CADENCE: Carboplatin And Docetaxel in Neoadjuvant Treatment of ER Negative, HER2 Negative Breast Cancer

A Co-clinical Trial with Genoproteomic Discovery

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with Genoproteomic Discovery

Protocol Revision History

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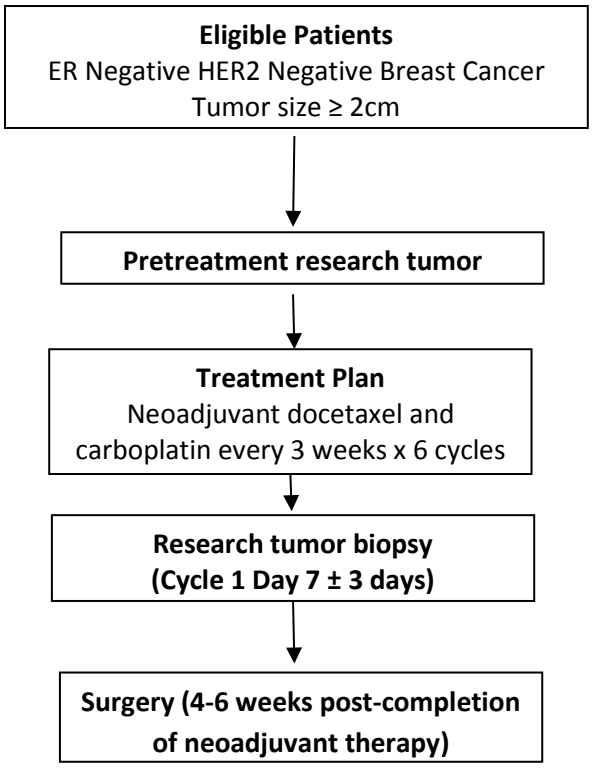
CADENCE TRIAL SCHEMA



Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BUN	Blood urea nitrogen
CALGB	Cancer and Leukemia Group B
CBC	Complete blood count
CFR	Code of Federal Regulations
CMP	Complete metabolic panel
CNS	Central nervous system
CR	Complete respose
CRF	Case report form
CS	Chemo sensitive
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DFS	Disease-free survival
DNA	deoxyribonucleic acid
DOB	Date of birth
DSM	Data and Safety Monitoring
EBCTCG	Early Breast Cancer Trialists' Cooperative Group
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
ER	Estrogen receptor
FDA	Food and Drug Administration
FWA	Federal wide assurance
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor, filgrastim (Neupogen)
HER2	Human epidermal growth factor receptor 2
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IND	Investigational New Drug

IRB	Institutional Review Board
IV	Intravenous
LD	Longest diameter
LDH	Lactate dehydrogenase
MRI	Magnetic resonance imaging
NCCN	National Cancer Center Network
NCI	National Cancer Institute
NIH	National Institutes of Health
NSABP	National Surgical Adjuvant Breast and Bowel Project
OHRP	Office of Human Research Protections
OS	Overall survival
pCR	Pathologic complete response
PD	Progressive disease
PDX	Patient-derived xenograft
PET	Positron emission tomography
PI	Principal investigator
PR	Partial response
PR	Progesterone receptor
PS	Performance status
QASMC	Quality Assurance and Safety Monitoring Committee
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RNA	Ribonucleic acid
SAE	Serious adverse event
SCC	Siteman Cancer Center
SCID	Severe combined immunodeficiency
SD	Stable disease
TNBC	Triple-negative breast cancer
UPN	Unique patient number
US	Ultrasound
WBC	White blood cell (count)
WHIM	Washington University Human in Mouse
WHO	World Health Organization

Table of Contents

1.0 BACKGROUND AND RATIONALE

1.1 Triple Negative Breast Cancer

Triple negative breast cancer (TNBC) represents approximately 10-15% of breast cancers worldwide and affects approximately 200,000 women annually [1]. It is defined by a lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) receptor. The disease is more common in young black women and in women with a deleterious mutation in the BRCA1 gene [2, 3].

Established targeted therapies in breast cancer are directed against a nuclear or a surface receptor, such as tamoxifen or trastuzumab, respectively. However, no established targets currently exist for TNBC, so patients receive chemotherapy for systemic control of their disease [4]. Despite neoadjuvant chemotherapy trials showing that TNBC has higher pathological complete response (pCR) rates compared to hormone-receptor-positive subtypes [5], TNBC remains extremely difficult to treat in many cases, and patients have high rates of relapse, particularly due to chemotherapy resistance within the first few years [6]. TNBC patients suffer from a worse initial prognosis among all other breast cancer subtypes, and there is a dire need to develop new treatment strategies for TNBC, particularly for those with disease that is resistant to standard chemotherapy.

