. Historical studies demonstrated 5-year disease-free survival (DFS) rates for LABC to be 41% with surgery alone and 29% with radiation therapy **(26)**. Patients with IBC did worse, with DFS rates of 3 to 10% with surgery or radiation alone **(27)**. In contrast, the combination of primary chemotherapy, surgical resection, and radiotherapy has significantly improved the clinical outcome of these patients **(28, 29)**. As a result of the favorable impact that preoperative chemotherapy has demonstrated on the survival rates of patients with locally advanced disease, numerous studies have evaluated its use in earlier-stage disease **(30-32)**. The largest of the randomized studies comparing preoperative to postoperative chemotherapy was the NSABP B-18 study. This study randomized 1,523 women with operable stage I-IIIA breast cancer to receive four cycles of Adriamycin (doxorubicin) and cyclophosphamide (AC) either preoperatively or postoperatively. Although the disease free and overall survival rates at 5 years of follow up were similar between the two cohorts (DFS: 66.7% vs. 67.3% and OS: 72.3 vs. 73.2%), it became clear that preoperative chemotherapy improved the breast conservation rate. There was an overall response rate of 79% in the patients after preoperative chemotherapy with an increase in the number of conservative surgeries (67% vs. 60%). More importantly, the pathologic complete response (CR) rate was directly proportional to DFS and overall survival (OS) rates **(33, 34)**. For patients who experienced a pCR, DFS and overall survival were significantly improved, with 84% of patients with pCR disease free at 5 years compared to 60% in patients who had no clinical response.

A similar association between pathologic CR rate and survival has been demonstrated in numerous other studies **(35-40)**. The pCR is increasingly an accepted surrogate marker in neoadjuvant trials for overall survival and outcomes. The majority of published studies of preoperative chemotherapy have used anthracycline-based regimens with pCR rates between 10-15%. However, owing to the efficacy of the single agent taxanes (paclitaxel and docetaxel) in the metastatic setting, both taxanes have been included in numerous trials over the past decade. Based on the theoretical benefit of combining alternating cross-resistant chemotherapy agents, the addition of paclitaxel and docetaxel to anthracycline-based regimens have increased pCR rates to between 20-25%. This was confirmed in a large randomized neoadjuvant study, NSABP-

27, comparing the addition of docetaxel to an anthracycline based regimen. The addition increased pCR from 13.7 % to 26.1 % at the cost of increasing G4 toxicity from 10.3 % to 23.4 %, with an incidence of neutropenic fever 7.3 % vs. 21.2 %. Ongoing studies with longer follow-up will clarify the role of preoperative chemotherapy in earlier stage disease and the most effective sequence or combination of chemotherapeutic agents. As it is described below, it has become apparent that not all breast cancer tumors have the same response to neoadjuvant chemotherapy; as an example, patients with estrogen receptor negative tumors have higher pathological complete responses to neoadjuvant chemotherapy than those with estrogen receptor positive tumors. Hence, pathologic CR rate is considered to be the most important study end point to assess the efficacy of preoperative chemotherapy regimens.

Neoadjuvant chemotherapy has multiple practical and theoretic advantages, enhancing our ability to care for patients with breast cancer. This treatment approach remains a standard of care for patients with IBC or LABC and a safe option for patients with early-stage breast cancer. Neoadjuvant chemotherapy can downstage both the primary tumor and metastatic disease in the axilla, therefore improving the ability to perform breast-conserving therapy without increasing the risk for local recurrence. This approach also allows the physician to evaluate the in vivo response to treatment and potentially to avoid ineffective therapies. The pathologic CR to a particular neoadjuvant therapy regimen can predict survival, allowing this end point to be used as a surrogate that can identify treatments that will improve survival much earlier than traditional trial designs. The future of neoadjuvant chemotherapy will be to develop classification or predictive markers to tailor therapy to an individual and enhance survival. Neoadjuvant chemotherapy is no longer limited to patients with stage III cancer or IBC but is an approach that will continue to develop new uses as research continues to move forward. Preoperative chemotherapy in primary breast cancer patients is now accepted as an effective treatment, showing that the DFS and overall survival are similar to those of patients in whom systemic therapy is used after surgery.

- Neoadjuvant hormonal therapy for operable breast cancer

One of the most important factors influencing the achievement of pathological CR with neoadjuvant chemotherapy is hormone receptor status. **ER positive patients achieve pathological CR rates of 8-12% (or lower) while ER negative patients have a pathological CR rates of 20-40%**. Additionally, these chemotherapy regimens are associated with significant toxicity including neutropenic fever, cardiomyopathy and alopecia **(94-96)**. Thus, post-menopausal women with ER positive or PR positive (Her-2-neu negative) tumors may benefit from alternative, less toxic neo-adjuvant therapy. Several phase II and III studies conducted with tamoxifen have shown that it is possible to obtain good clinical responses with pre-operative endocrine therapy. Initial studies in which endocrine therapy was compared with surgery demonstrated initial reduction in tumor size for the majority of the older patients receiving endocrine treatment without impact in the overall survival for both groups; however, long-term local disease control was poor for those patients receiving endocrine therapy alone **(41, 42)**. This might not be the case when endocrine therapy is used not “in substitution of” but “before” optimal local control with surgery.

While tamoxifen is well tolerated, its adverse events are of important clinical consequences to the patient. Thus, the AIs became an alternative treatment in the neoadjuvant setting. In a phase I-II trial conducted in Scotland, a clinical response rate of 92% was seen in 24 postmenopausal estrogen receptor positive patients whose locally advanced breast cancer was treated with letrozole for 3 months prior to surgery. Of note, all 15 patients not eligible for breast conservation surgery had sufficient tumor regression to allow breast conservative surgery **(43)**. This pilot study led to the conduct of a randomized, double-blind study designed to compare efficacy of preoperative letrozole versus preoperative tamoxifen for postmenopausal women with estrogen receptor and/or progesterone receptor positive breast cancer ineligible for breast conservative surgery **(44)**. Overall objective response rate was statistically significantly superior in the letrozole group (55%) compared with the tamoxifen group (36%). In addition, 45% of patients in the letrozole group underwent breast conservative surgery compared with 35% in the tamoxifen group. Clinical complete response was 10% for the group receiving letrozole compared with 4% for the group receiving tamoxifen. Neoadjuvant letrozole was very tolerable as the most common side effect was hot flashes (20%) and nausea (5%). Semiglazov et al presented to the 2004 ASCO Annual Meeting and the 5th European Breast Cancer Conference a study randomizing postmenopausal, ER positive patients to neoadjuvant AI (anastrozole or exemestane) vs. an anthracyline/taxane combination chemotherapy regimen. This trial demonstrated similar response rates between the two cohorts with a similar disease free survival at 3 years of follow-up. However there was a trend towards increased breast conservation surgery in the AI group. Neoadjuvant hormonal therapy also has similar response rates in both Her2 negative (106/154; 64%) and Her2 positive (11/18; 64%) breast cancers.

The optimal duration of neoadjuvant AI therapy is unknown, with current published trials using AI for 3-4 months prior to surgery. Renshaw et al (San Antonio Breast Cancer Meeting 2004, abstract # 405) evaluated 142 women treated with neoadjuvant AI, and demonstrated in the first 3 months of therapy, 100/142 pt had sufficient tumor regression to allow BCS with a median tumor regression of 52%. The 42 remaining patients continued to received letrozole, and between 3 and 6 months of therapy, had an additional 57% median tumor regression (CR = 12/42; 29%). After 6 months of therapy, 22/42 patients continued neoadjuvant letrozole for an additional 6 months. Between 6 and 12 months of therapy there was an additional 66% median tumor regression with only 1 patient having progressive disease (CR = 8/22; 36%). Extended therapy of neoadjuvant AI up to 12 months is safe with continued tumor regression. In addition, Paepke et al (2003 ASCO Annual Meeting, abstract #321) conducted a trial in which 33 patients received letrozole for a minimum of 4 and a maximum of 8 months prior to surgery. They reported that longer treatment resulted in a statistically significant increase in tumor size reduction. Moreover, 90% of the patients receiving therapy for longer than 4 months had CR or PR compared with 57% of patients with CR or PR receiving therapy for up to 4 months. **Thus, these studies strongly suggest that patients whose tumors respond to letrozole after 3 months can expect a further reduction in tumor volume with continued therapy.**

- Hormone resistance

While the hormonal agents have demonstrated adequate anti-tumoral activity, the development of resistance is at the core of the problem and must be overcome to make additional progress. Consequently, efforts aimed at gaining a better understanding of the mechanisms underlying hormone resistance are warranted and could lead to strategies to prevent or circumvent resistance. Although the mechanisms responsible for tamoxifen resistance remain unknown, over the last 20 years, a number of mechanisms for hormone resistance have been proposed including increased levels of intracellular antiestrogen binding sites; alterations in the estrogen receptor; increased agonistic properties of tamoxifen and ligand independent activation of the estrogen receptor; activation of ERβ by antiestrogens and constitutive growth factor signaling conferring ER+ breast cancer cells with estrogen independent and hormone resistant phenotypes **(45)**. In addition, a number of biological factors have demonstrated to be related with a favorable or poor response to endocrine therapies and they can be used to understand mechanisms of resistance and to develop new therapeutic strategies to overcome resistance to these agents in breast cancer patients.

Angiogenesis represent a fundamental step in tumor progression **(46)**; multiple studies have shown that node negative as well as node positive breast cancer patients with high intra-tumoral vascular endothelial growth factor (VEGF, a potent angiogenic factor) concentrations have a significantly shorter relapse-free survival **(47, 48)**. In addition, the expression of tyrosine kinases such as epidermal growth factor receptor or its family member HER2, the tumor suppressor gene TP53, and VEGF have been associated with a poor response to tamoxifen **(49, 50)**. Recent studies have demonstrated that oncogenes and tumor suppressor genes associated with neoplastic cell transformation play an integral part in activating the angiogenic switch. Alterations of these genes, including RAS, SRC, HER2, and TP53, have been characterized as inducers of VEGF expression (as an example, mutant TP53 has demonstrated the ability of up-regulate VEGF) **(51)**.

Furthermore, studies in UAB Breast Cancer Spore using the MCF-7 tamoxifen murine xenografts model by Dr. Fran Kern and collaborators indicate that paracrine effects of VEGF over-expression by ERα+ breast cancer cells can reverse the effectiveness of tamoxifen as a cytostatic or cytotoxic agent **(52-58)**. In this MCF-7 model, they demonstrated that increased expression of VEGF causes an acquired Tamoxifen resistance and tumor loss of estrogen dependence *in vivo*. MCF-7 cells with VEGF co-expression (stably transfected or regulated expression) had *in vitro* tumor cell proliferation and estrogen dependence identical to non-transfected MCF-7, while MCF-7/VEGF cells *in vivo* displayed increased tumor growth rates, higher rates of metastases, and estrogen independence. These survival and proliferation signals may be an indirect effect of VEGF presumably reflecting the enhanced vascularity of tumors with secondary growth factor and signal pathway modulation. Reversal of VEGF over-expression *in vivo* returned tumors to estrogen dependent growth.

In addition, Berns and collaborators evaluated the predictive value of TP53 gene mutation status and VEGF levels for response to tamoxifen therapy in 160 advanced breast cancer patients with ER+ tumors **(59)**. In a univariate analysis, both TP53 gene mutation and VEGF levels above the median value were significantly associated with a short progression-free survival, post-relapse

overall survival and a poor response to Tamoxifen. In a Cox multivariate regression analysis including the traditional predictive factors, the addition of TP53 gene mutation and VEGF status, alone or in combination significantly predicted a poor response to tamoxifen. When the two factors were combined, a significantly decreased odds ratio was seen for response (odds ratio, 0.27). Similarly, an increased hazard ratio was seen for progression-free survival (2.32) and post-relapse overall survival (1.68) in the group with mutant TP53 and high VEGF compared with the group with booth risk factors absent.

Dr. Zhican Qu, Ph.D., a recipient of a career development award of the UAB-Breast SPORE, conducted preclinical efficacy studies of tamoxifen and bevacizumab in combination for the treatment of breast cancer using a novel mouse model. In her previous studies she showed that elevated levels of VEGF increases tumor growth, and promotes metastasis and tamoxifen resistance; thus, she hypothesized that a combination of agents targeting both ER and VEGF signaling may be a more effective treatment than the use of either drug alone. She tested this hypothesis using a combination of tamoxifen and Avastin in a xenograft nude mouse model with regulated-VEGF expression. The efficacy of the treatment was tested in two groups of mice, one with early and one with late stage ER-positive breast cancer. All mice received orthotopic implantation of 10^7 tumor cells at mammary fat pat and a 17β-estradiol pellet supplement on Day 1. Doxycycline was given to all mice to induce expression and secretion of VEGF from tumor cells. Animals in the early stage group started therapy (tamoxifen or Avastin or placebo or tamoxifen-Avastin in combination) on Day 7 when the tumor size reached about 100 mm^3. The Avastin-tamoxifen combined treatment demonstrated better control of primary tumor growth than therapy with tamoxifen or Avastin alone. The survival rate was superior in the group of mice treated with combination therapy compared with the groups treated with single agents or placebo. Animals in the late stage group started therapy on Day 32 when the tumor size reached about 1000 mm^3. The combined treatment showed again a better reduction of tumor growth rate and a significant better survival. 83% of mice survived in the combined treatment group, while only about 50% of mice survived in the monotherapy groups. These results demonstrated that the Avastin-tamoxifen combination is more effective on inhibiting tumor growth of ER positive breast cancer and increasing survival rate than either drug treatment alone (2005 AACR Annual Meeting, abstract #1091).

1.2 Vascular Endothelial Growth Factor

A novel approach to the treatment of solid tumors, including breast cancer, is to inhibit the growth of new vessels supplying the tumor. Folkman and others have provided compelling evidence linking tumor growth and metastases with angiogenesis **(60)**. Human cancer cells placed in avascular sites in animals form tumors that growth only up to 1-2 mm in diameter **(61)**. The same cells implanted in vascular sites grow rapidly to form large, bulky tumors. Several authors have shown a correlation between microvessel density and clinical outcome. Weidner and coworkers have shown a statistically significant correlation between the number and density of microvessels in histologic specimens of human breast cancer and clinical outcome, including the incidence of metastases as well as overall survival and relapse-free survival **(62, 63)**. A study of a large cohort of patients (836) with operable, invasive breast cancer demonstrated that angiogenesis, as measured by Chalkley count, is an independent prognostic indicator of recurrence-free and overall survival **(64)**. Obermair and co-workers have also demonstrated the

microvessels density correlates inversely with disease-free survival in breast cancer **(65)**. In addition, Fox and coworkers have shown an association between tumor angiogenesis and the presence of bone marrow micrometastases in breast cancer patients prior to surgery **(66)**.

Recognition that angiogenesis is essential to the growth of solid tumors has led to identification of angiogenic factors responsible for stimulation of new blood vessel formation. Of the identified angiogenic factors, vascular endothelial growth factor (VEGF; also known as vascular permeability factor) is the most potent and specific and has been identified as a crucial regulator of both normal and pathologic angiogenesis **(67)**. VEGF is a highly conserved, homodimeric, secreted, heparin-binding glycoprotein whose dominant isoform has a molecular weight of approximately 45,000 **(67, 68)**. VEGF produces a number of biological effects, including endothelial cell mitogenesis and migration, induction of proteinases leading to remodeling of the extracellular matrix, increased vascular permeability, and maintenance of survival for newly form blood vessels **(67)**. VEGF expression is regulated by hypoxia via molecular pathways similar to those regulating erythropoietin gene expressions **(67)**. The biologic effects of VEGF are mediated through binding and stimulation of two receptors on the surface of endothelial cells: Flt-1 (fms-like tyrosine kinase) and KDR (kinase domain region) **(67)**. Increased expression of VEGF has been measured in most human tumors examined to date, including tumors of lung, breast, thyroid, gastrointestinal tract, kidney, bladder, ovary, and cervix, as well as angiosarcomas and glioblastomas **(67)**. In breast cancer, VEGF mRNA and protein are highly expressed in invasive ductal carcinoma, metastatic ductal carcinoma, and comedo-type DCIS but not as highly in lobular carcinoma **(69)**. Normal breast ductal epithelium expresses VEGF mRNA at low levels and VEGF receptor mRNA expression has not been detected. Increased VEGF mRNA and protein levels have been shown to correlate with shortened relapsed-free survival **(70, 71)**.

1.3 Anti-VEGF Monoclonal Antibody

Inhibition of VEGF using an anti-VEGF monoclonal antibody blocks the growth of a number of human cancer cell lines in nude mice **(67)**. The human cancers represented by these cell lines that are growth-inhibited by anti-VEGF antibody include non-small cell lung cancer (Calu-6), colorectal cancers (LS174T), HM-7, LSLiM6), breast cancer (MCF-7), prostate cancer (D-145), head and neck cancer (KB), ovarian cancer (SK-OV-3), and others **(67)**. In addition, the combination of anti-VEGF antibody and chemotherapy in nude mice injected with human cancer xenografts results in an increased anti-tumor effect compared with antibody or chemotherapy treatment alone **(72)**.

To test the hypothesis that inhibition of VEGF in patients with cancer results in clinical benefit, a recombinant humanized version of a murine anti-human VEGF monoclonal antibody, named rhuMAb VEGF (Bevacizumab or Avastin®), was developed **(73)**. In cynomolgus monkey studies, twice weekly IV treatment with rhuMAb VEGF (doses of 2, 10, and 50 mg/kg) for either 4, 13, or 26 weeks was well tolerated with no overt signs of acute toxicity **(74, 75)**. There were no effects on body weight, food consumption, blood pressure, EKGs, rectal body temperature, respiratory rate, ophthalmologic observations, or clinical pathology parameters. In all active treatment groups, animals with open growth plates showed physeal dysplasia. Focal to diffuse

chondroid necrosis and linear fissuring of the cartilaginous growth plate were also observed. Females treated with 10 to 50 mg/kg twice weekly had decreased ovarian and uterine weights, which were associated with the absence of corpora lutea. Minor changes in some organ weights were noted in the 4-week study but were not produced in the 13- or 26- week study. No antibodies against rhuMAb VEGF were detected. To assess the effects on wound healing, rabbits were given a partial thickness dermal wound on the ear or on the back and treated with rhuMAbVEGF every other day for two weeks at doses from 0.5 to 50 mg/kg per day **(76)**. Dose-related inhibition of wound healing was exhibited following treatment with the antibody, with maximal inhibition observed at a dose level of 10 mg/kg per day. No specific tissue cross-reactivity with human, cynomolgus monkey, or rabbit tissue was observed with 400 μg/ml of the antibody. The results of non-clinical efficacy and toxicology studies supported entering clinical development.