1.2 Neoadjuvant Chemotherapy in Triple Negative Breast Cancer

Neoadjuvant chemotherapy describes primary systemic therapy that is utilized prior to surgery. It was initially used for patients with locally advanced, inoperable breast cancer in an effort to improve local control and decrease distant metastases when used in combination with surgery [7]. A few years later, the National Surgical Adjuvant Breast and Bowel Project (NSABP) began a clinical trial (B-18) to evaluate the efficacy of neoadjuvant chemotherapy compared to adjuvant therapy in patients with operable breast cancer. Approximately 1500 women were randomized to receive chemotherapy either pre- or post-operatively [8]. Although there were no survival differences or differences in rates of ipsilateral tumor recurrence after lumpectomy, preoperative chemotherapy led to an improvement in rates of breast conservation. Since then, numerous studies have confirmed the utility of neoadjuvant chemotherapy in patients with locally advanced inoperable disease as well as those with operable disease who desire breast-conserving therapy [9, 10]. In addition, the neoadjuvant platform is an excellent model to assess pathologic tumor response and for serial tissue acquisition. Ultimately, this allows for the identification of molecular changes induced by treatment, and most importantly, it permits determination of predictors of resistance and sensitivity to therapy, which may lead to more individualized management.

While several clinical trials involving neoadjuvant traditional chemotherapy, biologics, or different dosing schedules have recently been launched in an attempt to improve the outcome of patients with TNBC, the initial phase of neoadjuvant studies did not restrict entry criteria to only patients with TNBC. However, a large proportion (41%) of the study population in NSABP protocol B-40 was classified as having TNBC [11]. The goals of this study were to determine if the addition of capecitabine or gemcitabine to docetaxel followed by doxorubicin and cyclophosphamide (AC) would increase pCR rates in patients with palpable and operable HER 2-negative disease and also to determine whether the addition of bevacizumab to docetaxel-based regimens followed by AC will increase pCR rates. In the chemotherapy alone arms, the addition of capecitabine or gemcitabine to docetaxel versus docetaxel alone did not increase the pCR rates (29.7% and 31.8%, respectively, vs. 32.7%; P=0.69). Similarly, the Gepar Trio study reported by Huober et al. showed a pCR rate of 39% in the TNBC patients in a trial that sought to determine the effect of switching neoadjuvant chemotherapy depending on mid-course response on pCR at time of surgery [12]. Patients received 2 cycles of docetaxel, doxorubicin, and cyclophosphamide (TAC), followed by either 4 or 6 more cycles of TAC in responders or 4 cycles of TAC versus capecitabine plus vinorelbine in non-responders. In non-responders, pCR rates were less than 10% in both groups, suggesting a lack of benefit of switching chemotherapy to these non-standard agents or even providing further chemotherapy in the adjuvant setting to that subset of patients.

In contrast to the lack of data that capecitabine, vinorelbine or gemcitabine improve pCR rates, there is increasing data indicating a role for platinum agents in the treatment of TNBC. CALGB 40603, presented at San Antonio Breast Cancer Symposium 2013, evaluated the impact of the addition of carboplatin and/or bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC on pCR rates in TNBC. This was a 2 X 2 factorial design in which patients on Arm A received weekly paclitaxel at 80 mg/m^2 for 12 weeks followed by dose dense AC for 4 cycles, patients on Arm B received weekly paclitaxel 80 mg/m^2 for 12 weeks plus bevacizumab followed by dose dense AC for 4 cycles, patients on Arm C received weekly paclitaxel 80 mg/m^2 for 12 weeks plus carboplatin followed by dose dense AC for 4 cycles, and patients on Arm D received weekly paclitaxel 80 mg/m^2 for 12 weeks plus carboplatin and bevacizumab followed by dose dense AC for 4 cycles. While there was no clear benefit from the addition of bevacizumab, pCR rates increased from 41% to 54% in patients receiving carboplatin. Similarly, a significant improvement in pCR rate (58.7% vs 37.9%, $p<0.05$) in TNBC was observed with the addition of carboplatin (AUC 1.5 weekly) to 18 weeks of weekly paclitaxel (80mg/m^2) plus non-pegylated liposomal doxorubicin (20mg/m^2) in the GeparSixto trial [13].

In this trial, we therefore aim to develop predictors of response (pCR vs. non-pCR) to neoadjuvant taxane and carboplatin. If sensitive tumors could be identified prospectively, a trial designed to show that the addition of platinum improves survival could be considered. This could lead to a simplified regimen whereby the doxorubicin-based component could be possibly avoided in the treatment of TNBC.