- Clinical Trials with Avastin (Bevacizumab)

Avastin has been studied in a multitude of Phase I, II, and III clinical trials in more than 5000 patients and in multiple tumor types. In addition, data are available from 3,863 patients enrolled in two post-marketing studies in metastatic colorectal cancer (CRC). Approximately 130,000 patients have been exposed to Avastin as a marketed product or in clinical trials. The following discussion summarizes Avastin's safety profile and presents some of the efficacy results pertinent to this particular trial. Please refer to the Avastin Investigator Brochure for descriptions of all completed Phase I, II, and III trials reported to date.

Phase I studies: Two phase I trials were conducted. The first study was a dose escalation, single agent trial in patients with advanced malignancies to evaluate safety and pharmacokinetics **(77)**. Five dose levels were evaluated (0.1 to 10 mg/kg) and subjects received a total of four doses over a 42-day treatment period. The second study evaluated the combination with different chemotherapeutic agents in patients with advanced solid malignancies **(78)**. The antibody was administered as 8 weekly doses of 3 mg/kg. In general, Avastin was well tolerated in both studies (see Avastin toxicity profile below). No antibodies to Avastin were detected in either study. The pharmacokinetics of Avastin appeared to be linear for doses greater than 1 mg/kg, with a half-life of 15 days. The combination with chemotherapy agents did not modify the pharmacokinetic behavior of the antibody. Thus, these phase I studies demonstrated that Avastin was well tolerated and had predictable pharmacokinetics when used either as a single agent or in combination with cytotoxic agents.

Phase II studies: Four studies were conducted in NSCLC in combination with carboplatin and paclitaxel, hormone-refractory prostate cancer as a single agent, relapsed metastatic breast cancer as a single agent, and metastatic colorectal cancer in combination with 5-FU and Leucovorin **(79-81)**. Doses varied from 5 to 20 mg/kg every other week. Antitumor activity has been demonstrated in multiple tumor types and with both single agent Avastin and combination chemotherapy. In the prostate study, no patient had an objective response; however there were signs of modest activity. The NSCLC trial reported increased response rates and prolongation of time to disease progression compared with chemotherapy alone. In the colorectal trial the hazard of experiencing disease progression was reduced by 57% in patients receiving the combination of Avastin and 5-FU/Leucovorin compared with those receiving

chemotherapy alone. Improvements in response rate and median survival were also seen in these colorectal patients.

Phase III studies; *pivotal trials*: Two trials were conducted in metastatic colorectal cancer. The first one was a phase III randomized study of irinotecan/5-FU/Leucovorin (IFL) with or without Avastin in patients with newly diagnosed metastatic colorectal cancer **(82)**. Patients received up to 48 doses of Avastin on an every 2-week infusion schedule. Nine hundred twenty five patients were enrolled in the trial. The results for this study confirmed that the addition of Avastin to chemotherapy resulted in an increase in overall survival, response rate, and duration of response compared with patients who received chemotherapy alone. The combination resulted in a statistically significant prolongation of survival (p=0.00004) with a corresponding increase in median duration of survival from 15.6 months for patients treated with chemotherapy alone to 20.3 months for patients treated with the combination of Avastin and chemotherapy (hazard ratio of death of 0.660). The addition of Avastin to chemotherapy also resulted in a significant improvement in progression-free survival; median of 6.24 months for patients treated with chemotherapy alone and 10.5 months for patients treated with the combination. Similar increases were seen in overall response rate (35% vs. 45%), and duration of response (7.1 vs. 10.4 months). The addition of Avastin to the chemotherapy regimen resulted in a small increase in the incidence of grade 3 and 4 adverse events (from 74% to 85%). No increase was seen in adverse events leading to death or study discontinuation or in 60-day mortality. Grade 3 and 4 adverse events that were increased in the combination arm compared with the chemotherapy arm included deep thrombophlebitis, hypertension, diarrhea, and leucopenia. No significant differences were observed in the incidence of infusion-related events, thromboembolic events, grade 3 and 4 bleeding, or proteinuria of all grades. The addition of Avastin to chemotherapy appeared to increase the risk of the uncommon event of gastrointestinal perforation.

The second trial was a double-blind, randomized trial of 5-FU/Leucovorin with or without Avastin in patients with metastatic colorectal cancer who were not candidates for treatment with irinotecan as a first line **(83)**. The trial enrolled 209 patients. Patients received up to 48 doses of Avastin (5 mg/kg) on an every-2-week infusion schedule. The addition of Avastin resulted in a statistically significant prolongation of progression-free survival and trends towards improved survival, response rate, and duration of response compared with chemotherapy alone. The addition of the antibody was well tolerated. Grade 3 hypertension and gastro-intestinal perforation were increased by the addition of the antibody. Proteinuria and arterial thromboembolic events were also observed.

The results of these trials led to the approval of Avastin in combination with intravenous 5-FU-based chemotherapy by the FDA in 2004 for first-line treatment of patients with newly diagnosed colorectal cancer.

Additional data from Phase III trials in metastatic CRC (E3200), non–small cell lung cancer (NSCLC; E4599), and metastatic breast cancer (E2100) have also demonstrated clinical benefit from Avastin when added to chemotherapy. In Study E3200, the addition of Avastin to FOLFOX chemotherapy resulted in improved overall survival compared with FOLFOX alone

(13.0 vs. 10.8 months, respectively, HR = 0.75; $p < 0.01$) in a population of previously treated CRC patients.

There was also improved overall survival in first-line NSCLC patients (E4599) treated with carboplatin/paclitaxel + Avastin compared with chemotherapy alone (12.3 vs. 10.3 months, respectively; HR = 0.80; $p = 0.003$). The results from this trial were the basis for FDA approval of Avastin for use in combination with carboplatin + paclitaxel as first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic, non-squamous NSCLC in October 2006. Finally, patients with untreated metastatic breast cancer (E2100) who received Avastin in combination with weekly paclitaxel had a marked improvement in PFS compared with chemotherapy alone (13.3 vs. 6.7 months, respectively; HR = 0.48; $p < 0.0001$) (see the Avastin Investigator Brochure for additional details).

- Avastin in the Treatment of Breast Cancer

The monoclonal antibody has been evaluated in two clinical trials **(80, 83)**. The first trial was a phase II, dose-escalation study evaluating the safety, efficacy, and pharmacokinetics of Avastin in patients with metastatic breast cancer that relapsed after at least one conventional chemotherapy agent. 18 patients were enrolled at 3 mg/kg every other week, 41 patients were enrolled at 10 mg/kg every other week, and 16 patients were enrolled at 20 mg/kg every other week. The overall response rate was 9.3% (7 out of 75 patients). A PR in a cervical lymph node was observed in 1 out of 18 patients treated at the 3 mg/kg dose cohort. One CR and 4 PRs were observed among the 41 patients enrolled in the 10 mg/kg dose cohort. One PR was observed in the group of patients treated in the 20 mg/kg dose cohort. Toxicity observed in this trial was not different from the toxicity in all other trials. Pharmacokinetic analysis suggested that the profile was similar to that in the colorectal and NSCLC phase II trials. Thus, this trial showed evidence of single-agent activity of Avastin in metastatic breast cancer and provided the rationale for a phase III trial using Avastin in combination with chemotherapy in this setting.

The first Phase III trial in breast cancer was a randomized, open-label study that evaluated safety and efficacy of Avastin in combination with capecitabine in patients with metastatic disease previously treated. Patients received up to 35 doses of Avastin on an every-3-week infusion schedule (15 mg/kg per dose). The trial enrolled 462 patients. The results of the primary efficacy analysis showed that the treatment with the combination of Avastin and capecitabine did not result in a statistically significant prolongation in progression-free survival compared with capecitabine alone. There was a statistically significant increase in the secondary endpoint of objective response rate; however, this did not result in an improvement in progression-free survival. The adverse event profile was similar to that for the completed phase III trials.

More recently Dr. K Miller presented the results of an ECOG phase III trial (E2100) in which patients with untreated metastatic breast cancer were randomized to paclitaxel versus paclitaxel and Avastin were presented (Proc ASCO 2005 and Breast Cancer 94(1): Abstract #3, 2005). A total of 722 women with recurrent or metastatic breast cancer who had not previously received systemic chemotherapy for their recurrent or metastatic disease were enrolled in this study between December 2001 and May 2004. Patients whose tumors overexpressed HER-2 were not included in the study unless they had previously received trastuzumab (Herceptin^{TM}) or were

unable to receive trastuzumab. Also excluded were patients who had received adjuvant chemotherapy treatment with paclitaxel within the previous 12 months, as well as patients with a prior history of blood clots or who were anticoagulated. In this trial the combination showed statistically significant prolongation of disease free survival (10.9 months vs. 6.1 months) and response rate (28.2% versus 14.2%). The tolerance of the combination was excellent. Avastin combination was well tolerated and now is being evaluated in the adjuvant setting. Thus, efficacy of Avastin has been demonstrated in breast cancer.

A pilot trial using the combination of Letrozole and Avastin (15 mg/kg every 3 weeks) in the neoadjuvant setting for post-menopausal, ER positive patients with locally advanced breast cancer was initiated in our institution at the end of 2005. Although it is early to evaluate efficacy at this time (August 2006), the combination has showed to be safe in the 18 patients enrolled (same toxicity profile of Letrozole and Avastin). Of the 18 patients, 8 have completed therapy per protocol and of those 2 had a pCR, 3 had a very good partial response, 2 had stable disease and 1 progressed within the first 12 weeks of therapy and was taken off the study at that time. Of the other 10 patients, 2 came out of the study due to refractory hypertension and 8 are in active therapy now. Of the 8 patients that had surgery, none has had a problem with healing. The Avastin dose selected for this trial was based on the pharmacokinetic data provided by Genentech (similar to 10 mg/kg every 2 weeks) as well as the experience in previous clinical trials. It is anticipated that preliminary results will be presented at the 2007 ASCO annual meeting.

The safety of the combination between Letrozole and Avastin has also been demonstrated by Traina et al (2006 proceedings of the ASCO annual meeting, abstract #3050). Metastatic breast cancer patients were eligible for the trial. Therapy consisted of letrozole (2.5 mg daily) and Avastin (15 mg/kg IV q3 weeks). The primary endpoint was frequency of Grade (Gr) 4 toxicity. Thirty two patients were enrolled and at the time of publication 28 were evaluable. After a median of 8 cycles, therapy-related toxicities observed were: Gr 2: hypertension 4, headache 4, proteinuria 3, fatigue 6, joint pain 5, hot flashes 1, epistaxis 1; Gr 3: hypertension 5, headache 1, proteinuria 1. There was no therapy-related Gr 4/5 toxicity. They concluded that the combination was safe and well tolerated.

- Avastin Safety Profile

In the initial Phase I and II clinical trials, four potential Avastin-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of Avastin as well as spontaneous reports have further defined the safety profile of this agent. Avastin-associated adverse events identified in phase III trials include congestive heart failure (CHF), gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). These and other safety signals are described in further detail as follows and in the Avastin Investigator Brochure.

Hypertension: Hypertension has been commonly seen in Avastin 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) (Ozcan et al., 2006; Glusker et al., 2006). 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 Avastin.

There is no information on the effect of Avastin in patients with uncontrolled hypertension at the time of initiating Avastin therapy. Therefore, caution should be exercised before initiating Avastin therapy in these patients. Monitoring of blood pressure is recommended during Avastin therapy. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without Avastin.

Temporary interruption of Avastin therapy is recommended in patients with hypertension requiring medical therapy until adequate control is achieved. If hypertension cannot be controlled with medical therapy, Avastin therapy should be permanently discontinued. Avastin should be permanently discontinued in patients who develop hypertensive crisis or hypertensive encephalopathy.

Proteinuria: An increased incidence of proteinuria has been observed in patients treated with Avastin compared with control arm patients. In the Avastin-containing treatment arms of clinical trials (across all indications), the incidence of proteinuria (reported as an adverse event) was up to 38% (metastatic CRC Study AVF2192g). The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been grade 1. NCI-CTC Grade 3 proteinuria was reported in up to 3% of Avastin-treated patients, and Grade 4 in up to 1.4% of Avastin-treated patients. The proteinuria seen in Avastin clinical trials was not associated with renal impairment and rarely required permanent discontinuation of Avastin therapy. Avastin should be discontinued in patients who develop Grade 4 proteinuria (nephrotic syndrome).

Patients with a history of hypertension may be at increased risk for the development of proteinuria when treated with Avastin. There is evidence from the dose-finding, Phase II trials (AVF0780g, AVF0809s, and AVF0757g) suggesting that Grade 1 proteinuria may be related to Avastin dose.

Proteinuria will be monitored by urine protein:creatinine (UPC) ratio at least every 6 weeks. If the UPC ratio is not available, a dipstick urinalysis may be used to allow treatment to proceed.

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 Avastin in the colorectal cancer trials and, to a lesser extent, in patients treated with Avastin in NSCLC and breast cancer trials.

Venous thromboembolism (including deep venous thrombosis, pulmonary embolism, and thrombophlebitis: 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 Avastin plus chemotherapy compared with chemotherapy alone (19% vs. 16%).

In Study AVF2107g, a Phase III, pivotal trial in metastatic CRC, VTE events, including deep venous thrombosis, pulmonary embolism, and thrombophlebitis, occurred in 15.2% of patients receiving chemotherapy alone and 16.6% of patients receiving chemotherapy + Avastin.

The incidence of NCI-CTC Grade ≥ 3 venous VTE events in one NSCLC trial (E4599) was higher in the Avastin-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%). One event (0.2%) was fatal in the Avastin-containing arm; not fatal events were reported in the carboplatin/paclitaxel arm (see Avastin Investigator Brochure). In metastatic CRC clinical trials, the incidence of VTE events was similar in patients receiving chemotherapy + Avastin and those receiving the control chemotherapy alone.

In clinical trials across all indications the overall incidence of VTE events was 2.8%–17.3% in the Avastin-containing arms compared with 3.2%–15.6% in the chemotherapy control arms. The use of Avastin with chemotherapy does not substantially increase the risk of VTE event compared with chemotherapy alone. However, patients with metastatic CRC who receive Avastin and experienced a VTE event may be at higher risk for recurrence of VTE event.

Arterial Thromboembotic Events: An increased incidence of ATE events was observed in patients treated with Avastin compared with those receiving control treatment. ATE events include cerebrovascular accidents, myocardial infarction, transient ischemic attacks (TIAs), and other ATE events. In a pooled analysis of data from five randomized Phase II and III trials (mCRC [AVF2107g, AVF2192g, AVF0780g]; locally advanced or metastatic NSCLC [AVF0757g]; metastatic breast cancer [AVF2119g]), the incidence rate of ATE events was 3.8% (37 of 963) in patients who received chemotherapy + Avastin compared with 1.7% (13 of 782) in patients treated with chemotherapy alone. ATE events led to a fatal outcome in 0.8% (8 of 963) of patients treated with chemotherapy + Avastin and 0.5% (4 of 782) of patients treated with chemotherapy alone. Cerebrovascular accidents (including TIAs) occurred in 2.3% of patients treated with chemotherapy + Avastin and 0.5% of patients treated with chemotherapy alone. Myocardial infarction occurred in 1.4% of patients treated with chemotherapy + Avastin compared with 0.7% of patients treated with chemotherapy alone (see the Avastin Investigator Brochure for additional details).

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, similarly 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 (Hambleton et al., 2005). Further analyses of the effects of concomitant use of Avastin 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 Avastin and chemotherapy. Avastin should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory process and gastrointestinal perforation to Avastin has not been established. Nevertheless, caution should be exercised when treating patients with intra-abdominal inflammatory processes with Avastin. Gastrointestinal perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, breast, and NSCLC) and may be higher in incidence in some tumor types.

Fistula: Avastin use has been associated with serious cases of fistulae including events resulting in death. Fistulae in the GI tract are common (1%–10% incidence) in patients with metastatic CRC, but uncommon (0.1%–1%) or rare (0.01%–0.1%) in other indications. In addition, fistulae that involve areas of the body other than the GI tract (e.g., tracheoesophageal, bronchopleural, urogenital, biliary) have been reported uncommonly (0.1%–1%) in patients receiving Avastin in clinical studies and postmarketing reports. Events were reported at various timepoints during treatment, ranging from 1 week to > 1 year following initiation of Avastin, with most events occurring within the first 6 months of therapy.

Permanently discontinue Avastin in patients with tracheoesophageal fistulae or any Grade 4 fistula. Limited information is available on the continued use of Avastin in patients with other fistulae. In cases of internal fistula not arising in the GI tract, discontinuation of Avastin should be considered.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in patients receiving Avastin. 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 Avastin did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone (Scappaticci et al., 2005). Surgery in patients currently receiving Avastin is not recommended. No definitive data are available to define a safe interval after Avastin exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of Avastin is 20 days. Avastin should be discontinued in patients with severe wound healing complications.

If patients receiving treatment with Avastin require elective major surgery, it is recommended that Avastin be held for 4–8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin or restart Avastin until 4 weeks after that procedure (in the case of high-risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 weeks and Avastin no earlier than 8 weeks after surgery).

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

Tumor-Associated Hemorrhage: Major or massive pulmonary hemorrhage or hemoptysis has been observed primarily in patients with NSCLC. Life-threatening and fatal hemoptysis was identified as a Avastin-related adverse event in NSCLC trials. Tumor-associated hemorrhage was observed in phase I and phase II Avastin 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 Avastin. 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.

GI hemorrhages, including rectal bleeding and Melena have been reported in patients with CRC, and have been assessed as tumor-associated hemorrhages.

Mucocutaneous Hemorrhage: Across all Avastin clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with Avastin. These were most commonly NCI-CTC grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in Avastin treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Reversible Posterior Leukoencephalopathy Syndrome: There have been rare reports of Avastin-treated patients developing signs and symptoms that are consistent with RPLS, a rare neurologic disorder that can present with the following signs and symptoms (among others): seizures, headache, altered mental status, visual disturbance, or cortical blindness, with or without associated hypertension. Brain imaging is mandatory to confirm the diagnosis of RPLS. In patients who develop RPLS, treatment of specific symptoms, including control of hypertension, is recommended along with discontinuation of Avastin. The safety of reinitiating Avastin therapy in patients previously experiencing RPLS is not known (Glusker et al. 2006; Ozcan et al. 2006).

Congestive heart failure: CHF has been reported in Avastin 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 Avastin for metastatic breast cancer, 7 subjects (3.1%) who received capecitabine plus Avastin 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 Avastin. All but one of these subjects had significant prior exposure to anthracyclines as well (Karp et al., 2004). Other studies are ongoing in this patient population. Patients receiving anthracyclines or

with prior exposure to anthracyclines should have a baseline MUGA or ECHO with a normal ejection fraction.

In a randomized, Phase III trial of patients with previously untreated metastatic breast cancer (E2100), the incidence of LVEF decrease (defined as NCI-CTC Grade 3 or 4) in the paclitaxel + Avastin arm was 0.3% for the paclitaxel alone arm.

No information is available on patients with preexisting CHF of New York Heat Association (NYHA) Class II-IV at the time of initiating Avastin therapy, as these patients were excluded from clinical trials.

Two additional studies investigated concurrent administration of anthracyclines and Avastin. In 21 patients with inflammatory breast cancer treated with neoadjuvant docetaxel, doxorubicin, and Avastin, no patients developed clinically apparent CHF; however, patients had asymptomatic decreases in LVEF to < 40% (Wedam et al. 2004). In a small Phase II study in patients with soft tissue sarcoma, 2 of the 17 patients treated with Avastin and high-dose doxorubicin (75 mg/m^2) developed CHF (one Grade 3 event after a cumulative doxorubicin dose of 591 mg/m^2, one Grade 4 event after a cumulative doxorubicin dose of 420 mg/m^2); an additional 4 patients had asymptomatic decreases in LVEF (D'Adamo et al. 2004).

Other studies in patients with various tumor types and either a history of anthracycline exposure or concomitant use with Avastin are ongoing.

Patients receiving concomitant anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA scans or echocardiograms (ECHOs) with a normal LVEF.

Neutropenia: Increased rates of severe neutropenia, febrile neutropenia, or infection with severe neutropenia (including some fatalities) have been observed in patients treated with some myelotoxic chemotherapy regimens plus Avastin in comparison to chemotherapy alone (Sandler et al. 2006).

Additional Adverse Events: See the Avastin Investigator Brochure for additional details regarding the safety experience with Avastin.

1.4 Hypothesis for This Study

Preclinical and clinical data (described in the background of this protocol) have demonstrated that up-regulation of tumor cell VEGF is an important mechanism to subvert estrogen dependence in hormone responsive breast cancer resulting in reduced therapy response or tumor resistance to hormonal therapy; thus, we hypothesize that the combination of an anti-VEGF agent (Avastin, an anti-VEGF monoclonal antibody) and hormonal therapy should be more effective than hormonal therapy alone for the treatment of breast cancer.

1.5 Study Rationale

Despite the enormous advances in hormonal therapy for breast cancer, impaired response and development of resistance to endocrine manipulation is at the core of the problem and must be overcome to make additional progress; thus, a number of endocrine resistant modulators are now under development (e.g., signal transduction inhibitors, agents to down-regulate VEGF, etc). Developing different combination of agents is a challenge because we still do not have a clear understanding of the molecular basis of resistance.

The development of anti-VEGF therapy to overcome resistance to endocrine therapies is of potentially great importance. The unique mechanism of action, limited toxicity and preclinical and clinical data generated with Avastin, and the pre-clinical and clinical observations by Kern, Berns and Qu demonstrating correlation between VEGF expression and response to hormonal therapy, support the hypothesis that VEGF expression by breast cancers is a potential mechanism for estrogen independence of breast tumors. Interference with VEGF activity would thus improve and/or restore response anti-estrogen drug therapy. **We therefore hypothesize that the efficacy of hormonal therapy in women with ER+ and/or PR+ breast cancer can be augmented by means of Avastin, a recombinant humanized antibody to vascular endothelial cell growth factor. As described below, neoadjuvant therapy is a very attractive model to evaluate the hypothesis.**

2.0 OBJECTIVES

2.1 Primary

In post-menopausal patients with pathologically confirmed invasive ductal carcinoma or invasive lobular carcinoma of the breast (T2, T3, T4a-c, N0-2, and M0, excluding patients with T4d or inflammatory breast cancer) whose tumors are estrogen receptor and/or progesterone receptor positive, Her-2-neu negative, we will determine pathological complete response to a maximum of 24 weeks of neoadjuvant therapy using a combination of letrozole (Femara®) and Avastin or letrozole alone.

2.2 Secondary

1. Determine the clinical objective response rate to a maximum of 24 weeks of neoadjuvant therapy using letrozole and Avastin or letrozole alone.

2. Determine the tolerability and toxicity of the combination of letrozole and Avastin compared to letrozole alone.

3. Evaluate specific biomarkers for prognostic value and as markers for response/resistance to the combination of letrozole and Avastin or letrozole alone.

3.0 STUDY DESIGN

3.1 Description of the Study

This will be an open label, randomized, Phase II study of the combination of Letrozole and Avastin versus Letrozole alone in post-menopausal patients with newly diagnosed and pathologically confirmed invasive breast cancer (T2, T3, T4a-c, N0-2, and M0), estrogen receptor and/or progesterone receptor positive, Her-2-neu negative (0, 1 or 2 + by IHC or non-amplified by FISH). Patients with inflammatory breast cancer will not be included (T4d). The primary objective for this randomized phase II trial is to estimate the pathological complete response rates, defined as the percentage of patients with a complete pathological response at the time of surgery, for each of the two arms in the trial (see Section 11 – Statistical Section).

Patients must be post-menopausal (no peri- or pre-menopausal) and 50 years or older, have measurable disease by mammogram and/or ultrasound (in special cases with a clear clinical indication, dedicated breast MRI may be used – not required per protocol), and have a performance status of ECOG 0 or 1. Patients previously treated or patients with no measurable disease or patients with metastatic disease will be excluded.

Patients will be registered through the Clinical Trials Network (CTN) Office of the UAB Comprehensive Cancer Center where the CTN Manager will register the patient in the trial (will randomize and complete the randomization forms). Monthly accrual will be communicated to the Breast Cancer Research Consortium and Genentech. Within eight weeks before the initiation of the therapy patients will have a PET/CT scan or [CT of the chest, abdomen, pelvis, with a bone scan]. Patients with metastatic disease will not be enrolled in the trial. Additionally, blood counts, UA, urinary protein/creatinine ratio (24-hour urine collection if indicated), INR and CMP will be obtained within 4 weeks before initiation of therapy. Echocardiogram and ultrasound of the breast within 8 weeks before therapy will be also obtained, and they will be used as baseline evaluations. Core needle biopsies will be performed in order to provide adequate tissue for standard diagnosis (H&E, ER/PR receptors, Her-2-neu status) as well as to provide tissue for the biomarker assays (tumor blocks and fresh tissue). If the patient agrees to consent, a core needle biopsy of the upper quadrant of the unaffected breast will be obtained for comparison of biomarkers.

Patients with palpable axillary lymph nodes or patients with FNA positive axillary lymph nodes will be treated as having at least N1 disease and are not required to undergo sentinel lymph node biopsy prior to initiation of neoadjuvant therapy; axillary lymph node dissection will be performed in those patients at the time of definitive surgery. Sentinel lymph node biopsy is to be performed at the discretion of the treating physician according to institution protocol. A sentinel node procedure will not be required on non-palpable nodes before initiation of protocol therapy. However, if a sentinel node procedure is performed for non-palpable modes, then Letrozole can be started immediately post sentinel node procedure with Avastin being able to begin 2 weeks post sentinel node procedure. Post-surgical adjuvant therapy will be administered at the investigator's discretion (chemotherapy is allowed).

Patients meeting the eligibility criteria and who have signed the consent form will start Letrozole 2.5 mg PO a day and Avastin 15 mg/kg IV every 3 weeks or Letrozole 2.5 mg PO alone. At six weeks patients will have the first evaluation of response with physical exam, and ultrasound of the breast. In addition, core biopsies will be obtained for evaluation of biomarkers. Patients with objective response or stable disease in the first evaluation (physical evaluation and

ultrasound) according to the RECIST criteria will continue the same regimen with restaging every 6 weeks for a total of 24 weeks or until progression is observed. If during the second evaluation (12 weeks) an objective response or stable response is observed, the patient will complete therapy, but if during the 12 week evaluation the patient has disease progression the patient will be removed from the protocol and will be treated at the investigator's discretion. If a patient has a clinical response with tumor size decreasing to less than 2 cm, a metal coil will be placed within the tumor to aid in identification of the primary site at the time of the operation. Patients with disease progression according to the RECIST criteria will be removed from the study and will be treated at the discretion of the treating physician. Definitive surgery of the primary tumor will be performed at the discretion of the surgeon no sooner than 4 weeks after the last Avastin dose (fourth evaluation on week 24). Patients will continue letrozole during those 4 weeks before the surgery. No dose reductions are planned.

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies: 24 weeks of therapy, disease progression, intercurrent illness that prevents further administration of treatment, any toxicity that results in a treatment delay of > 3 weeks, unacceptable grade 3 or 4 non-hematologic adverse events(s), patient decides to withdraw from the study for any reason, or general or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

3.2 Rationale for Study Design

Neoadjuvant endocrine therapy is an appealing context to conduct research in this area because clinical outcomes can be obtained within a few months of treatment, and repeated images and tumor sampling for biomarker analysis can be readily achieved. There is now substantial evidence that the responses seen in the neoadjuvant setting can be used to accurately predict disease free survival, dramatically shortening the typical time it takes to derive information from a clinical trial of a novel agent/regimen from years to 4-6 months.

In this trial, we will use a novel combination of hormonal agent (letrozole) and an anti-VEGF monoclonal antibody (Avastin) in the neoadjuvant setting based on pre-clinical and clinical observations previously described in the background and the data will be compared with that obtained from patients treated with letrozole alone. In choosing this combination we hope the combination will be synergistic and will demonstrate higher efficacy than the use of the hormonal agent alone. We will investigate modulation of breast cancer biomarkers during neoadjuvant therapy for newly diagnosed breast cancer (see appendix I and section 6.2 for a detailed description). Tissue will be obtained before initiation of therapy, and 6 weeks after day 0 of therapy. Specific markers of interest in the paraffin embedded tissue include: marker of proliferation (Ki-67), apoptotic markers (Tunnel, caspase 3, bcl-2 and bax), and a novel prognostic factor (Zinc finger protein GKLF/KLF-4, a novel oncogene recently described by investigators at UAB). In addition, we will plan on coding, processing, and banking fresh frozen tissue; this stored tissue will be transferred to Dr. Charles Perou (University of North Carolina at Chapel Hill). Tissue stored in this fashion will be used for examination of differential gene expression patterns. In addition, circulating tumor cells will be evaluated before and after therapy (These assays will be performed by Dr. Hope Rugo, University of California at San Francisco).

Although the majority of the clinical trials published at this time have used neoadjuvant hormonal therapy for a period of 3 to 4 months before surgery, we have decided to allow the patients enrolled in this trial to receive therapy for up to 6 months based on: 1. the published data (described in the background) which suggested that extended therapy with an AI in the neoadjuvant setting for up to 12 months is safe with continued tumor regressions, and that patients whose tumors respond to letrozole after 3 months can expect a further reduction in tumor volume with continued therapy; 2. The fact that tumor response to biological agents is slow; and 3. The safety rule included in this trial in which patients who have not had a response at the 12 week evaluation will be taken off the study. In addition, the Avastin dose of 15 mg/kg every three weeks was selected based on: 1. previous clinical studies in which the dose demonstrated to be safe and effective, 2. Pharmacokinetic data which demonstrated that this dose is pharmacologically equivalent to the 10 mg/kg every two weeks.

We believe this approach will allow a relatively rapid assessment of a novel regimen (translational research) and aid in identifying surrogate endpoint markers which will enhance our ability to select patients who are likely to benefit from these interventions.

3.3 Outcome Measures

3.3.1 Primary Outcome Measures

Pathological complete response is defined as no evidence of residual invasive tumor in the breast or axillary lymph nodes or only residual ductal carcinoma in-situ.

3.3.2 Secondary Outcome Measures

The clinical response will be based on the Response Evaluation Criteria in Solid Tumors (RECIST Criteria) described in Appendix H.

4.0 SAFETY PLAN

Based on the toxicity profile of Avastin (see section 1.3), a number of measures will be taken to ensure the safety of the patients enrolled in this trial. These measures will be addressed through exclusion criteria (see section 5.3) and routine monitoring as follows:

Patients enrolled in the trial will be carefully monitored during the entire treatment period and will be followed as is appropriate. Safety evaluations will consist of medical interviews, collection of adverse events, PE, VS, and laboratory measurements (see Section 8). Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of the participation in the study. Patients discontinued from treatment for any reason will be evaluated approximately 30 days (28-42 days) after the last dose of Avastin (see section 8.3). Specific monitoring procedures are as follow:

- Hypertension will be monitored through routine evaluation of blood pressure prior to each Avastin treatment. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without Avastin.
- In patients with bleeding, hemostasis evaluation should be performed as clinically indicated.
- Proteinuria will be monitored through regular urinalysis and urinary protein/creatinine ratio or dipstick at least every 6 weeks (before the administration of Avastin).
- Patients who have an ongoing Avastin-related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed (see Section 7).
- If patients on treatment with Avastin require major surgery or choose elective major surgery, it is recommended that Avastin be held for 4-8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin/restart Avastin until 4 weeks after that procedure (in the case of high risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 weeks and that Avastin be restarted no earlier than 8 wk after surgery).

Please see Section 7 for detailed instructions for the management of study drug–related toxicities.

4.1 Protocol Management and Oversight of Participating Affiliate Sites

Dr. Andres Forero functions as the sponsor of the trial at UAB and at each affiliate. Each affiliate will utilize their respective IRBs of record. Appendix J provides guidelines for off-site conduct of protocols sponsored by UAB Cancer Center faculty.

5.0 STUDY SUBJECTS

5.1 Subject Selection and Registration

At the time of study entry, the patient will be evaluated by a multidisciplinary team of medical oncologists, radiation oncologists, and surgical oncologists. Before registration investigators are required to indicate the type of operation they intend to perform (lumpectomy or mastectomy). Patients will be consented by the investigator and the coordinator of the trial.

Patients will be registered through the Clinical Trials Network (CTN) Office of the UAB Comprehensive Cancer Center where the CTN Manager will register the patient in the protocol, complete the randomization forms, and notify the sponsor-investigator. Prior to any treatment, patients will be randomized and registered with the CTN Manager (Pam Dixon, Tel: 205-975-5387; Fax: 205-975-9875; Email: pam.dixon@ccc.uab.edu), between 8:00 A.M. and 4:30 P.M. central time, Monday through Friday.

5.2 Inclusion Criteria

All patients must meet the following criteria to be eligible for study entry:

5.2.1 Pathologically confirmed invasive ductal carcinoma or invasive lobular carcinoma of the breast, T2-T3 / T4a-c / N0-2 / M0, with positive estrogen and/or progesterone receptors, and Her-2-neu negative. Patients with inflammatory breast cancer will not be included (T4d). Patients previously treated patients with no measurable disease or patients with metastatic disease will be excluded.

5.2.2 Give written informed consent prior to study specific screening procedures, with the understanding that the patient has the right to withdraw from the study at any time, without prejudice (see Appendix B – consent form).

5.2.3 Patients must be postmenopausal, defined as one of the following:

- Patients $\geq$ 50 years of age with no spontaneous menses for at least 12 months,
- Bilateral oophorectomy

5.2.4 Be ambulatory (outpatient) and have an ECOG PS $\leq$1 (Appendix F).

5.2.5 Patients must have measurable disease by mammogram and/or breast ultrasound (in special cases a dedicated breast MRI may be clinically indicated). The target lesion must not have been previously irradiated.

5.2.6 No prior chemotherapy.

5.2.7 Patients must have adequate organ and marrow function as defined as follows: absolute neutrophil count $\geq$ 1,500/mm^3, hemoglobin $\geq$ 8.0 g/dl, platelets $\geq$ 75,000/mm^3, total bilirubin $\leq$ 2 mg/dl, serum creatinine $\leq$ 2 mg/dl, Transaminases (AST, ALT) may be up to 2 x institutional upper limit of normal. In addition < 1 gr of protein in 24 hr urine collection and urine protein/creatinine ratio $\leq$ 1.0 (see Appendix E). No evidence of proteinuria as evidenced by either urine protein/creatinine ratio <1 or urine proteinuria greater than 2+. Patients who have $\geq$ 2+ proteinuria should have a 24-hour urine collection and must demonstrate $\leq$ 1g of protein in 24 hours to be eligible.

5.2.8 No life threatening parenchymal disease or rapidly progressing disease warranting cytotoxic chemotherapy.

5.2.9 Hypertension must be controlled (<150/100 mmHg).

5.2.10 Ejection Fraction $\geq$ 50% by echocardiogram/MUGA. (LVEF greater than 75% at baseline should be reviewed and/or the test repeated as it may be falsely elevated).