1.3 Chemotherapy Agents

Chemotherapy can substantially reduce the risk of breast cancer recurrence and death in early stage breast cancer [14], and there are many chemotherapy regimens with established efficacy and safety data. The value of chemotherapy has been consistently demonstrated from the data of individual randomized trials and affirmed in the Early Breast Cancer Trialists' Collaborative Group's (EBCTCG) 15-year meta-analyses combining data from the individual chemotherapy trials. The meta-analyses have shown that anthracycline-containing therapies, such as sequential doxorubicin and cyclophosphamide (AC) followed by taxanes, and combination regimens such as docetaxel, doxorubicin, and cyclophosphamide (TAC), reduce the risk of recurrence by 11% and the risk of death by 16% compared with cyclophosphamide, methotrexate, and fluorouracil (CMF) combinations [15].

CALGB 9344 and NSABP B-28 established the role of taxanes as a component of adjuvant therapy of breast cancer. Between May 1994 and April 1999, the CALGB randomized 3121 patients in a 3x2 factorial design to cyclophosphamide combined with one of three doses of doxorubicin given every 3 weeks for 4 cycles followed by paclitaxel on an every-3-week schedule or observation. No differences in efficacy based on doxorubicin dose were observed. However, adding paclitaxel to the AC regimens led to reductions in hazard for recurrence and death of 17% and 18%, respectively [16]. NSABP B-28 randomized 3060 women with resected, node-positive breast cancer to receive 4 cycles of AC followed by 4 cycles of paclitaxel on the every-3-week schedule vs. 4 cycles of AC. The addition of paclitaxel significantly reduced the hazard for disease-free survival (DFS) events by 17% (relative risk 0.83; 95% CI, 0.72– 0.95; p=0.006), but overall survival (OS) was similar for both groups [17].

NSABP B-27 evaluated the potential benefit of administering docetaxel (T) following AC as preoperative therapy for 2411 women with palpable, operable breast cancer. The addition of preoperative or postoperative T after preoperative AC showed a non-significant trend toward improving DFS, primarily by decreasing the incidence of local recurrences, but did not improve OS. Concurrent use of tamoxifen may have limited the impact of adding T [18].

ECOG E1199 employed a 2x2 factorial design to compare paclitaxel to docetaxel following 4 cycles of AC and a weekly schedule vs. an every-3-week schedule of the taxanes in nearly 5,000 women with node-positive or high-risk node-negative breast cancer. Patients were randomly assigned to receive one of the following taxane treatments: docetaxel 35 mg/m^2 once a week; docetaxel 100 mg/m^2 once every 3 weeks; paclitaxel 80 mg/m^2 once a week; or paclitaxel 175 mg/m^2 once every 3 weeks. The primary comparisons showed no differences between the taxanes (paclitaxel vs. docetaxel: HR 1.032; p=0.61) or schedule (q3w vs. q1w: HR 1.062; p=0.33). However, a Cox proportional hazards model, which included the taxane administered, the taxane schedule and their interaction, showed the interaction of docetaxel and the weekly schedule was significant for both DFS (p=0.003) and OS (p=0.01). This complicated the interpretation of the primary endpoints so comparisons were made between the standard every-3-week

paclitaxel schedule and each of the other three arms. As compared with the group receiving paclitaxel every 3 weeks, there was better DFS in the group receiving weekly paclitaxel (HR 1.27; p=0.006) and in the group receiving every-3-week docetaxel (HR 1.23; p=0.02). However, while OS was significantly better in the group receiving weekly paclitaxel (HR 1.32; p=0.01), it was not evident in the group receiving docetaxel every 3 weeks (HR 1.13; p=0.25). Serious adverse effects of treatment developed in 71% of patients treated with docetaxel every 3 weeks, and only 28% of patients treated with paclitaxel once a week. The increase in adverse events with docetaxel was primarily due to febrile neutropenia and neutropenia-associated infections which can be substantially diminished with the use of primary prophylactic G-CSF [19, 20]. Most recently, SWOG 0221, compared weekly paclitaxel to dose-dense (every 2 week) scheduling as adjuvant breast cancer treatment and showed no difference in efficacy endpoints with either method of delivery. Patients had higher rates of grade 3 and 4 neuropathy (17% vs. 10%) with dose-dense delivery and higher rates of hematologic toxicity with weekly scheduling[21].