5.2.11 No history of thrombosis during the previous 12 months.

5.3 Exclusion Criteria

5.3.1 Current, recent (within 4 weeks of the first infusion of this study), or planned participation in an experimental drug study other than this sponsor-investigator Avastin cancer study.

5.3.2 Uncontrolled high blood pressure (>150/100 mmHg).

5.3.3 Prior history of hypertensive crisis or hypertensive encephalopathy

5.3.4 Unstable angina

5.3.5 New York Heart Association (NYHA) Grade III or greater congestive heart failure (see Appendix G)

5.3.6 History of myocardial infarction or unstable angina within 12 months

5.3.7 History of stroke or TIA

5.3.8 Clinically significant peripheral vascular disease
5.3.9 History of a bleeding disorder

5.3.10 Presence of central nervous system or brain metastases

5.3.11 Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Day 0, anticipation of need for major surgical procedure during the course of the study

5.3.12 Minor surgical procedures (excluding fine needle aspirations or core biopsies) within 5 days prior to Day 0

5.3.13 Pregnant (positive pregnancy test) or lactating

5.3.14 Urine protein: creatinine ratio ≥1.0 at screening and patients demonstrating > 1 gr of protein in 24 hr urine collection within 4 weeks prior to study entry will not participate in the trial.

5.3.15 History of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within 6 months prior to Day 1

5.3.16 Serious, non-healing wound, ulcer, or bone fracture

5.3.17 Unwilling or unable to comply with the protocol for the duration of the study.

5.3.18 Psychiatric illness/social situations that would limit compliance with study requirements.

5.3.19 History of another malignancy within the last five years except non-melanoma skin cancer and carcinoma in–situ of uterine cervix. Second primary breast cancers are allowed regardless of the number of years since they were first diagnosed.

5.3.20 Patients with metastatic disease.

5.3.21 Life expectancy of less than 12 weeks

5.3.22 Known hypersensitivity to any component of Avastin

6.0 STUDY DESIGN

6.1 Treatment Plan

This will be an open label, randomized, Phase II study of the combination of Letrozole and Avastin versus letrozole alone in patients with newly diagnosed breast cancer (randomization 2:1). It is anticipated that with 50 evaluable patients receiving the combination therapy and 25 evaluable patients receiving letrozole as a single agent (total 75 evaluable patients). Accrual is expected to be completed in 24 months (see Section 11 on statistics). Patients meeting the eligibility criteria and who have signed the consent form will start Letrozole 2.5 mg PO daily alone or in combination with Avastin 15 mg/kg IV every 3 weeks for 24 weeks. After neoadjuvant therapy, patients will undergo surgical treatment and will receive adjuvant therapy according to the treating physician. It is anticipated an accrual rate of 4 patients a month.

6.2 Biopsy Specimens and Research Blood Samples (See Appendix I for detailed description of sample timing, processing and shipping)

Core biopsies will be obtained before initiation of therapy and 6 weeks after initiation of therapy. Biopsy samples will preferably be obtained using a 14-18 gauge core needle; at least two core biopsies will be obtained for frozen research biopsies and two for the preparation of the paraffin blocks. If blocks from original diagnosis (before enrolling in the trial) are available, they will be obtained and then only samples for the frozen research biopsies will be obtained. Processing of the specimens is described in Appendix I. Paraffin blocks will be sent to UAB while frozen research biopsies will be sent to Dr. Charles Perou (Departments of Genetics and Pathology, Lineberger Comprehensive Cancer Center, University of North Carolina Chapel Hill, NC).

Tissue sections obtained as described in Appendix I from paraffin-embedded material will be sectioned and stained for a marker of proliferation (Ki67), apoptosis markers (TUNEL, caspase 3 bcl-2, bax), and a novel prognostic factor (zinc finger protein GKLF/KLF4, a novel oncogene recently described by investigators at UAB) by conventional IHC. Frozen research biopsies will be used for gene expression array analysis and will be performed as previously described (97). Briefly, total RNA will be isolated from the research biopsies. Dr. Perou has successfully analyzed specimens using tissue from ½ of a core biopsy (approximately 5 mg tissue). The isolated RNA from the breast tumors will be reverse transcribed to cDNA using the incorporation of a fluorescently tagged “red” dye (Cy5). A "common reference" sample cDNA will be prepared from RNA pooled from 11 diverse human cell lines, and fluorescently tagged with a “green” dye (Cy3). The red-tagged cDNA from the breast tumor will be hybridized on a microarray with the green-tagged "common reference". The ratio of fluorescence intensity at each gene on the array will be measured for the data analysis. The DNA microarray will be obtained from UNC/LCCC Genomics Core Facility. The analysis of gene expression profiles based on the DNA microarray data will be done using statistical analysis and hierarchical

clustering. Hierarchical clustering analysis allows individual tumors to group together based on common and recurrent gene expression patterns. Identification of the breast cancer subtype using previously established criteria (97) and comparison of changes in profile induced by the research agents will be performed.

Gene expression Profiling Analyses. In this study, patients will be randomized to receive letrozole, or letrozole plus Avastin. To examine the possibility that gene expression patterns might be able to identify those patients who would gain a benefit from Avastin, we propose to perform DNA microarray analyses on the pretreatment biopsies and perform supervised analyses to identify gene patterns that might correlate with response to Avastin. We will perform an exploratory study of the available microarray data in order to identify a set(s) of genes that are predictive of response or resistance to Avastin. The clinical endpoint used will be pCR vs. no pCR, and clinical response classified as SD+PD vs. CR+PR measured after the first phase of treatment, and after all neoadjuvant treatments are complete. To predict response, the expression data for the pre-treatment samples will be used and the "supervising parameter" will be pathologic complete response (pCR). Four statistical classification methods are used to develop predictors of response when using the pre-treatment gene expression data: a k-Nearest Neighbor Classifier (k-NN with k=1, 3, 5, or 7) with either Euclidean distance or one-minus-Spearman-correlation as the distance function, and a Class Nearest Centroid (CNC) classifier with either Euclidean distance or one-minus-Spearman-correlation as the distance function.

To evaluate the gene expression predictor accuracy, each of the four prediction methods undergoes 10-fold cross validation (CV) where in a given round of CV, each predictor uses *n* genes (with the *n* genes selected using the ratio of between-group to within-group sums of squares {105}) obtained from training on 90% of the samples, which are then used to make predictions on the remaining 10%. This procedure is repeated 9 more times such that every sample is "left out" exactly once. The prediction accuracies on the withheld samples for each of the 10 iterations are averaged and this average prediction accuracy recorded for each prediction method with each *n* gene set. *n* is increased and decreased for many subsequent rounds of CV. For each supervising parameter/response variable, the set of *n* genes that gives the highest average prediction accuracy during CV is determined and reported for each prediction method. Using this approach, we have created centroid predictors that could classify tumors (1) into intrinsic subtypes that predict outcomes and show high concordance with the GHI RS and NKI 70-gene profiles, (2) according to novel patterns of estrogen-regulated genes that predict outcomes in tamoxifen-treated patients, and (3) according to lymph node metastasis status. Thus, we will be able to determine if gene expression patterns are associated with response to Avastin. Lastly, we will also compare pretreatment samples versus during treatment, and post treatment samples if they exist, in order to identify a set of genes, or sets of genes, that change in response to the administration of letrozole, and the addition of letrozole and Avastin.

Circulating tumor cells (CTC) from whole blood will be processed for immunohistochemistry and RNA-based studies and compared to results obtained using the automated Veridex method for enumeration of circulating tumor cells.

Patients will be allowed to start Avastin therapy next day after core biopsies.

7.0 STUDY MEDICATION

7.1 Avastin Dosage and Formulation

Avastin will be supplied by Genentech as a clear to slightly opalescent, colorless to pale brown, sterile liquid concentrate for parenteral administration. Avastin will be supplied in 5 cc (100 mg), 20 cc (400 mg), and 50 cc (1000 mg) glass vials with a 4 ml, 16 ml, or 40 ml of Avastin, respectively (all at 25 mg/ml). The formulation contains sodium phosphate, trehalose, polysorbate 20, and sterile water for injection, USP. Vials contain no preservative and are suitable for single use only. The monoclonal antibody (Avastin) being administered is the commercially marketed drug (Avastin). For further details and molecule characterization, see the Avastin Investigator Brochure.

The dose of Avastin administered in this study is 15 mg/kg given by intravenous infusion once every 3 weeks for up to 24 weeks. The patient's weight at screening will be used to determine the dose of Avastin to be used for the duration of the study; however, if a patient's weight changes by 10% during the study, the dose of Avastin will be recalculated. Treatment will be administered on an outpatient basis.

7.1.1 Avastin Administration

Avastin will be diluted in 0.9% sodium chloride injection, USP, to a total volume of 100 ml. Avastin will be administered as a continuous IV infusion. Anaphylaxis precautions should be observed during the study drug administration. The initial Avastin dose should be delivered over 90±15 minutes. If the first infusion is tolerated without infusion-associated adverse events, the second infusion may be delivered over 60±10 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be delivered over 30± 10 minutes. A rate-regulating device should be used for all Avastin infusions. When the Avastin IV bag is empty, an additional 50 ml of 0.9% sodium chloride injection should be added to the IV bag and the infusion should be continued for a volume equal to that of the tubing to ensure complete delivery of the Avastin. An alternative method of flushing the infusion line would be to replace the empty Avastin infusion bag with a 50 ml bag of 0.9% Sodium Chloride Injection and infuse a volume equal to that of the tubing to ensure complete delivery of the Avastin. This additional saline flush is not included in the specified infusion times.

If a patient experiences Avastin infusion-associated adverse events, the patient may receive pre-medication at the investigator's discretion prior to the next Avastin infusion. If pre-medication is required, the infusion time may not be decreased for the subsequent infusion. However, if the next infusion is well tolerated with pre-medication, the subsequent infusion time may then be decreased by 30 minutes per infusion to a minimum infusion time of 30±10 minutes as long as the patient continues to receive the same pre-medication. If a pre-medicated patient experiences infusion-associated adverse events with the 60-minute infusion, all subsequent doses should be given over 90±15 minutes. Similarly, if a pre-medicated patient experiences infusion associated adverse events with the 30-minute infusion, all subsequent doses should be given over 60±10 minutes.

Anaphylaxis Precautions:
Anaphylaxis precautions should be observed during Avastin administration. The patient's blood pressure and heart rate should be monitored every 15 minutes during the infusion. Emergency agents including oxygen, oral and endotracheal airways, intubation equipment, epinephrine, antihistamines and corticosteroids should be available. In the event of a suspected anaphylactic reaction during infusion of Avastin, the infusion should be interrupted for subjects who develop dyspnea or clinically significant hypotension. Subjects who experience a NCI CTCAE v. 3.0 Grade 3 or 4 allergic reaction / hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from Avastin treatment.

The infusion should be slowed to 50% or less or interrupted for subjects who experience any infusion-associated symptoms not specified above. When the subject's symptoms have completely resolved, the infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle. Administer antihistamines, epinephrine, or other medications at the investigator's discretion.

Avastin Infiltration:
Should infiltration of the Avastin infusion occur the following steps are to be taken: Discontinue the IV. If a significant volume of the Avastin infusion remains, restart the IV and complete the infusion. Treat the infiltration according to institutional guidelines for infiltration of a non-caustic agent.

7.1.2 Avastin Storage

Upon receipt of Avastin, vials are to be refrigerated at 2°C-8°C (36°F-46°F) and should remain refrigerated until just prior to use. DO NOT FREEZE. DO NOT SHAKE. Opened vials must be prepared for use within 8 hours. Vials should be protected from light. VIALS ARE FOR SINGLE USE ONLY. Vials used for one patient may not be used for any other patient. Once Avastin has been added to a bag of sterile saline, the solution must be administered within 8 hours.

7.1.3 Avastin Dose Modification and Toxicity Management

There are no reductions in the Avastin dose. If adverse events occur that require holding Avastin, the dose will remain the same once treatment resumes. Any toxicities associated with or possibly associated with Avastin treatment should be managed according to standard medical practice. Avastin has a terminal half-life of 2 to 3 weeks; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for Avastin. Patients randomized in the combination arm will receive a total of 9 cycles of Avastin even if delays occur between doses; for these patients, the total time of participation in the trial may be longer than 24 weeks. For these patients requiring Avastin infusions delays, evaluations scheduled by weeks will be changed to evaluations by cycle as follow:

Week 0 or cycle 1
Week 3 or cycle 2
Week 6 or cycle 3
Week 9 or cycle 4
Week 12 or cycle 5
Week 15 or cycle 6
Week 18 or cycle 7
Week 21 or cycle 8
Week 24 or cycle 9

Adverse events requiring delays or permanent discontinuation of Avastin are listed in Table 1. Regardless of the reason for holding study drug treatment, the maximum allowable length of treatment interruption is 2 months.

Table 1: Avastin Dose Management Due to Adverse Events

Event	Action to be Taken
Hypertension	
No dose modifications for grade 1/2 events	
Grade 3	If not controlled to ≤ 150/100 mmHg even with adjustments of the medications, discontinue Avastin.
Grade 4 (including RPLS (confirmed by MRI) or hypertensive encephalopathy)	Discontinue Avastin
Hemorrhage	
No dose modifications for grade 1/2 nonpulmonary and non-CNS events	
Grade ≥ 2 pulmonary or CNS hemorrhage	Discontinue Avastin
Grade 3 nonpulmonary and non-CNS hemorrhage	Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving Avastin. All other subjects will have study treatment held until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence. Subjects who experience a repeat Grade 3 hemorrhagic event will be discontinued from receiving Avastin.
Grade 4 nonpulmonary or non-CNS hemorrhage	Discontinue Avastin
Grade 1 pulmonary or CNS hemorrhage	Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving Avastin. All other subjects will have study treatment held until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.
Grade 2, 3, or 4 pulmonary or CNS hemorrhage	Discontinue Avastin
Venous Thrombosis	
No dose modifications for grade 1/2 events	

Table 1: Avastin Dose Management Due to Adverse Events

Event	Action to be Taken
Grade 3/ Asymptomatic Grade 4	Hold study drug treatment. If the planned duration of full-dose anticoagulation is <2 weeks, study drug should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is >2 weeks, Avastin may be resumed during the period of full-dose anticoagulation if all of the following criteria are met: • The subject must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin (or other anticoagulant) prior to restarting study drug treatment. • The subject must not have had a Grade 3 or 4 hemorrhagic event while on anticoagulation. • The subject must not have had evidence of tumor involving major blood vessels on any prior CT scan.
Symptomatic Grade 4	Discontinue Avastin.
Arterial Thromboembolic event (Angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event)	
Any grade	Discontinue Avastin
Congestive Heart Failure (Left ventricular systolic dysfunction) No dose modifications for grade 1/2 events	
Grade 3	Hold Avastin until resolution to Grade ≤ 1
Grade 4	Discontinue Avastin
Proteinuria No dose modifications for grade 1/2 events	
Grade 3 **(UPC> 3.5,** **24- hr urine >3.5 g/24 hr)**	Hold Avastin treatment until ≤ Grade 2, as determined by either UPC ratio ≤ 3.5 or 24 hr collection ≤ 3.5 g
Grade 4 (nephrotic syndrome)	Discontinue Avastin
GI Perforation	Discontinue Avastin (Any grade)
Fistula	
Any grade (TE fistula	Discontinue Avastin
Grade 4 fistula	Discontinue Avastin
Bowel Obstruction	
Grade 1	Continue patient on study for partial obstruction NOT requiring medical intervention.

Table 1: Avastin Dose Management Due to Adverse Events

Event	Action to be Taken
Grade 2	Hold Avastin for partial obstruction requiring medical intervention. Patient may restart upon complete resolution.
Grade 3/4	Hold Avastin for complete obstruction. If surgery is necessary, patient may restart Avastin after full recovery from surgery, and at investigator's discretion.
Wound dehiscence requiring medical or surgical therapy	Discontinue Avastin
Reversible Posterior Leukoencephalopathy Any grade (confirmed by MRI)	Discontinue Avastin
Other Unspecified Avastin-Related Adverse Events	
Grade 3	Hold Avastin until recovery to ≤ Grade 1
Grade 4	Discontinue Avastin

7.2 Letrozole

Letrozole (Femara®) is the hormonal agent that will be used in this trial. Patients will receive 2.5 mg PO daily until 1 day before surgery. Dose of the hormonal agent will not be modified for this protocol. Adverse events associated with these medications are provided in the package inserts (www.fda.gov/cder/foi/label/2001/20726S006LBL.PDF). The hormonal agent is commercially available and will not be provided by the study.

7.3 Other Concomitant Medications

The reason(s) for treatment dosage, and dates of treatment should be recorded in the source documents. Concomitant medications should be reported to the investigator and will be recorded as instructed in the study specific case report forms. Patients who experience Avastin-related temperature elevations or other infusion-related symptoms may be treated symptomatically with acetaminophen, dyphenhydramine, meperidine, or other medications as clinically indicated, including < 48 hours of treatment with corticosteroids. Patients should receive full supportive care, including transfusions, antibiotics, anti-emetics, etc. when appropriate. Pre-trial supportive care will continue.

Unless there is a contraindication, patients with a history of atherosclerosis or at high risk for atherosclerosis should be taking aspirin (81 mg PO a day) and/or clopidrogel (75 mg PO a day) to prevent arterial thrombosis. Unless there is a contraindication, patients with a central venous line should be receiving warfarin (1 mg PO a day) to prevent venous thrombosis. Patients

receiving warfarin 1 mg a day should have their INR checked every week for 4 weeks to make sure that it does not increase to > 1.5.

The following therapies are excluded during active protocol therapy: radiotherapy, hormonal therapy different from the agent prescribed in the protocol, chemotherapy, and cancer immunotherapy or other biologic agents.

8.0 CLINICAL AND LABORATORY EVALUATIONS

Patients will be monitored for safety and tolerability during and after the treatment period. Patients will undergo an assessment of tumor status at baseline (screening) and at the completion of the first 6 weeks of therapy. Further tumor status evaluation will be done every 6 weeks until disease progression or for 24 weeks. If there is any doubt about whether disease progression has occurred based on clinical evaluation or images, treatment on study should continue until the next tumor assessment, as appropriate for patient care.

8.1 Pre-Treatment Evaluations

Informed consent will be obtained before study-specific screening evaluations are performed. Screening evaluations must be performed within 8 weeks prior to day 0 (except blood and urine tests which should be obtained within 4 weeks). Results of tests or examinations done as standard of care prior to obtaining informed consent and within 56 days prior to day 0 may be used rather than repeating tests.

The following evaluations and procedures will be performed during the **screening period**:

- Signed informed consent.

- Clinical evaluations: medical history, demographics, weight and height, complete physical examination, vital signs and performance status.

- Laboratory assessment: UA, urinary protein/creatinine ratio (24-hour urine collection if indicated), CBC with differential and platelet count, serum chemistries (glucose, BUN, creatinine, total protein, albumin, bilirubin, calcium, alkaline phosphatase, AST, and ALT), serum electrolytes (Na, K,Cl), INR.
- Core needle biopsy of the tumor and of the unaffected breast (optional); samples for circulating tumor cells (Appendix I).

- Sentinel lymph node biopsy when required.

- Estrogen and Progesterone receptor status.
- Her-2-neu by immunohistochemistry (Negative: IHC 0, 1 or 2+ or non-amplified by FISH).

- Tumor assessments: Mammogram and breast ultrasound are required. PET/CT scan or [CT scan of chest, abdomen, pelvis, with bone scan] will be obtained. If the scan shows disease outside the breast and/or the axilla, the patient will not be enrolled in the trial.

- Evaluation of the ejection fraction with an echocardiogram/MUGA.

- Concomitant medication assessment.

- Patients who have satisfied basic eligibility criteria will be assigned with a study patient number and the patient can proceed to be consented. After screening procedures are completed, the patient will be randomized.

8.2 Evaluations During Treatment

The following evaluations and procedures will be performed during the **treatment period**: (All assessments should be performed within 5 working days of the protocol-specified date).

- Physical examination, vital signs, weight, and performance status on weeks 6, 12, 18, and 24 or until disease progression.

- For patients randomized to the Avastin arm: urine protein/creatinine ratio or urine dipstick before every administration of Avastin (every 6 weeks). Patients found to have $\geq$ 2+ or greater proteinuria and/or urine protein/creatinine ratio $\geq$ 1.0 must undergo a 24-hour urine collection Patients who develop grade 3 proteinuria during treatment will not receive additional doses of Avastin unless the proteinuria improves to grade 2 or better and the patient has not missed more than 2 scheduled sequential doses (see Appendix F).

- Laboratory assessments: CBC with differential and platelet count, serum chemistries (glucose, BUN, creatinine, total protein, albumin, bilirubin, calcium, alkaline phosphatase, AST, and ALT), and serum electrolytes (Na, K, Cl) on weeks 1, 3, 6, 9, 12, 15, 18, 21, and 24 for the combination arm and weeks 1, 6, 12, 18, and 24 or until disease progression is documented in the Letrozole alone arm. The last evaluation is at 24 weeks.

- Core needle biopsy of the tumor and of the unaffected breast (optional) at week 6 only; samples for evaluation of circulating tumor cells (Appendix I).

- Tumor assessments: Ultrasound of the breast will be obtained on week 6, 12, 18, and 24. Mammogram will be obtained on week 24. Additional PET scans or CT of the chest, abdomen, and pelvis, or bone scans are not required except as clinically indicated. (see Appendix H for RECIST criteria).

- Evaluation of the ejection fraction with an echocardiogram/MUGA (Week 24 only)

- Surgical evaluation at the end of neoadjuvant therapy. There must be at least four weeks between the last dose of Avastin and surgery. Patients will continue letrozole until the day before surgery.

- Concomitant medication assessment.

- Assessment of adverse events (Appendix C and section 13).

8.3 Post-Treatment Evaluations

Patients will complete participation in the protocol at the time of surgery and will not be followed unless the patient requires following of adverse events. The following evaluations and procedures will be performed at **early termination**:

- Clinical evaluations: Weight, physical examination (complete), vital signs and performance status. There must be a complete and clear documentation of objective response or disease progression using the RECIST criteria. Reason(s) for discontinuation must be documented.

- Laboratory assessment: UA (subjects found to have $\geq$ 2+ or greater proteinuria and/or urine protein/creatinine ratio $\geq$ 1.0 must undergo a 24-hour urine collection), CBC with differential and platelet count, serum chemistries (glucose, BUN, creatinine, total protein, albumin, bilirubin, calcium, alkaline phosphatase, AST, and ALT), and serum electrolytes (Na, K, Cl).

- Tumor assessments: Mammogram and breast ultrasound. Spiral CT scans of chest, abdomen, and pelvis, bone scan, and any other procedure are not required per protocol but will be obtained if clinically indicated.

- Concomitant medication assessment.

- Assessment of adverse events (Appendix C and section 13). If the patient has an ongoing toxicity, the patient should be followed until resolution or stabilization. Serious and non-serious adverse events occurring within 30 days of day 0 of the last treatment should be reported on the appropriate adverse event CRF. Patients with ongoing hypertension at termination visit will have their BP and use of anti-hypertensive medications monitored every 4 months for up to 1 year or until BP returns to normal limits. Patients who have ongoing proteinuria (grade 3) at the termination visit will be monitored with 24-hour urine collection every 4 months for up to 1 year or until urine protein improves to grade 2.

9.0 SUBJECT DISCONTINUATION

Patients may withdraw from this study at any time. Any patient who withdraws will be encouraged to return to the study center for the post-dose evaluations. The termination visit consists of all evaluations scheduled for the termination visit. The primary reason(s) for discontinuation must be recorded on the appropriate CRF page. The PI may discontinue a patient from treatment. Reasons may include, but are not limited to, the following: clinically significant deterioration, noncompliance, persistent grade 3 or 4 adverse event or any significant adverse event that compromises the patient's ability to participate in the study, requirement of a significant surgical procedure or radiation therapy during the treatment period of the study, investigator's determination that it is not in the patient's best interest to continue participation, proteinuria greater than 2 gr in 24 hours, grade 4 hypertension, nephrotic syndrome, grade 2 or higher pulmonary or CNS hemorrhage and any other grade 4 hemorrhage, grade 4 venous thromboembolic event, any grade arterial thromboembolic event, grade 4 congestive heart

failure, gastrointestinal perforation, wound dehiscence requiring medical or surgical intervention, development of brain metastases, pregnancy.

Patients who have an ongoing Avastin-related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible (see Section 7.1.3).

10.0 STUDY DISCONTINUATION

The principal investigator and/or Genentech and/or the Breast Cancer Research Consortium have the right to terminate the study for the following reasons: the incidence or severity of adverse events indicates potential health hazard for patients, subject enrollment is unsatisfactory, or data recording is inaccurate or incomplete.

11.0 STATISTICAL METHODS

11.1 Sample Size Determination

The design utilized will be an open label phase II randomized trial of neoadjuvant Avastin and letrozole or single agent letrozole in post-menopausal patients with new diagnosed stage II/III, ER/PR positive, Her-2 negative breast cancer. The randomization will be 2:1 combination versus single agent letrozole with a total of 75 patients (50 receiving the combination and 25 receiving single agent. The intent of the trial is not a comparison of the two arms of the study but rather to estimate the pathologic complete remission rate (pCR) of the combination therapy. Previous studies have demonstrated that the pCR rate of single agent letrozole is <1% (44 and recent analysis by Ellis, *et al*, *J. Natl. Cancer Inst* 2008, 100:1380-1388). A recent pilot trial of neoadjuvant therapy of this population using Avastin and letrozole had a pCR of 12% (breast and nodes) and breast pCR of 16%. In order to achieve a more accurate analysis of pCR rate, this trial in 50 patients would have standard errors of $\pm$ 0.052 and using the Blyth-Still-Cosella method provide a pCR rate and two-sided confidence interval of 5.4% - 23.3% based on the pilot study of 12% pCR. The single agent letrozole 25 patient arm with projected <1% pCR rate would be expected to have no pCR events and the probability of observing one pCR event would be 22%. The purpose of the single agent letrozole arm is to characterize the pCR response as similar to the prior (historical control) trials of single agent neoadjuvant letrozole.

11.2 Statistical Analysis

Descriptive statistics will be used to summarize baseline characteristics of all patients by randomized arm in order to assess relative comparability of the two arms of the study. Mean, standard deviation and range will be summarized for continuous variables, and frequency (percentages) will be calculated for categorical variables. The difference between the pathological complete responses and clinical responses will also be determined along with 95% exact confidence intervals about these differences in proportions.

Descriptive statistics will be calculated to summarize biomarker values from biopsies during pre- and post-therapy (6 weeks) for all patients. The change between pre- and post-therapy for each biomarker will also be calculated and summarized for each arm together with 95% confidence intervals. All toxicities will be recorded and summarized by calculating frequencies and proportions of toxicity grades for each arm.

12. ADMINISTRATIVE RULES OF THE PROTOCOL

12.1 Compliance with the Protocol and Protocol Revisions

The study must be conducted as described in this approved protocol. Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UAB. The written amendment will be sent to investigators and must be submitted to the IRB at the investigator's site for approval. All revisions to the protocol will be provided to Genentech by UAB. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients. Documentation of approval signed by the chairperson or designee of the IRB(s) must be sent tothe UAB Clinical trial Network Coordinator (Pam Dixon fax 205-975-9875). If the revision is an Administrative Letter, Investigators must inform their IRB(s)/IEC(s).

12.2 Informed Consent

The Investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

12.3 Records and Reports

An Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the investigational product or entered as a control in the investigation.

12.4 Study Management

12.4.1 Institutional Review Board (IRB) Approval and Consent

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the IRB for the protocol, consent form, patient recruitment materials/process (e.g., advertisements), and any other written information to be provided to patients. The Investigator should provide the IRB with reports, updates, and other information (e.g., Safety Updates, Amendments, Administrative Letters) according to regulatory requirements or Institution procedures. Copies of the initial IRB approval as well as annual re-approvals must be submitted to UAB. UAB will provide copies of IRB approval to Genentech.

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki. Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient or the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. Documentation of IRB approval must be on file before any patient can be registered. Registration begins with signed informed consent.

12.4.2 Study Monitoring

Data from every site will be captured on manual CRFs, and supporting documents will be faxed to UAB. Personnel from the University of Alabama at Birmingham will monitor the trial and may periodically visit the investigative site to ensure proper conduct of the trial and proper collection of the data. The investigators at each site will allow the monitor to review all source documents used in the preparation of the case reports.

12.4.3 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the UAB Clinical Trials Network Office:

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any associate investigators who will be involved in the study

• A copy of the IRB-approved consent form and HIPAA authorization
• CLIA Laboratory certification and institution lab normal values

In addition, an executed subcontract must be in place at UAB. A separate agreement with Genentech may be required for Avastin shipment.

12.4.4 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol. Any deviation from the protocol must have prior approval by the Principal Investigator and must be recorded and explained.

12.4.5 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approved signed patient consent forms). Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and conduction of the clinical research study. Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug pursuing regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study. If the Investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB). Notice of such transfer will be given in writing to Genentech. Records of the patient's participation in this study will be kept confidential so far as permitted by law. However, the patient's doctor and his/her staff, representatives of Genentech, representatives of the Breast Cancer Research Consortium, the U.S. Food and Drug Administration, and the IRB will be able to inspect patient records and have access to confidential information which identifies the patient by name. Any publication of data will not identify the patient by name. Should the patient's medical record need to be reviewed by a foreign regulatory agency, a member of the IRB staff will observe the review so that the record is not removed, copied, or identifiable information recorded in any manner.

12.4.6 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must ensure that all study site personnel, including subinvestigators and other study staff members, adhere to the study protocol and all

FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for ensuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper data entry. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

13.0 ASSESSMENT OF SAFETY

13.1 Adverse Event Reporting and Definitions (See also Appendix D)

In the event of an adverse event the first concern will be for the safety of the subject.

13.1.1 Adverse Events (AEs)

Adverse events should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to patients experiencing AEs that cause interruption or discontinuation of investigational product or those experiencing AEs that are present at the end of their participation in the study. Such patients should receive post-treatment follow-up as appropriate as it has been described throughout the protocol. If an ongoing AE changes in its severity or in its perceived relationship to study drug, a new AE should be completed.

13.1.2 Serious Adverse Events (SAEs)

SAEs require expeditious handling and reporting to UAB to comply with regulatory requirements. Investigators are required to report within 24 hours of investigator's knowledge (MedWatch Form 3500 – Appendix D) to the principal investigator (UAB – Andres Forero), who will report to the FDA, UAB IRB and Genentech Drug Safety, ANY serious treatment emergent adverse event (SAE) as soon as possible. Affiliate SAEs must be reported to local IRB according to their institutional guidelines. All SAEs must be collected and reported until 30 days following patient discontinuation of dosing; if only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

A SAE is any sign, symptom or medical condition that emerges during Avastin treatment or during a post-treatment follow-up period that (1) was not present at the start of Avastin treatment and it is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of Avastin treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization

- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

13.2 Reporting of Serious Treatment Emergent Adverse Events

All SAEs should be recorded on a MedWatch 3500 Form (see Appendix D - can be accessed at: https://www.accessdata.fda.gov/scripts/MedWatch) and faxed to UAB:
Pam Dixon
Phone: 205-975-5387
Fax: 205-975-9875

And
Andres Forero
Phone: 205-934-7167
Fax: 205-975-6911

Once forms are reviewed, UAB will report to:

MedWatch
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)

And

Genentech Drug Safety
Fax: (650) 225-4682 or (650) 225-5288

And:

UAB IRB

MedWatch 3500a Reporting Guidelines:
In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500 form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:
Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500a report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500a form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B., initials, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)

Occasionally the principal investigator and/or Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom an adverse event was reported.

Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that Avastin caused or contributed to an adverse event. The following general guidelines may be used.

Yes: if the temporal relationship of the clinical event to Avastin administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to Avastin administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

14. REFERENCES/BIBLIOGRAPHY

1. American Cancer Society. Cancer facts and figures. Atlanta, GA: American Cancer Society, 2005.
2. Forbes JF. The control of breast cancer: the role of tamoxifen. Semin Oncol 1997;24(1):S15–9.
3. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma; suggestions for a new method of treatment with illustrative cases. Lancet 1896; ii: 104–7.
4. Morgan LR Jr, Schein PS,Woolley PV, et al. Therapeutic use of tamoxifen in advanced breast cancer: correlation with biochemical parameters. Cancer Treat Rep 1976; 60:1437–43.
5. Henderson IC. Endocrine therapy of metastatic breast cancer. Breast Dis 1991; 2:559–603.
6. Jordan VC. The role of tamoxifen in the treatment and prevention of breast cancer. Curr Probl Cancer 1992; 16:129–76.
7. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet 1998; 351:1451–67.
8. Current Trials Working Party of the Cancer Research Campaign Breast Cancer Trials Group. Preliminary results from the cancer research campaign trial evaluating tamoxifen duration in women aged fifty years or older with breast cancer. J Natl Cancer Inst 1996; 88(24):1834–9.
9. Adjuvant tamoxifen in the management of operable breast cancer: the Scottish Trial. Report from the Breast Cancer Trials Committee, Scottish Cancer Trials Office (MRC), Edinburgh. Lancet 1987; ii:171–5.
10. Stewart HJ, Forrest AP, Everington D, et al. Randomized comparison of 5 years of adjuvant tamoxifen with continuous therapy for operable breast cancer. The Scottish Cancer Trials Breast Group. Br J Cancer 1996; 74:297–9.
11. Randomized trial of two versus five years of adjuvant tamoxifen for postmenopausal early stage breast cancer. Swedish Breast Cancer Cooperative Group. J Natl Cancer Inst 1996; 88:1543–9.
12. Swedish Breast Cancer Cooperative Group. Randomized trial of 2 versus 5 years of adjuvant tamoxifen in postmenopausal early-stage breast cancer. Proc Am Soc Clin Oncol 1996;15:126. Surg 1978; 187:475–84.
13. Buzdar A, Douma J, Davidson N, et al. Phase III, multicenter, double-blind, randomized study of letrozole, an aromatase inhibitor, for advanced breast cancer versus megestrol acetate. J Clin Oncol 2001;19:3357–66.
14. Buzdar AU, Jonat W, Howell A, et al. Anastrozole versus megestrol acetate in the treatment of postmenopausal women with advanced breast carcinoma: results of a survival update based on a combined analysis of data from two mature phase III trials. Arimidex Study Group. Cancer 1998;83:1142–52.

15. Buzdar A, Jonat W, Howell A, et al. Anastrozole, a potent and selective aromatase inhibitor, versus megestrol acetate in postmenopausal women with advanced breast cancer: results of overview analysis of two phase III trials. Arimidex Study Group. J Clin Oncol 1996;14: 2000–11.

16. Dirix L, Piccar MJ, Lohrisch C, et al. Efficacy of and tolerance to exemestane (E) versus tamoxifen (T) in first-line hormone therapy (HT) of postmenopausal metastatic breast cancer (MBC) patients (pts): a European Organisation for the Research and Treatment of Cancer (EORTC Breast Group) Phase II trial with Pharmacia and Upjohn. Proc Am Soc Clin Oncol 2001;20:29a.

17. Dombernowsky P, Smith I, Falkson G, 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;16:453–61.

18. Gershanovich M, Chaudri HA, Campos D, et al. Letrozole, a new oral aromatase inhibitor: randomised trial comparing 2.5 mg daily, 0.5 mg daily and aminoglutethimide in postmenopausal women with advanced breast cancer. Letrozole International Trial Group (AR/BC3). Ann Oncol 1998;9:639–45.

19. Kaufmann M, Bajetta E, Dirix LY, 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;18:1399–411.

20. Milla-Santos A, Milla L, Portella J, et al. Anastrozole vs tamoxifen as first-line therapy in postmenopausal patients with hormone-dependent advanced breast cancer: a prospective, randomized, phase III study. Breast Cancer Res Treat 2001; 69(3):257.