As sporadic TNBC is clinically similar to BRCA-1 associated breast cancer, there has been significant interest in using platinum compounds in TNBC. BRCA-1 associated breast cancers are sensitive to these agents since they cause double-strand breaks in DNA and these cancers are deficient in homologous recombination mediated DNA repair mechanisms. Several neoadjuvant trials have evaluated platinum agents in TNBC patients. Alba et el. investigated whether the addition of carboplatin to standard chemotherapy in patients with TNBC would lead to an increase in the pCR rates in the neoadjuvant setting.[22] Patients received epirubicin plus cyclophosphamide (EC) followed either by docetaxel or docetaxel plus carboplatin. The addition of the platinum did not improve pCR rates (35% versus 30%) in this study. Silver et al. evaluated the efficacy of neoadjuvant cisplatin in 28 patients with TNBC.[23] All patients received 4 cycles of cisplatin preoperatively, followed by definitive surgery, and then adjuvant chemotherapy and/or radiation as per their treating physicians. The pCR rate was 21% (6 of 28 patients), while 64% (18 of 28) achieved either a clinical complete or partial response. The efficacy of neoadjuvant cisplatin in TNBC versus non-TNBC was also compared in a small retrospective study by Sirohi et al.[24] Complete response rates by clinical exam were higher for those with TNBC (88%) versus the non-TNBC group (51%). Paradoxically survival outcomes were worse for the TNBC group despite higher rates of initial response to chemotherapy. This has also been seen in a study that showed despite a higher rate of chemosensitivity, patients with TNBC had a worse outcome than those with ER positive disease.[25] Multiple other small studies have also evaluated neoadjuvant platinum-based therapy in patients with TNBC with varying results.[26-33] While current data provides insufficient evidence for the routine use of platinums in patients with TNBC, the pCR improvements observed in the neoadjuvant setting have created a conundrum and some physicians are now routinely adding carboplatin. However, until survival data for the addition of carboplatin is available, the use of carboplatin is still considered investigational by other physicians. Since the most significant issue remains the identification of platinum sensitivity, this protocol is therefore designed to have high clinical and scientific impact.

1.4 Study Rationale

While improvements in adjuvant therapy have remarkably improved the outcome of women with resectable breast cancers over the past two decades, biologically defined subsets of patients at a high risk of recurrence and death continue to exist. Improving upon our ability to eradicate micrometastases and prevent recurrences in these high risk individuals, particularly those with TNBC remains a challenge. The relapse rate for patients treated with adjuvant therapy on clinical trials has declined, due, in part, to improvements in treatment. Unfortunately, testing new drugs in the adjuvant setting in breast cancer requires large number of patients as well as long follow up periods. The neoadjuvant platform allows the opportunity to assess clinically meaningful responses in vivo, therefore enabling more rapid identification of effective drugs and consequently changes to established standards of care. In fact, to facilitate early access of potentially active drugs for patients with high risk disease such as TNBC, the Food and Drug Administration (FDA) has recently outlined a pathway for accelerated drug approval based on pathologic complete response (pCR) from neoadjuvant trials. Additionally, the neoadjuvant platform allows us to obtain serial tissue and blood samples before and after treatment, which may allow identification of subsets of patients less or more likely to benefit from a given treatment.

As TNBC lacks an identified therapeutic target, most individuals receive chemotherapy as part of their systemic management. Unfortunately a large proportion develops recurrences due to chemotherapy resistance, therefore there is a need to develop more effective chemotherapy regimens and also identify predictors of CR and chemotherapy sensitivity to better individualize management. The use of proteomic and genomic analysis in the design of trials evaluating mechanisms of CR are lacking. By being able to assess pathologic tumor response and molecular changes induced by treatment, the neoadjuvant platform creates an excellent model for such a study. Typical clinical trial experimentation recruiting individuals to adjuvant drug studies, and then assessing survival may not be the most efficient method. Additionally, although cell lines are informative, they do not accurately reflect tumor morphology or heterogeneity in vivo. To address this, PDX are being explored as surrogates for originating tumors.

This research proposal investigates a potential new therapeutic option for patients with curable TNBC using a combination of docetaxel and carboplatin in the neoadjuvant setting. We propose to treat patients with a similar backbone as the CALGB 40603 regimen by administering six 3-week cycles of docetaxel in combination with 6 cycles of carboplatin to improve pCR. If successful, this strategy may help avoid anthracyclines with the associated cardiotoxicity and leukemia risk, and will develop evidence for a regimen that is more convenient to administer than the Gepar Sixto protocol of 18 weeks of weekly carboplatin and paclitaxel.