21. Mouridsen H, Gershanovich M, Sun Y, 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; 19:2596–606.

22. Baum M, Buzdar AU, Cuzick J, et al (ATAC Trialists' Group). Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausalwomen with early breast cancer: first results of the ATAC randomised trial [published erratum appears in Lancet 2002; 360:1520]. Lancet 2002; 359:2131–9.

23. Buzdar AU, ATAC Trialists' Group. The ATAC ('Arimidex', Tamoxifen, Alone or in Combination) trial in postmenopausal women with early breast cancer: updated efficacy results based on a median follow-up of 47 months. Breast Cancer Res Treat 2003; 77(3):295.

24. Sainsbury R and the ATAC Trialists' Group. Beneficial side effect profile of anastrozole compared with tamoxifen confirmed by additional 7 months of exposure data: a safety update from the 'Arimidex,' Tamoxifen, Alone or in Combination (ATAC) trial [abstract]. Breast Cancer Res Treat 2002; 76 (Suppl 1):S156.

25. Goss PE, Ingle JN, Martino S, et al. A Randomized Trial of Letrozole in Postmenopausal Women after Five Years of Tamoxifen Therapy for Early-Stage Breast Cancer. New Eng J of Med 2003; 349 (19).

26. Hortobagyi GN. Comprehensive management of locally advanced breast cancer. Cancer 1990; 66:1387–91.

27. Jaiyesimi I, Buzdar A, Hortobagyi G. Inflammatory breast cancer: a review. J Clin Oncol 1992;10: 1014–24.

28. Ueno N, Buzdar A, Singletary S, et al. Combined modality treatment of inflammatory breast cancer: twenty years of experience at M.D. Anderson Cancer Center. Cancer Chemother Pharmacol 1997;40:321–9.

29. Buzdar A, Singletary S, Booser D, et al. Combined modality treatment of stage III and inflammatory breast cancer. Surg Clin North Am 1995;4:715–34.

30. Gunduz N, Fisher B, Saffer EA. Effect of surgical removal on the growth and kinetics of residual tumor. Cancer Res 1979;39:3861–5.

31. Fisher B, Gunduz N, Saffer EA. Influence of the interval between primary tumor removal and chemotherapy on kinetics and growth of metastases. Cancer Res 1983;43:1488–92.

32. Hortobagyi GN, Blumenschein GR, Spanos W, et al. Multimodal treatment of locoregionally advanced breast cancer. Cancer 1983;51:763–8.

33. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol 1997; 15:2483–93.

34. Wolmark N, Wang J, Mamounas E, et al. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from NSABP B-18. J Natl Cancer Inst Monogr 2001;30:96–102.

35. Feldman L, Hortobagyi G, Buzdar A, et al. Pathologic assessment of response to induction chemotherapy in breast cancer. Cancer Res 1986;46:2578–81.

36. Kuerer H, Sahin A, Hunt K. Incidence and impact of documented eradication of breast cancer axillary lymph node metastases before surgery in patients treated with Neoadjuvant chemotherapy. Ann Surg 1999;230:72–8.

37. McCready D, Hortobagyi G, Kau S, et al. The prognostic significance of lymph node metastases after preoperative chemotherapy for locally advanced breast cancer. Arch Surg 1989;124:21–5.

38. Kuerer H, Newman L, Buzdar A, et al. Residual metastatic axillary lymph nodes following neoadjuvant chemotherapy predict disease-free survival in patients with locally advanced breast cancer. Am J Surg 1998;176:502–9.

39. Pierga J, Mouret E, Dieras V, et al. Prognostic value of persistent node involvement after neoadjuvant chemotherapy in patients with operable breast cancer. Br J Cancer 2000; 83:1480–7.

40. Kuerer H,Newman L, Smith T, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol 1999;17:460–9.

41. Bates T, Riley DL, Houghton J et al. Breast Cancer in elderly women: A cancer research campaign trial comparing treatment with tamoxifen and optimal surgery with tamoxifen alone. Br J Surg 1991; 78(5): 591-4.

42. Kenny FS, Robertson JFR, Ellis EO, et al. Long-term follow-up of elderly patients randomized to primary tamoxifen or wedge mastectomy as initial therapy for operable breast cancer. Breast 1998; 7: 335-9.

43. Dixon JM, Love CDB, Bellamy COC, et al. Letrozole as primary medical therapy for locally advanced and large operable breast cancer. Breast Cancer Res Treat 2001; 66: 191-9.

44. Eiermann W, Paepke S, Appfelstaedt J, et al. Preoperative treatment of postmenopausal breast cancer patients with letrozole: A randomized, double-blind multicenter study. Ann of Oncol 2001; 12: 1527-1532.

45. Johnston SR: Acquired tamoxifen resistance in human breast cancer – potential mechanisms and clinical implications. Anticancer Drugs 8:911-930, 1997.

46. Hanahan D, Folkman J: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86:353-364, 1996.

47. Gasparini G, Toi M, Gion M, Verderio P, Dittadi R, Hanatani M, Mastsubara I, Vinante O, Bonoldi E, Boracchi P, Gatti C, Suzuki H, Tominaga T: Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 89:139-147, 1997.

48. Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B, Heriksson R: 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 18:1423-1431, 2000.

49. Foekens JA, Peters HA, Grbenchtchikov N, Look MP, Meijer-van Gelder ME, Geurts-Moespot A, van der Kwast TH, Sweep CGJ, Klijn JGM: High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. Cancer Res 61:5407-5414, 2001.

50. Berns EM, Foekens JA, Vossen R, Look MP, Devilee P, Henzen-Logmans SC, van Staveren IL, van Putten WLJ, Inganas M, Meijer-van Gelder ME, Cornelisse C, Claassen CJC, Portengen H, Bakker B, Klijn JGM: Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer.

51. Toi M, Matsumoto T, Bando H: Vascular endothelial growth factor: Its prognostic, predictive, and therapeutic implications. Lancet Oncol 2:667-673, 2001.

52. Bullocks J, Zhang L, Ding IYF, McLeskey SW, Tobias CA, Miller DL, Kern FG: Overexpression of vascular endothelial growth factor (VEGF) in MCF-7 breast carcinoma cells facilitates growth in tamoxifen-treated nude mice and tumor cell dissemination. Proc Am Assoc Cancer Res 38:A3521, 1997.

53. Qu Z, Thottassery JV, Manuvakhova M, Van Ginkel S, Bullocks J, Kern FG: Exploring the mechanism of tamoxifen resistance induced by angiogenic growth factor overexpression using athymic nude mouse xenograft models with doxycycline regulated expression of VEGF165. 9th SPORE Investigators' Workshop, Washington, DC, p 137, 2001.

54. Qu Z, Thottassery JV, Van Ginkel S, Westbrook L, Manuvakhova M, Bullocks J, Zhang L, Kern FG: Use of an improved doxycycline-regulated transactivator to study the mechanism of tamoxifen resistance mediated by increased angiogenesis in breast cancer xenografts. Proc Am Assoc Cancer Res 43:A5230, 2002.

55. Qu Z, Van Ginkel S, Thottassery JV, Nasrin M, Carey D, Frost AR, Grizzle WE, Kern FG: Regulatable overexpression of VEGF-A165 in MCF-7 breast carcinoma cells demonstrates the effects of increased angiogenesis on estrogen stimulated tumor growth, tamoxifen sensitivity, and macrometastatic potential. 10th SPORE investigators' Workshop, Washington, DC, p 7, 2002.

56. Qu Z, Thottassery JV, Van Ginkel S, Westbrook L, Kern FG: Improved performance of a doxycycline-regulated gene expression system in a breast carcinoma cell line using a modified Tet-on transactivator and an insulator-flanked TRE vector. Proc Am Assoc Cancer Res 44:A2290, 2003.

57. Qu Z, Van Ginkel S, Thottassery JV, Nasrin M, Maxuitenko Y, Carev D, Frost AR, Grizzle WE, Kern FG: Doxycycline-regulated expression of VEFG-A165 in MCF-7 breast cancer cells demonstrates its ability to enhance estrogen-stimulated tumor growth, tamoxifen resistance and macrometastatic potential. Proc Am Assoc Cancer Res 44:A4972, 2003.

58. Qu Z, Frost AR, Moore CS, Maxuitenko Y, Thottassery JV, Li R, Kern FG: VEGF induces desmoplastic response, tamoxifen resistance, and metastasis in breast cancer. Proc Am Assoc Cancer Res 45:A, 2004, In Press.

59. Berns EM, Klijn JG, Look MP, Grebenchtchikov N, Vossen R, Peters H, Geurts-Moespot A, Portengen H, van Staveren IL, Meijer-van Gelder ME, Bakker B, Sweep FC, Foekens JA: Combined vascular endothelial growth factor and TP53 status predicts poor response to tamoxifen therapy in estrogen receptor-positive advanced breast cancer. Clin Cancer Res 9:1253-1258, 2003.

60. Folkman J: Antiagiogenic therapy. In: DeVita VT Jr, Hellman S, Rosenberg SA, (eds.). Cancer: Principles and practice of oncology, fifth edition. Philadelphia: Lippincott-Raven 3075-3085, 1997.

61. Folkman J: What is the evidence that tumors are angiogenesis dependent? J Nat Cancer Inst 82:4-6, 1990.

62. Weidner N, Semple JP, Welch WR, Folkman J: Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. N Engl J Med 324:1-8, 1991.

63. Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, et al.: Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 84:1875-1887, 1992.

64. Hansen S, Grabau DA, Sorensen FB, Bak M, Vach W, Rose C: The prognostic value of angiogenesis by Chalkley counting in a confirmatory study design on 836 breast cancer patients. Clin Cancer Res 6:139-146, 2000.

65. Obermair A, Kucera E, Mayerhofer K, Speiser P, Seifert M, Czerwenka K, et al.: Vascular endothelial growth factor (VEGF) in human breast cancer: Correlation with disease-free survival. Int J Cancer 74:455-458, 1997.

66. Fox SB, Leek RD, Bliss J, Mansi JL, Gusterson B, Gatter KC, et al.: Association of tumor angiogenesis with bone marrow micrometastases in breast cancer patients. J Natl Cancer Inst 89:1044-1049, 1997.

67. Ferrara N, Davis-Smyth T: The biology of vascular endothelial growth factor. Endocr Rev 18:4-25, 1997.

68. Dvorak HF, Brown LF, Detmar M, Dvorak AM: Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 146:1029-1039, 1995.

69. Brown LF, Berse B, Jackman RW, Tognazzi K, Guidi AJ, Dvorak HF, et al.: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. Hum Pathol 26:86-91, 1995.

70. Gasparini G, Toi M, Gion M, Verderio P, Dittadi R, Hanatani M, et al.: Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 89:139-147, 1997.

71. Relf M, LeJeune S, Scott PA, Fox S, Smith K, Leek R, et al.: Expression of the angiogenic factors vascular endothelial growth factor, acidic and basic fibroblast growth factor, tumor growth factor β-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 57:963-969, 1997.

72. Borgstrom P, Gold DP, Hillan KJ, Ferrara N: Importance of VEGF for breast cancer angiogenesis in vivo: Implications from intravital microscopy of combination treatments with an anti-VEFG neutralizing monoclonal antibody and doxorubicin. Anticancer Res 19:4203-4214, 1999.

73. Presta LG, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, et al.: Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 57:4593-4599, 1997.

74. Christian B: 26-week intravenous toxicity study with rhuMAb VEGF in cynomolgus monkeys with a 12-week recovery. South San Francisco (CA): Genentech; Report No. 97:194-1751, 1999.

75. Ryan AM, Eppler DB, Hagler KE< Bruner RH, Thomford PJ, Hall RL, et al.: Preclinical safety evaluation of rhuMAbVEGF, an antiangiogenic humanized monoclonal antibody. Toxicol Pathol 27:78-86, 1999.

76. Leach W, Shopp G: Safety evaluation of rhuMAb VEGF in a rabbit dermal wound model. South San Francisco (CA): Genentech; Report No.:96:407-1751, 1997.

77. Gordon MS, Margolin KA, Talpaz M, Sledge GW Jr, Holmgren E, Benjamin R, et al.: A Phase I safety and pharmacokinetic study of recombinant human anti-vascular

endothelial growth factor antibody in patients with advanced cancer. J Clin Oncol 19:843-850, 2001.

78. Margolin K, Gordon MS, Holmgren E, Gaudreault J, Novotny W, Fyfe G, et al.: Phase 1b trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: Pharmacologic and long-term safety data. J Clin Oncol 19:851-856, 2001.

79. Yang JC, Haworth L, Steinberg SM, Rosenberg SA, Novotny W: A randomized double-blind placebo-controlled trial of Bevacizumab (anti-VEGF antibody) demonstrating a prolongation time to progression in patients with metastatic renal cancer [abstract]. J Clin Oncol 21:15a, 2002.

80. Cobleigh MA, Langmuir VK, Sledge GW, Miller KD, Handey L, Novotny WF, Reinmann JD, Vassel A: A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. Semin Oncol 30(5 Suppl 16):117-124, 2003.

81. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E: Phase II, randomized trail comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 21:60-65, 2003.

82. Kabbinavar F. Hurwitz HI. Fehrenbacher L. Meropol NJ. Novotny WF. Lieberman G. Griffing S. Bergsland E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. Journal of Clinical Oncology. 21(1):60-5, 2003 Jan 1.

83. Folprecht G. Kohne CH. The role of new agents in the treatment of colorectal cancer. Oncology. 66(1):1-17, 2004.

84. Ohta M, Tokuda Y, Suzuki Y, Kubota M, Makuuchi M, Tajima T, Nasu S, Suzuki Y, Yasuda S, Shohtsu A: Whole body PET for the evaluation of bony metastases in patients with breast cancer: Comparison with 99TcM-MDP bone scintigraphy. Nuclear Medicine Communications 22:875-879, 2001.

85. Peterson J, Kransdorf M, O'Connor M: Diagnosis of occult bone metastases: Positron emission tomography. Clinical Orthopaedics and Related Research 415S:S120-S128, 2003.

86. Weber WA, Peterson F, Schmidt B, Tyndale-Hines L, Link T, Peschelo C, Schwaiger M: Positron emission tomography in non-small cell lung cancer: Prediction of response to chemotherapy by quantitative assessment of glucose use. J Clin Onc 21:2651-2657, 2003.

87. Cerfolio RJ, Ohja B, Mukherjee S, Pask AH, Bass CS, Katholi CR: Positron emission tomography scanning with 2-fluoro-2-deoxy-d-glucose as a predictor of response to neoadjuvant treatment for non-small cell carcinoma. J Thor Cardiovasc Surg 125:938-944, 2003.

88. Smith I, Welch A, Hutcheon AW, Miller ID, Payne S, Chiloctt F, Waikar S, Whitaker T, Ah-See AK, Eremin O, Heys DS, Gilbert FJ, Sharp P: Positron emission tomography using I18F1-fluorodeoxy-d-glucose to predict the pathologic response of breast cancer to primary chemotherapy. J Clin Onc 18:1676-1688, 2000.

89. Mortimer JE, Dehdashti F, Siegel BA, Trinkaus K, Katzeenellenbogen JA, Welch M: Metabolic flare: Indicator of hormone responsiveness in advanced breast cancer. J Clin Onc 19:2797-2803, 2001.

90. Mortimer J, Dehdashti F, Siegel B, Katzenellenbogen J, Fracasso P, Welch M: Positron emission tomography with 2-[17F]fluoro-2deoxy-d-glucose and 16α-{18F}-17β-estradiol in breast cancer: Correlation with estrogen receptor status and response to systemic therapy. Clin Cancer Res 2:933-939, 1996.

91. Oyama N, Akino H, Suzuki Y, Kanamaru H, Ishida H, Tanase K, Sadato N, Yonekura Y, Okada K: FDG PET for evaluation of the change of glucose metabolism in prostate cancer after androgen ablation. Nuclear Medicine Communications 22:963-969, 2001.

92. Oyama N, Ponde DE, Dence C, Kim J, Tai Y-C, Welch MJ: Monitoring of therapy in androgen-dependent prostate tumor model by measuring tumor proliferation. J Nuclear Med 45:519-525, 2004.

93. Frost AR, Sparks D, Grizzle WE. Methods of antigen recovery vary in their usefulness in masking specific antigens in immunohistochemistry. Applied Immunohistochemistry and Molecular Morphology 2000; 8: 236-34.

94. Guarneri V, Broglio K, Kau SW, Cristofanilli M, Buzdar A, Valero V, Buchholz T, Meric F, Middleton L, Hortobagyi G, Gonzalez-Angulo AM: Prognosis value of pathologi complete response after primary chemotherapy in relation to hormone receptor status and other factors. J Clin Oncol 24; 7: 1037-1044, 2006.

95. Gianni L, Baselga J, Eiermann W, *et al*: Feasibility and tolerability of sequential doxorubicin/paclitaxel followed by cyclophosphamide, methotrexate, and fluorouracil and its effects on tumor response as preoperative therapy. Clin Cancer Res 11; 24: 8715-8721, 2005.

96. Kuerer H, Newman LA, Smith TL, *et al*: Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol 17; 2: 460-469, 1999.

97. Drubin D, Smith S, Liu W, Zhao W, Chase GA, Clawson GA. Comparison of cryopreservation and standard needle biopsy for gene expression profiling of human breast cancer specimens. Breast cancer Research & Treatment 90: 93-96, 2005.

98. Glusker P, Recht L, Lane B. Reversible posterior leukoencephalopathy syndrome and bevacizumab [letter]. New Engl J Med 354:980–1, 2006.

99. Hambleton J, Skillings JR, Kabbinavar F, et al. Safety of low-dose aspirin (ASA) in a pooled analysis of 3 randomized, controlled trials (RCTs) of bevacizumab (BV) with chemotherapy (CT) in patients (pts) with metastatic colorectal cancer (mCRC). J Clin Oncol 23 Suppl 16S:3554, 2005.

100. Miller KD, Chap LI, Holmes FA, et al. Randomized Phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 23:792–9, 2005.

101. Ozcan C, Wong SJ, Hari P. Reversible posterior leukoencephalopathy syndrome and bevacizumab [letter]. N Engl J Med 354:981–2, 2006.

102. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. New Engl J Med 355(24):2542–50, 2006.

103. Scappaticci FA, Fehrenbacher L, Cartwright T, et al. Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. J Surg Oncol 91:173–80, 2005.

104. Wedam SB, Low JA, Yang X, et al. A pilot study to evaluate response and angiogenesis after treatment with bevacizumab in patients with inflammatory breast cancer. J Clin Oncol 22 Suppl 14S:578, 2004.

105. Dudoit, S., Yang, Y. H., Callow, M. J. and Speed, T. P. (2002). Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments. Statistica Sinica 2002: 12 (1): 111—139.

APPENDIX A

Study Flowchart

	Screening Days -56 to -1	Treatment At each Avastin dose	Termination Week 24	End of Study Visit
Signed Informed Consent	X			
Medical History/Demographics	X			
Weight	X	X[a]	X	
Height	X			
Physical Exam	X	X[i]	X	
Vital Signs	X	X[b]	X	
ECOG Performance Status	X	X[a]	X	
Core Needle Biopsies [tumor and normal breast (optional)]	X	X (only on week 6)		
UA[k], protein/creatinine ratio[c, i] (24 hr urine collection if indicated) or urine dipstick[k]	X[k]	X[i]	X	
Urine Protein[c]	X[k]	X	X	
CBC (complete)[l]	X[k]	X	X	
Circulating Tumor Cells[g]	X	X	X	
Serum Chemistries/ Electrolytes[l]	X[k]	X	X	
INR	X[k]			
FSH[d]	X			
Mammogram	X		X	
Breast Ultrasound[e]	X	X (every 6 weeks)	X	
PET/CT scan or [CT of the chest, abdomen, pelvis, with Bone Scan]	X			
Ejection Fraction – Echocardiogram/MUGA[h]	X		X	
Sentinel LN biopsy as per institution protocol	X			
Avastin Administration		X	X	
Hormonal Therapy[f]		X		
Concomitant Medications	X	X	X	
Adverse Events	X	X	X	X[j]
Surgery				X
Pathology Report				X

a. Prior to each dose of Avastin.
b. Record complete vital signs before and after the Avastin infusion. BP and HR every 15 minutes during Avastin administration.
c. See Appendix E.
d. If needed to confirm post-menopausal status.
e. First evaluation at 6 weeks; then every 6 weeks until week 24 or tumor progression.
f. Daily by mouth until surgery -1 day or disease progression (Letrozole, 2.5 mg PO a day). Letrozole can start immediately post sentinel node biopsy procedure
g. See Appendix I.
h. LVEF calculated only by echocardiogram/MUGA.
i. Weeks 6, 12, 18, 24.
j. Follow according to Section 8.3.
k. U/A, protein/creatinine ratio (24 hr urine collection if indicated) or urine dipstick within 4 weeks of Day 0.
l. For the Letrozole arm alone; to be done every 6 weeks.

APPENDIX B

NCI Common Toxicity Criteria, Version 3.0
http://www.ctep.cancer.gov/forms/ctcaev3.pdf

APPENDIX C

FDA MedWatch 3500 Form
http://www.accesdata.fda.gov/scripts/MedWatch

SAE Reporting

- **SAE reporting period begins at the signing of informed consent.**
- **All SAEs, regardless of attribution must be reported to UAB by fax within 24 hrs of investigator's knowledge:**
  - **MedWatch Form 3500 must be submitted, regardless of completeness of information.**
    - **Fax to Pam Dixon at 205-975-9875.**
  - **If only limited information is available, follow-up reports are required.**
- **Affiliate SAEs must be reported to local IRB according to their institutional guidelines.**
- **UAB is responsible for submitting all SAE MedWatch forms to FDA.**
- **UAB is responsible for submitting all MedWatch forms to Genetech.**
- **All SAEs must be collected and reported until 30 days following patient discontinuation of dosing.**
  - **If only limited information is initially available, follow-up reports are required.**
  - **The original SAE form must be kept on file at the study site.**

APPENDIX D

Evaluation of Proteinuria

EVALUATION OF PROTEINURIA

At Screening

- Urinalysis is required for all subjects
- Urinary protein/creatinine ratio is required for all subjects
- 24 hour urine collection if indicated

Subjects must demonstrate < 1g of protein or a urinary protein/creatinine ratio < 1.0 to allow participation in the study.

During Study (every 6 weeks for patients randomized to the Avastin arm)

- Urinalysis with urinary protein/creatinine ratio or urine dipstick is required for all subjects

Subjects found to have ≥ 2+ proteinuria by urinalysis or dipstick and/or a urine protein/creatinine ratio ≥ 1.0 must undergo a 24 hour urine collection for quantitation of protein and determination of creatinine clearance (urine volume required)

Proteinuria	
Grade 3 protein/24 hour	If urinalysis indicates ≥ 2+ protein, perform 24-hr urine collection for quantitation of protein. If Grade 3 proteinuria discontinue Avastin until proteinuria grade 2 or better.
Grade 4 (nephrotic syndrome)	Discontinue the subject from Avastin.

Study Termination

- Urinalysis with urinary protein/creatinine ratio or urine dipstick is required for all subjects
is required for all subjects

Subjects found to have ≥ 2+ proteinuria by urinalysis and/or urine protein/creatinine ratio ≥ 1.0 must undergo a 24 hour urine collection for quantitation of protein and determination of creatinine clearance (urine volume required)

Follow-Up

For subjects who have ongoing grade 3 protein/24 hour urine at the termination visit will be monitored by 24 hour urine collection every 4 months for up to 1 year or until total 24 hour urine protein improves to grade 2 or better of protein/24 hour, whichever comes first.

PROCEDURE FOR OBTAINING A URINE PROTEIN / CREATININE RATIO

1) **Obtain at least 4 ml of a 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 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 24hr urine collection)

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

APPENDIX E

ECOG Performance Status Scale

ECOG PERFORMANCE STATUS SCALE

GRADE	DESCRIPTION
0	Full activity, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework or office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours.
3	Capable of only limited self-care, confined to a bed or chair >50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

APPENDIX F

NYHA GUIDELINES
http://www.hcoa.org/hcoacme/chf-cme/chf00070.htm

NEW York Heart Association (NYHA) Classification:

A functional and therapeutic classification for prescription of physical activity for cardiac patients.

- Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities.
- Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.
- Class III: patients with marked limitation of activity; they are comfortable only at rest.
- Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

APPENDIX G

RECIST Criteria

J Natl Cancer Ins; 92 (3): 205, 2000

http://ctep.cancer.gov/guidelines/recist.html

APPENDIX H

Blood and Biopsy Procedures

UAB 0648 - Blood Draw and Biopsy Schedule
Paraffin blocks to UAB, Frozen biopsies to UNC, all other blood to UCSF

Collect in Specified Tubes	Pre-Therapy Day -56 to Day 1	Post-Therapy 6 week	Post-Therapy Week 18	Post-Therapy Week 24
10 ml EDTA – Lavender Top	X	X	X	X
22.5 ml – Cell Saver	X	X	X	X
Research Biopsies	X	X		

A. TO UCSF

Whole blood samples for circulating tumor cell (CTC) analysis will be obtained at:

1. Pre-therapy;
2. On weeks 6, 18, and 24 during therapy

The following samples will be collected at each time point listed above:

1. Pre-therapy: 10 ml of whole blood (1-10 ml tubes, 2-4 ml tubes) collected in a lavender top EDTA tubes and 22.5 ml collected into three Cell Saver tubes (provided to each site).
2. At subsequent time points: 10 ml of whole blood in lavender top EDTA tubes and 22.5 ml collected into three Cell Saver tube (provided to each site).

All samples will be labeled with an indelible marker to include:

1. The study identification number (UAB 0648);
2. The subject's study number; and
3. The date of collection.

Shipping samples as diagnostic specimens must be in compliance with IATA packing instruction 650 (see below). Ship whole blood samples at room temperature **overnight** in appropriate containers. **Blood samples may be drawn Monday through Thursday, but not on Fridays or the day before a holiday.** Any samples collected on Thursday should be collected early in the day to ensure they are sent the same day for overnight shipment. Notification that a sample will be sent should occur on the day of shipping or before if possible by email to: jscott@cc.ucsf.edu and hrugo@medicine.ucsf.edu. Blood should be shipped attention to:

Park Laboratory
2340 Sutter Street, 4th floor, Room S471
San Francisco CA 94115
(Phone: 415/514-3969)

UAB Pam Dixon (pam.dixon@ccc.uab.edu or 205-975-5387) will inform UCSF that all regulatory documents have been received and are in order. Then UCSF will provide cell saver tubes to each site. Ten (10) tubes will be shipped to sites upon said

notification. Requests for additional cell saver tubes when initial shipment of tubes is exhausted should be directed to Hope Rugo (hrugo@medicine.ucsf.edu or 415/353-7428) and Lauren Metzroth (Lauren.Metzroth@ucsfmedctr.org or 415/885-7213). The study coordinator for UCSF will be the secondary contact (in lieu of Lauren Metzroth) when that contact information is made available.

B. To UAB - Paraffin blocks (all patients)

Paraffin blocks for immunohistochemistry studies will be obtained at:

1. Pre-therapy;
2. At week 6

Fixed formalin paraffin embedded blocks (FFPE) will be obtained from the original diagnostic specimen from all patients. These blocks will be shipped by the registering institution to UAB for sectioning. Approximately fifteen sections will be cut from these blocks, and the blocks will be returned to registering institution. If the registering institution is not able to provide tumor blocks for sectioning, please contact Kathy Sexton at the University of Alabama at Birmingham Comprehensive Cancer Center's Tissue Procurement Core Facility (sexton@uab.edu; phone 205-934-6071) for procedures to obtain tumor slides.

All specimen blocks will be labeled with an indelible marker to include:
1. The study identification number (UAB 0648);
2. The subject's study number; and
3. The date of acquisition., and
4. Timepoint of sample acquisition (pre-therapy, 6 week evaluation)

Shipping samples as diagnostic specimens must be in compliance with IATA packing instruction 650 (see below). Wrap FFPE blocks in bubble wrap and place in zip lock bags. Have the cassettes and bags appropriately labeled and include a specimen tracking form with shipment. Ship the slides by overnight carrier at ambient temperature. Samples may be sent monthly on the Monday or Tuesday of a non-holiday week. The receiving laboratory should be notified that a shipment is being sent by email to sexton@uab.edu (phone 205-934-6071). The shipping address is:

University of Alabama Comprehensive Cancer Center
Tissue Procurement Core Facility
703 South 19th Street
Zeigler Research Building, Room 449
Birmingham, AL 35294
(Phone: 205-934-6071)

C. **To UNC** - Frozen research biopsies (all patients)
Preferably, at least 2 core biopsies will be obtained at:
1. Pre-therapy;
2. At week 6

Biopsy samples, preferably obtained with a 14-18 gauge core needle, should be flash (snap) frozen (close cap tightly), ideally within 5 minutes but not greater than 30 minutes, and stored in liquid nitrogen or at -80°C until shipping. If research biopsies prove inadequate, up to two additional biopsies may be requested.

All cryovials (NALGENE® Cryoware™ 5000-0000) will be labeled with an indelible marker to include:

1. The study identification number (UAB 0648);
2. The subject's study number;
3. The date of acquisition; AND
4. Timepoint of sample acquisition (pre-therapy, 6 week evaluation).

Each subject's biopsy specimen's cryovials should be placed in an individual ziploc bag with the same identifying information written on the ziploc bag. Samples should be shipped on dry ice with a specimen tracking form in an approved container using overnight shipping. The amount of dry ice to use for each shipment will depend on how many specimens are included. The box should be full. The container for shipping on dry ice is 12.25 inches on the inside. If, for example, you have 3 baggies with 2 to 3 vials each, then you would put 5-6 inches of dry ice on the top and bottom. Remember the net weight of dry ice included in the shipping container must be declared on the outside of the container. Samples may be sent monthly or quarterly, depending on quantity, on the Monday or Tuesday of a non-holiday week. The receiving laboratory should be notified that a shipment is being sent by contacting Xiaping He (Xiaping@med.unc.edu). The shipping address is:

Charles Perou (attention Xiaping He)
Lineberger Comprehensive Cancer Center
102 Mason Farm Road
CB# 7295 Room 12-020
Chapel Hill, NC 27599-7925
(Phone: 919-843-5717)

Preparing and Shipping Tissue in Frozen Cryovials

Snap Freezing:
Do not Cut the tissue and simply ship as is. Place each core of tissue into a labeled individual 2.0 ml NALGENE® Cryoware™ cryovials. The label should contain the UAB study number (UAB 0648) followed by the subject's study number, as well as the date the specimen was collected. Do not place the patient's name or medical record number on the label. The container will become very cold. Do not handle with unprotected hands. Safety goggles should be use throughout the procedure. Snap freeze samples in liquid nitrogen. If not available, you may use an alcohol/dry ice bath. Fill pan about 1-2 inches deep with methanol. The depth should be enough to cover the height of the vial. Slowly add crushed dry ice until the boiling stops.

The bath is now read for use. Drop the sealed vials directly into the liquid nitrogen (LN_2) carefully. Leave the vials in the liquid nitrogen for at least one minute. Once frozen, transfer the samples using tongs or a large spoon to remove the vials from the liquid nitrogen and transfer to a –80 °C freezer until shipped.

Shipping Frozen Tissue:

Shipping frozen tissue on dry ice must be in compliance with IATA packing instruction 904 (see below). Dry ice is considered dangerous goods and must always be declared by marking the box appropriately with labeling and documentation according to Class 9 procedures. Proper training is required under federal and/or state regulations to handle dangerous goods. All persons responsible for shipping dangerous goods must comply with all federal regulations, including but not limited to the specific training requirements of 49 CFR (172.700-172.704).

Specimens should be shipped in dry ice safe containers, such as styrofoam (not metal) and using a secondary packaging (e.g. styrofoam box inside cardboard box). For shipping specimens on dry ice the "Special Handling" box of the Airbill (FedEx) should be used. Proper shipping name box should be checked, the Class Number 9 (misc.) is printed on the form – fill in amount of dry ice, the UN Number (UN1845) should be on the form, and mark "yes (Shippers declaration not required)".

D. IATA Packing Instructions

650 GENERAL REQUIREMENTS

Shippers of diagnostic specimens where a relatively low probability exists that infectious substances are present must comply with Packaging Instruction 650 of these regulations. Diagnostic specimens being transported for the purpose of initial diagnosis may be considered to fall under this category where a low probability exists that infectious substances are present. The shipper must also ensure that shipments are prepared in such a manner that they arrive at their destination in good condition and that they present no hazard to persons or animals during shipment.
Inner packaging contains:

- A watertight primary receptacle(s)- for diagnostic specimens the maximum quantity must not exceed 500mL
- A watertight secondary packaging -the maximum quantity per outer packaging for diagnostic specimens must not exceed 4L
- An absorbent material- must be placed between the primary receptacle and the secondary packaging. No absorbent material is required when shipping solid substances.

If multiple primary receptacles are placed in a single secondary packaging they must be wrapped individually or for those transported in liquid nitrogen, separated and supported to ensure that contact between them is prevented. The absorbing material,

for example cotton or wool, must be sufficient to absorb the entire contents of all primary receptacles.

- An outer packaging of adequate strength for its capacity, weight and intended use.

The primary receptacle or the secondary packaging used for liquid diagnostic specimens must be capable of withstanding, without leakage, an internal pressure which produces a pressure differential of not less than 95 kPa (0.95 bar, 13.8lb/in2) in the range of -40.oC to + 55.oC(-40.oF to 130.oF). It is not necessary for the primary or secondary packaging to be capable of withstanding 95 kPa pressure differential when solid diagnostic specimens are being shipped.
Packages consigned as freight must be at least 100 mm (4 in) in the smallest overall external dimension.

An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. Each package and the "Nature and Quantity of Goods" box of the airbill must show the text "DIAGNOSTIC SPECIMEN PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650" (example below). A Shipper's Declaration for Dangerous Goods is not required.

650 SPECIFIC REQUIREMENTS
Substances shipped at ambient temperatures or higher- Primary receptacles include those of glass, metal or plastic. Positive means of ensuring a leak-proof seal, such as heat seal, skirted stopper or metal crimp seal must be provided. If screw caps are used these must be reinforced with adhesive tape or parafilm.

Substances shipped on dry ice must be placed outside the secondary packaging(s) or alternatively in an overpack with one or more completed packagings. Interior support must be provided to secure the secondary packaging(s) in the original position after the dry ice has dissipated. The packaging must be leak-proof. The outer packaging must permit the release of carbon-dioxide gas. The primary receptacle must maintain its containment integrity at the temperature of the refrigerant as well as at the temperatures and pressure of air transport to which the receptacle could be subjected if refrigeration were to be lost. New shipping regulations effective January 1, 2005: Package must have both the shipper and recipient’s name, address and phone number.

Package must have the new UN3373 symbol and the words “Diagnostic Specimen” adjacent to that symbol; keep the biohazard sticker in place as well.

labeled box should be inserted into the FedEx Diagnostic Shipping Bag as usual for shipment via FedEx.

904 GENERAL REQUIREMENTS

Carbon dioxide, (dry ice, LN_2), when offered for transport by air, must be in packaging designed and constructed to permit the release of carbon dioxide gas and to prevent a build-up of pressure that could rupture the packaging.

The net weight of the Carbon dioxide, solid (dry ice) must be marked on the outside of the package.

Arrangements between shipper and operators must be made for each shipment to ensure ventilation safety procedures are followed. When a Shipper's Declaration is not required, the information as required by 8.2.3 for dry ice (LN_2) must be contained in the "Nature and Quantity of Goods" box on the airbill, excluding the packing instruction number and packing group.