It has been postulated that DNA-damaging agents like platinum salts may be particularly effective in TNBC due to its similarities with BRCA-1 associated breast cancer. Studies utilizing taxanes and platinum salts

have been performed in unselected breast cancer patients and have shown higher response rates than single agent taxanes and good tolerance with a low incidence of serious side effects.

In addition, PDX models will be developed from tumors from individual patients on this trial. This study is therefore based on the hypotheses that neoadjuvant docetaxel and carboplatin will achieve a pCR rate of 54%, similar to the CALGB 40603 carboplatin-containing arms, and that PDX are accurate replicas of originating tumors and can recapitulate chemotherapy response. The promise of this novel strategy is that PDX may be used as drug discovery platform for chemotherapy-resistant patients.

To understand the potential clinical impact of this study, it is valuable to consider a clinical scenario. After diagnosis, a TNBC patient may be treated with neoadjuvant chemotherapy with the ultimate goal of minimizing the risk of disease recurrence. While these treatments are associated with a 20-38% decrease in breast cancer mortality amongst all subtypes [15], patients with TNBC that do not respond to chemotherapy continue to have a substantial risk of recurrent disease within the first 5 years. A novel personalized strategy to assess drug responsiveness will be to develop a PDX model from a particular patient, either shortly before or during neoadjuvant treatment, treat the PDX model with a view to determining chemotherapy response in the PDX. This then may be able to be a surrogate for whether the TNBC patient will likely respond to the drug being administered. In addition, drug discovery using the PDX platform as a living replica may also be utilized for those with chemotherapy resistant TNBC, thus allowing for individualized management.

1.5 Correlative Studies Background

1.5.1 Patient-Derived Xenografts (PDX) in Breast Cancer

Despite progress in our understanding of cancer biology, the translation of research findings into new therapies for cancer is still an enormous barrier. Recent data suggests a 90% rate failure rate for oncology products in clinical development [34]. In part, the development of new therapies in breast cancer is constrained by the scarcity of reliable *in vivo* models of human breast cancer with which to study the biology of tumors and how they spread. Breast cancer cell lines, although informative, do not reliably reflect breast cancer heterogeneity or morphology *in vivo*, and thus poorly predict how drugs will perform in the clinical setting [34-36]. To address this, patient-derived xenografts (PDX) are being explored as surrogates for originating tumors, as reviewed by Landis and colleagues [37].

Interestingly, several groups have found that the take rate (rate of engraftment) of PDX correlate with tumor grade, with the most aggressive TNBCs having higher take rates than ER+ tumors [38-41]. Marangoni and colleagues used estrogen-treated Swiss nude mice to subcutaneously transplant tumor into the subscapular fat pad [38]. A 12.5% rate of engraftment was observed (25 out of 200 samples), with almost all PDX displaying an aggressive phenotype. PDX response rate compared to patient response to treatment was concordant in 5/7 of cases, supporting the utility in these models to predict patient

response to therapeutics. Bergmaschi and colleagues used estrogen supplementation and transplantation into the interscapular fat pad of severe combined immunodeficiency (SCID) mice, resulting in a 7% take rate [39]. Of the 2 PDX established, both maintained concordance with the original tumors. Our group, in collaboration with others successfully established PDX directly from breast cancer patient samples, using epithelium-free mammary fatpad as the transplantation site [40]. Again, most patients yielded triple negative PDX, though lines from other subtypes were also established. PDX were phenotypically consistent with their tumor of origin, and of the 27 lines fully evaluated, 48% developed metastatic lesions to the lungs.

Investigators at Washington University in St. Louis have also successfully developed several panels of PDX from breast cancer patients. Six patients with TNBC have had successful PDX developed from their original tumors.

Whole genome sequencing of a breast cancer primary, brain metastasis, and PDX basal-like breast cancer in one patient demonstrated a PDX model that efficiently captured almost all of the genome-wide somatic mutations from the original tumor and was enriched for mutations present in the metastatic sample. They have also shown that comparative whole-genome sequencing demonstrates that PDX preserves the genomic structural similarities to originating TNBC tumors [42]. A panel of PDX from patients with treatment-resistant breast cancer was established to study the genomic, biological, and pharmacological characteristics of advanced breast cancer

This trial is based on the hypothesis that PDX are accurate replicas of originating tumors and can recapitulate chemotherapy response. The ability to effectively utilize innovative PDX models may be a unique new starting point for molecular pharmacology in TNBC patients. The development of these models, as outlined here, will allow an exploration of the relationship between PDX phenotype and clinical outcome of patients from whom tumors arose. It will elucidate the mechanisms of response to chemotherapy through analysis of serial biopsies using proteomic technologies, while also correlating molecular changes in PDX to those in originating tumors. The profound impact of this study to clinical breast cancer research is that ultimately, drug discovery using PDX as a living replica may be done more efficiently, especially for those with chemotherapy-resistant TNBC, thus allowing for uniquely individualized management.

1.5.2 Cell-free circulating tumor DNA

Cell-free circulating tumor DNA has been proposed as a surrogate biological material to define the genetic aberrations of a primary tumor and/or metastases in a given cancer patient, and to serve as a biomarker for diagnosis, prognostication, and monitoring of response to therapy. Recent studies have demonstrated that mutations in *PIK3CA* and/or *TP53* can be identified in the ctDNA from plasma samples of the vast majority of patients with advanced breast cancer and in approximately 50% of patients with early stage breast cancer.

Our hypotheses are that plasma ctDNA analysis may provide a means to predict relapse after primary breast cancer systemic therapy and to characterize the genetic composition of the resistant clones. Furthermore, ctDNA analysis has been shown to constitute a source of DNA that allows for the investigation of the repertoire of somatic mutations found in micrometastatic and metastatic disease. ctDNA may also prove useful as an indicator of pCR before surgery. This could improve surgical decision making and allow for de-escalation of local therapy.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether neoadjuvant docetaxel and carboplatin will increase the pCR rate in TNBC compared to historical controls. Pathologic complete response (pCR) will be defined as no residual invasive breast cancer in the breast and ipsilateral axillary lymph node ($ypT_{0\text{-}is}$ ypN_0).

2.2 Exploratory Objectives

1. To determine the xenografting rate from TNBC patients being treated with neoadjuvant chemotherapy.

2. To compare chemotherapy responses in PDX and TNBC patients being treated with neoadjuvant chemotherapy

3. To investigate genomic and proteomic molecular changes in PDX and corresponding host patients with the intent to identify predictors of drug response and resistance.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. All patients must be 18 years of age or older.

2. All patients must be diagnosed with invasive breast cancer.

3. Breast cancer must be ER-negative, and HER-2 negative according to CAP/ASCO biomarkers testing guidelines. Tumors may be PgR positive with an Allred score of less than 5.

4. Primary breast tumor size at least 2 cm in one dimension by clinical or radiographic exam. Patients who have multicentric breast cancer are eligible if each lesion is ER-negative and HER2-negative. In that case, one lesion needs to be identified as the index lesion to be followed for clinical response. The index lesion must also be the lesion from which core biopsies are obtained.

5. Patients with inflammatory breast cancer are eligible if they meet **both** of the following criteria:

   a. Patient has an underlying, clinically palpable breast mass of at least 2cm, AND

   b. a corresponding lesion is visualized on mammogram or ultrasound

6. Normal bone marrow and organ function as defined below:

   a. Leukocytes $\geq$ 3,000/mcL

   b. Absolute neutrophil count $\geq$ 1,200/mcl

   c. Platelets $\geq$ 100,000/mcl

   d. Serum bilirubin ≤ institutional 1.5 times ULN (***OR*** for patients with documented Gilbert Syndrome, total bilirubin ≤ 3.0 times ULN with direct bilirubin ≤ ULN)

   e. AST(SGOT)/ALT(SGPT) ≤ 2.5 times ULN

   f. Creatinine ≤ 1.5 ULN

7. Women of childbearing potential (defined as women under the age of 55 with intact ovaries and uterus) must agree to use adequate contraception prior to study entry and for the

duration of study participation. They must also have a negative urine pregnancy test within 7 days of starting treatment.

8. Ability to understand and willingness to sign an IRB approved written informed consent document and follow study procedures including willingness to undergo study biopsies.

3.2 Exclusion Criteria

1. Any prior systemic therapy for breast cancer within 5 years.
2. A history of other malignancy ≤ 5 years previous with the exception of basal cell or squamous cell carcinoma of the skin which were treated with local resection only or carcinoma *in situ* of the cervix.
3. Patients with known bilateral invasive breast cancer. Patients with contralateral in situ breast carcinoma are eligible.
4. Patients with confirmed stage IV disease.
5. Currently receiving any other investigational agents.
6. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to docetaxel or carboplatin.
7. Known to be seropositive for HIV, HCV, or HBV.
8. Any prior treatment with Taxotere or Carboplatin.
9. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
10. If the patient is otherwise not deemed a good study candidate by sole discretion of the principal investigator.
11. Patient is pregnant or breastfeeding.