APPENDIX I

IMPLEMENTATION PLAN FOR OFF-SITE CONDUCT OF UAB COMPREHENSIVE CANCER CENTER INVESTIGATOR-INITIATED PHASE I AND II STUDIES

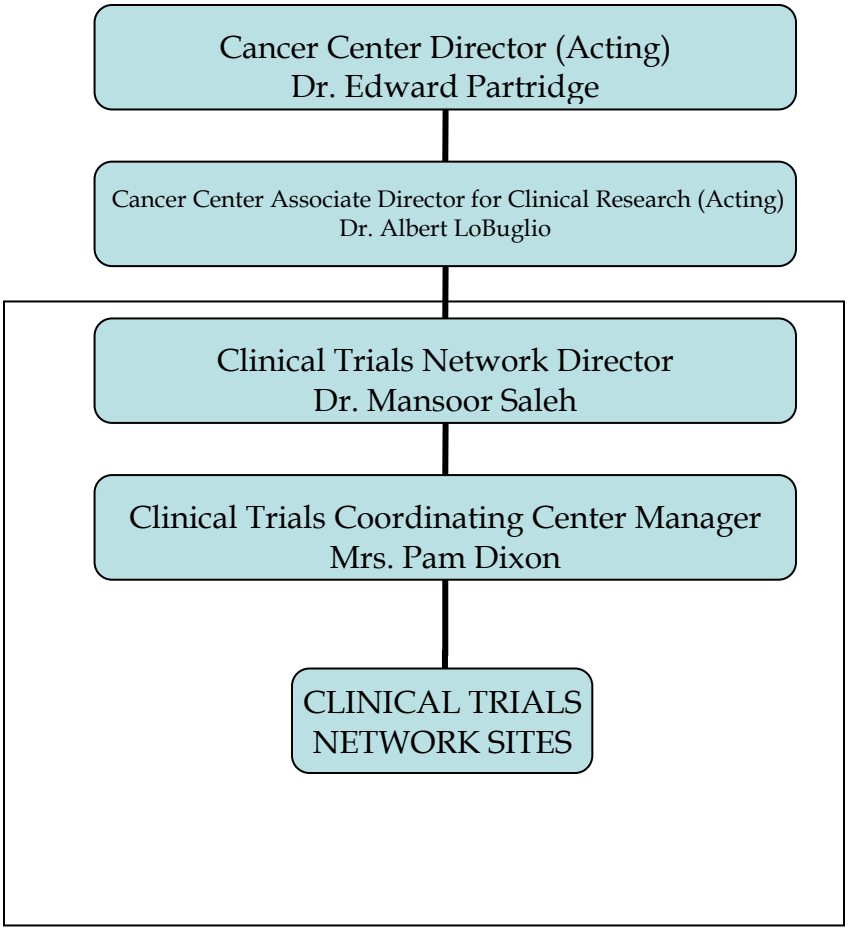
IMPLEMENTATION PLAN FOR OFF-SITE CONDUCT OF UAB COMPREHENSIVE CANCER CENTER INVESTIGATOR-INITIATED PHASE I AND II STUDIES

CLINICAL TRIALS NETWORK (CTN)

1.0 INTRODUCTION

The Clinical Trials Network (CTN) of the UAB Comprehensive Cancer Center (CCC) coordinates investigator-initiated clinical trials under Good Clinical Practice conditions at CTN sites to achieve timely study subject enrollment and to provide subjects at CTN sites with access to CCC investigator-initiated phase I and II studies. The CTN sites are required to have 1) an experienced staff of investigators, research nurses, and data managers; 2) a designated IRB and significant experience in undertaking clinical trials; and 3) accrual of at least five study subjects annually into CCC investigator-initiated CTN site studies, as well as successful completion of monitoring and annual audits. CTN sites must adhere to guidelines in the CCC Data Safety and Monitoring Plan (DSMP).

2.0 ADMINISTRATION OF THE CLINICAL TRIALS NETWORK



Overall responsibility for the CTN lies with the Director of the CCC with primary oversight by the CCC Associate Director for Clinical Research. The Director of the CTN coordinates the overall efforts of the CTN, while day-to-day activities are supervised by the CTN Coordinating Center Manager.

2.1 Current CTN Personnel

- CTN Director: Mansoor Saleh, MD
- CTN Coordinating Center Manager: Pam Dixon, RN, BSN, OCN, CCRP

3.0 IMPLEMENTATION PLAN FOR CTN SITE STUDIES

The P.I. will submit the phase I and/or phase II studies to the UAB IRB as identified protocols to be carried out at affiliate site (CTN). Once approved by the UAB IRB, the affiliate site P.I. will proceed with affiliate site IRB approval. The UAB P.I. will provide UAB IRB with an amendment to the approved UAB protocol which will provide the affiliate site IRB approval, consent documents and proposed date of initiation of the trial at the affiliate site. When the amendment is approved by the UAB IRB, the affiliate site can initiate patient accrual.

Serious Adverse Events (SAEs) are reported by the CTN site Lead Investigator within 24 hours to the CTN Coordinating Center Manager by email (pam.dixon@ccc.uab.edu) or by fax (205) 975-9875. The 24 hour paging number for the CTN Coordinating Center Manager is (205) 934-3411, beeper #5904. The CTN Manager is then responsible for reporting SAEs to the UAB IRB and protocol P.I. in accordance with study-specific requirements. SAEs occurring at CTN sites are reported to the UAB IRB as “non-UAB” events.

The CTN Coordinating Center Manager also ensures that the following are accomplished:

- Develop and implement plans for monitoring and auditing CTN site studies
- Provide initial protocol and consent documents to CTN sites
- Coordinate CTN site initiation visits / teleconferences
- Receive and evaluate documents from CTN sites and determine the need for further evaluation
- Submit required regulatory documentation to the UAB IRB
- Generate study-specific queries and develop resolutions
- Perform monitoring at CTN sites
- Provide for needed fiscal management including development of CTN site budgets and ensure sub-contracts are negotiated
- Provide for distribution of study-specific funds to CTN sites.

4.0 CTN SITE REQUIREMENTS

4.1 Personnel

A Lead Site Investigator is identified for each CTN site study. Adequate CTN site staff is provided for administration, data management, and research nurse activities. The administrative staff provides clerical assistance to support CTN site studies. The data management staff collects and manages necessary research data on study subjects enrolled onto CTN site studies. The research nurses coordinate the study subjects' care in conjunction with study-specific requirements to ensure patient safety, continuity of care and to provide ongoing assessment of the impact of the study on the CTN site and on patient resources. The CTN site staff has expertise in study management and data collection. Training in necessary computer applications, Human Subject Research, and HIPAA regulations is provided and documentation of this training is provided to the CTN Coordinating Center Manager. CTN site staff cooperates in all data management activities with the CTN Coordinating Center Manager, including participation in monitoring and audit activities.

4.2 Equipment and Facilities

CTN sites have equipment and facilities to conduct CTN site studies. These include:

- Patient care facilities, equipment, and supplies
- Required information systems
- Equipment and facilities for processing and storage of study-related tissues, tissue extracts, and blood products
- The capability to transport study-related tissues, tissue extracts, and blood products per study specifications
- Dedicated fax machine for transmitting and receiving study-specific correspondence.

The CTN site provides adequate facilities for secure storage of study documents and for study monitoring activities. A designated monitoring area is provided that includes a workstation, copier, and fax machine. The workstation is located in proximity to the workstations of the CTN site study-specific research nurse and data manager. A secure facility, with restricted access, is provided for storing study drugs, specimens, and other study-specific materials. Systems to assure maintenance of drug receipts and drug dispensing documents and to assure secure disposal of sensitive documents are in place.

CLINICAL TRIALS NETWORK (CTN)
STANDARD OPERATION PROCEDURES

1.0 PROCEDURES FOR INITIATING AND ACTIVATING CLINICAL TRIALS

1.1 Letter of Understanding

An Initial Study Offering Document addressed to the Lead Investigator at a CTN site is developed by the CTN Director. This document enables the CTN site to provide assurances that all responsibilities have been defined and that all necessary resources are available to conduct the proposed CTN site study. The document includes a synopsis of the study, enrollment targets, and estimated funding. The document is signed by the Lead Investigator at the CTN site and is on file at the CTN Coordinating Center before the study can be initiated at the CTN site.

1.2 Budget Development

Designated personnel at the CTN site coordinate budget development and budget negotiations for the study with the CTN Coordinating Center Manager. Acceptable payment terms are also addressed by both parties in conjunction with any sub-contracting requirements.

1.3 Regulatory Procedures

Prior to activation of a study at a CTN site, the following documents must be on file at the CTN Coordinating Center:

- A signed Sub-Contract
- A signed FDA Form 1572, with a current signed and dated CV for each CTN site investigator listed on the form and a copy of the investigators' current medical license
- Current resume and contact information for the study-specific CTN site research nurse and study-specific data manager
- Human Subjects Research Training certification for key CTN site study personnel
- Documentation of HIPPA Compliance Training for key CTN site study personnel
- A copy of all current CTN site laboratory certifications (CAP and / or CLIA) and current laboratory reference ranges for all tests specified in the study. Current CV and license of the CTN site laboratory director, if listed on the FDA Form 1572
- Name of and contact information for the CTN site's IRB Chairperson,

contact information and current CTN site IRB membership roster
- Assurance Identification / CTN site IRB Certification
- CTN site IRB approval of the study protocol and consent form
- CTN site IRB acknowledgement of receipt of the study-specific Investigational Brochure
- Any special study-specific approvals such as radiation licensing
- Signed final CTN site budget / contracting documents
- Yearly updates of the above information are required during the course of the CTN site study

All documents are mailed to the CTN Coordinating Center:

UAB Comprehensive Cancer Center
Clinical Trials Network Coordinating Center
c/o Pam Dixon, RN
1802 6th Avenue South
North Pavilion Room 2523
Birmingham, Alabama 35294

Tel: 205-975-5387
Fax: 205-975-9875
Email: pam.dixon@ccc.uab.edu

CTN sites are responsible for the timely submission of safety reports, protocol amendments, protocol exceptions and deviations, ancillary study documentation (such as advertisement materials), and annual renewals to their CTN site IRB. Copies of CTN site IRB approvals are forwarded to the CTN Coordinating Center.

1.4 Initiation

An initiation is conducted the first time a CTN site participates in a CCC investigator-initiated study. The initiation provides instruction and orientation and reviews the facilities and study staff to ensure that all study-specific requirements are satisfied. CTN site personnel who must attend the initiation include the research nurse, data manager, study investigators, research pharmacist, and other study-specific personnel.

During initiation, the CTN Coordinating Center Manager presents: 1) receipt, review and filing of the Investigator's Brochure; 2) maintaining up-to-date study-specific protocols; 3) study objectives and design; 4) participant screening log and participant identification log; 5) clinical and laboratory evaluations and schedule of evaluations; 6) specimen collection, processing, storage, and shipping; 7) informed consent process and randomization procedures; 8) missed evaluations and protocol deviations and violations; 9) adverse event reporting guidelines, procedures and forms for adverse event reporting, and toxicity

management; and 10) receipt, review, and filing of addendums to the Investigator's Brochure. Study specific data collection procedures are presented including: CRF guidelines; common errors and corrections; CRF update procedures; missed visits; data inquires; source documentation; record keeping; and disposition of CRFs. Investigational agent procedures are reviewed including: dissemination of information to the CTN site research pharmacist; agent storage and accountability; pharmacy guidelines; and quality assurance. A review of the CTN site Lead Investigator's responsibility is carried out with the CTN site Lead Investigator. The CTN site Regulatory Document Binder is reviewed including: CTN site IRB documentation, study-specific approval letter, study-specific informed consent, advertisements and participant information sheets when applicable, annual renewal, and roster. Assurance number, laboratory certification, laboratory norms, documentation of submission of the Investigator's Brochure, and signature logs are also reviewed.

A CTN site may not enroll a patient on a study until all of the regulatory documents have been received and CTN site IRB documentation is reviewed and approved by the CTN Coordinating Center manager and UAB IRB.

2.0 PROCEDURES FOR CONDUCTING CLINICAL TRIALS

All studies conducted at CTN sites must adhere to the study-specific protocol.

2.1 Patient Registration

The CTN site maintains an accurate screening log for the study and forwards this to the CTN Coordinating Center Manager on a monthly basis.

Once a study subject has been consented, screened and deemed eligible for study entry by the CTN site, a study-specific study subject eligibility checklist, a copy of the dated and signed consent form, and corresponding source documentation are faxed to the CTN Coordinating Center Manager for eligibility verification. Subsequently, a study-specific number is assigned to the study subject and sent to the CTN site. Finally, a Patient Registration Form is completed and faxed by the CTN site to the CTN Coordinating Center Manager.

Queries regarding data accuracy are forwarded from the CTN Coordinating Center Manager to the CTN site for clarification or correction. Once queries are addressed by the CTN site, any corrected data forms or copies of corrected source documentation are faxed to the CTN Coordinating Center Manager.

2.2 Study Monitoring

Each study subject is discussed at the weekly CCC Clinical Trials Monitoring Committee meeting by the CTN Coordinating Center Manager.

All questions and concerns regarding the conduct of a study at a CTN site are directed to the site Lead Investigator who consults with the CTN Director and / or the CTN Coordinating Center Manager when necessary. The P.I. (assisted by the CTN Coordinating Center Manager) is the primary contact for study-specific questions such as dose modifications, toxicities, and supportive care. The CTN Coordinating Center Manager is the primary contact for issues regarding patient registration, regulatory documents, completion of CRFs, data collection, and data submission.

The CTN office will maintain 100% of the CRF's with source documentation in the form of a shadow chart.

Monitoring of all CTN site studies (25% of patients) will be conducted "off-site" at the CTN Coordinating Center using the CRFs and supporting source documents that are transmitted from the CTN site on a monthly basis.

Following each monitoring exercise, queries and/or requests for additional documentation are generated by the CTN Coordinating Center Manager generally within 2 weeks of receipt of the CTN site documents. Subsequently, the CTN site reviews and responds to queries and implements the necessary corrective action(s). Within two weeks, the CTN site submits query responses and, if appropriate, a written summary of corrective actions to the CTN Coordinating Center Manager. When necessary, the CTN Coordinating Center Manager conducts "on-site" monitoring visits to provide staff education and to assist in implementing corrective action at the CTN site.

2.3 Auditing of CTN Site Studies by the CCC Quality Assurance Committee

Audits of all CCC investigator-initiated studies are conducted on a yearly basis, within two to three months of the selected study's UAB IRB renewal date. Ten percent of the patient charts are audited. Included in this audit are the CTN site "shadow charts" maintained at the CTN Coordinating Center. Following the audit of the CTN site "shadow charts", a summary of the audit findings are forwarded to the CTN site IRB, the UAB IRB, the CTN site Lead Investigator, the CTN Director and the CCC Associate Director for Clinical Research. The CTN site Lead Investigator, in consultation with the CTN director, has 2 weeks to respond to any deficiencies prior to formal UAB IRB action concerning the audit findings.

2.4 Data Collection and Submission

Data collection and submission is the responsibility of the CTN site Lead Investigator. Data collection forms are provided by the CTN Coordinating Center Manager. Each CTN site maintains a study-specific research data file (research chart) for study subjects enrolled into a study. The research chart includes completed CRFs and copies of all source documentation. Completed CRFs are reviewed, signed and dated by CTN site Lead Investigator. Any deviations from the study protocol are documented in the study subject's medical record and research chart. Missing data is documented in the research chart. Copies of the completed CRFs, other study-related documents, and source documents are faxed to the CTN Coordinating Center Manager on a monthly basis.

Failure to submit the required documents in a timely fashion (within one week of the due date) results in a Letter of Notification. Subsequent failure to comply can result in the following:

- Suspension of CTN site study subject accrual until all delinquent data is submitted to the CTN Coordinating Center
- Permanent suspension of study subject accrual to a CTN site
- Other action determined by unique circumstances.

2.5 Adverse Event Reporting

In cancer clinical trials, an Adverse Event (AE) is any unfavorable physical sign, symptom, significant laboratory test abnormality or disease that is temporally associated with the use of a medical treatment, procedure or device. All AEs must be recorded on study-specific CRFs. AE information is collected from the initiation of the treatment, procedure or use of a study device and continues until the treatment, procedure or use of a study device is stopped and follow-up is completed. Severity is graded according to Common Toxicity Criteria. The CTN site Lead Investigator must evaluate the severity of each AE, assess causal relationships, determine the action to be taken and document the outcome. The CTN site study-specific research nurse is responsible for documenting, filing, and reporting AEs on a weekly basis to the CTN Coordinating Center Manager, who in turns reports the AE at the next weekly CCC Clinical Trials Monitoring Committee meeting.

A Serious Adverse Event (SAE) is an AE that 1) results in patient hospitalization or prolongation of hospitalization; 2) results in persistent or significant disability or incapacity; 3) results in death; 4) is a cancer or congenital abnormality or 5) results in the development of drug dependence or abuse. An AE must be considered an SAE when the nature or severity of the event is not consistent with the current Investigator's Brochure. CTN site SAEs must be reported by the CTN site Lead Investigator to the CTN Coordinating Center Manager by email or by

fax. It is also the responsibility of the CTN site Lead Investigator to report SAEs to the CTN site IRB and to submit copies of that report to the CTN Coordinating Center Manager. It is the CTN Coordinating Center Manager's responsibility to report the SAE to the appropriate regulatory agency and / or industry sponsor.

3.0 PROCEDURES FOR STUDY CLOSURE

3.1 Closeout

A closeout at the CTN site is conducted by the CTN Coordinating Center Manager when a CTN site study reaches its accrual goal or is closed prematurely. This includes close out and storage of study subject records and resolution of all outstanding regulatory, data management, and pharmacy issues. The CTN site retains study documents pertaining to an investigational agent for two years when a marketing application is approved or, if a marketing application is not approved, for two years after shipment and delivery of the investigational drug is discontinued.

3.2 Manuscripts, Publications, Press Releases

Any reports or manuscripts describing study results are reviewed by the CTN Director and circulated to contributing CTN sites for their review prior to any presentation, submission for publication, or press release. The CTN Director, in consultation with the CCC Principal Investigator, determines the time and place of any presentation, submission for publication or press release and is the final arbiter of authorship.