4.0 REGISTRATION PROCEDURES

4.1 Guidelines for Lead Institution

Patients should be registered in the electronic database (available at: https://oncore.research.bcm.edu/) as soon as the patient has signed the informed consent. Following registration, the patient's enrollment status in the electronic database will be listed as pending. Prior to enrollment in the electronic database, the eligibility worksheet must be reviewed and signed by the study coordinator and a Breast Center physician, in accordance with Lester & Sue Smith Breast Center standard operating procedures. Eligibility data will then be entered in the electronic database and the subject will be designated as "on study".

Study treatment may begin only after eligibility has been confirmed by both a study coordinator and a Breast Center physician. Once eligibility has been reviewed, the study treatment must begin according to the timeframe designated by the screening procedures listed in the study calendar (Section 5.1).

4.2 Guidelines for Other Institutions

Participants MUST be registered with the Leading Institution prior to the start of protocol treatment. Subject registration will be managed by the lead study coordinator at the Lester & Sue Smith Breast Center (LSSBC) as described below.

Potential study subjects who have signed the Informed Consent document should be registered in the electronic database Oncore. ***Registration should occur as soon as possible after signed consent is obtained, in order to ensure that the potential subject is assigned the correct study number.*** The Lead Coordinator will then assign the subject with the study number.

Once a potential subject has completed all required screening procedures, the responsible study coordinator should complete the Eligibility Worksheet, which will be provided to sites upon site activation. The eligibility worksheet should be reviewed by both the site study coordinator and the local principal investigator for accuracy. Completed worksheets, along with de-identified source documentation, should be transmitted to the lead study coordinator at the Lester & Sue Smith Breast Center for confirmation of eligibility. Once eligibility data are entered in the electronic database and confirmed, the subject will be designated "on study". Study treatment may not begin until eligibility has been confirmed by a representative from the Lester & Sue Smith Breast Center.

Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These patients will not be eligible to receive study treatment.

4.3 Eligibility exceptions

Any requests for protocol exceptions must be approved in writing by Dr. Mothaffar Rimawi at the Lester & Sue Smith Breast Center. Request for protocol exceptions must be made via email or fax to the lead study coordinator. Both Dr. Rimawi and the local PI should be copied on all requests and associated correspondence. Once a determination has been made, the site study coordinator and local PI will be notified in writing. In the event that an eligibility exception is granted by the coordinating center, the local site must submit the exception to their local IRB in accordance with institutional standard operating procedures.

5.0 STUDY PROCEDURES

Patients enrolled on study will have the following study assessments and procedures performed as described. For a summary of required assessments, please refer to the study calendar located in Section 11.0.

5.1 Screening assessments

A signed, written informed consent must be obtained before any study-specific assessments are initiated. Results from assessments performed prior to written consent as part of a patient's routine diagnostic workup may be used for screening purposes. The following assessments will be obtained at screening, within 28 days prior to beginning study medication, unless otherwise noted:

- Signed, written informed consent
- Subject demographic information, to include age, ethnicity, and race
- Medical history, including past and current conditions, and past surgical history
- Physical exam, to include vital signs, weight, a clinical breast exam, and palpation of lymph nodes.
  - For patients with palpable breast masses, tumor measurements (as assessed by palpation)/calipers MUST be recorded in the clinic progress note 14 days prior to initiation or treatment
  - Please see Section 12.1 for tumor measurement guidelines
- Laboratory assessments to include:
  - CBC/differential/platelets
  - Serum chemistry profile (should include sodium, potassium, chloride, calcium, glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase, total bilirubin, total protein, and albumin)

- Serum β HCG (for pre- or perimenopausal subjects with an intact uterus). NOTE – If screening HCG performed more than 7 days prior to Day 1, serum HCG must be repeated on Day 1 prior to beginning study medication.

- Bilateral mammogram (may be obtained within 6 weeks prior to Day 1 of study treatment). A full, diagnostic mammogram must be performed to fulfill this requirement. Mammograms performed to verify clip placement, or mammograms containing only spot compression images, will not be accepted for study purposes.
- Breast ultrasound (unilateral, unless a bilateral ultrasound is clinically indicated; may be obtained within 6 weeks prior to Day 1 of study)
- Bone imaging, if patient is experiencing bone pain, or if bone imaging is otherwise clinically indicated. If bone imaging is not clinically indicated, the treating physician must document as such in the source documents.
- Chest imaging (A CT chest, Chest X-ray PA & Lateral, or PET/CT are acceptable imaging techniques), to be performed if clinically indicated
- Review of all underlying AEs taken within 28 days of planned treatment start date

If the above studies are within acceptable parameters as defined by the eligibility criteria, the participant will undergo a breast biopsy as described in Section 10.1 and research blood will be collected as descibed in Section 10.2. If eligible, the patient will begin the combination of docetaxel and carboplatin as descibed in Section 6.

5.2 Cycle 1 Day 1

The following assessments should be performed within 3 days prior to the start of study medication:

- Physical exam, to include vital signs, weight, a clinical breast exam, and palpation of lymph nodes.
  - For patients with palpable breast masses, bidimensional tumor measurements (as assessed by palpation)/calipers) MUST be recorded in the clinic progress note (may be performed up to 3 days prior to C1D1)
- Laboratory assessments to include a CBC/differential/platelets and serum chemistry panel, as defined in Section 5.1.
- Record compliance to study medications and infusion schedule
- Non-serious and serious adverse events should be recorded and graded appropriately as discussed in Section 8.
- Serum pregnancy test if not performed 7 days priot to initiation of study treatment.

5.3 Cycle 1 Day 7

The following assessments should be performed on day 7 after treatment initiation (+/- 3 days):

- Core needle biopsy (optional)
- Research blood draw (mandatory)

5.4 Cycles 2,3,4,5, and 6 Day 1

The following assessments should be performed within 3 days prior to the start of study medication:

- Physical exam, to include vital signs, weight, a clinical breast exam, and palpation of lymph nodes.
  - For patients with palpable breast masses, bidimensional tumor measurements (as assessed by palpation)/calipers) MUST be recorded in the clinic progress note (may be performed up to 3 days prior to each cycle)
- Laboratory assessments to include a CBC/differential/platelets and serum chemistry panel, as defined in Section 5.1.
- Record compliance to study medications and infusion schedule
- Non-serious and serious adverse events should be recorded and graded appropriately as discussed in Section 8.

5.5 End of Treatment

The following assessments should be performed within 2 weeks of the completion of last cycle. These assessments should also be performed for patients who discontinue treatment prematurely prior to the initiation of alternate treatment, if applicable.

- Physical exam, to include vital signs, weight, a clinical breast exam, and palpation of lymph nodes.
  - For patients with palpable breast masses, tumor measurements (as assessed by palpation)/calipers) MUST be recorded in the clinic progress note.
- Laboratory assessments to include a CBC/differential/platelets and serum chemistry panel as defined in section 5.1.
- Unilateral mammogram and breast ultrasound will be performed to further assess clinical response in the primary tumor and to document response to treatment. A diagnostic mammogram is necessary to fulfill the mammogram requirement.
- Collect research blood
- Non-serious and serious adverse events should be recorded and graded appropriately as discussed in Section 8 of the protocol.

At this point, patients will have completed study treatment and should proceed with definitive surgery. In the event of early discontinuation, patients who require additional neoadjuvant treatment will have an option to undergo a core needle biopsy for research purposes. For patients proceeding directly to surgery, surgery should be scheduled between 4 and 6 weeks after the last dose of study treatment. If surgery is

delayed for a time period greater than 65-weeks following study completion, a core needle biopsy may be performed in lieu of surgical specimen collection if the patient has residual disease amenable to biopsy.

5.6 Follow up procedures

The following assessments should be performed within 2 months (+/- 1 week) of the completion of last cycle, and then annually for five years or until disease recurrence, whichever comes first:

- Research blood collection

All subjects will be followed for survival at approximately 1 year intervals from the date of diagnosis. Survival follow-ups may be conducted by phone, email, or clinic visit, and will be discontinued in the event of subject death or withdrawal of consent. At each follow-up, disease and vital status will be documented. If patient has experienced disease recurrence or progression, the sites of recurrence/progression will be recorded.

6.0 TREATMENT PLAN

6.1 Premedication Administration

Patients receiving docetaxel should be premedicated according to institutional standards to prevent or decrease the chance of reactions. Premedications must include steroids orally or intravenously.

6.2 Agent Administration

Docetaxel will be administered intravenously at a dose of 75mg/m^2 on Day 1 of each 21-day cycle. Carboplatin AUC 6 will be administered intravenously on Day 1 of each 21-day. For carboplatin dosing, creatinine clearance (CrCl) will be calculated according to the modified Cockcroft-Gault equation using actual body weight. CrCl should be capped at 125 mL/min. A total of 6 cycles will be given. A 3 day window will be allowed for the 21 day cycle, if deemed appropriate by the treating physician.

Surgery will take place after the conclusion of the neoadjuvant regimen, ideally within 4-6 weeks. Further adjuvant chemotherapy may be given at the discretion of the treating physician.

6.3 Anthracycline-Based Adjuvant Chemotherapy

Patients who develop any of the following will have the option of receiving anthracycline-based chemotherapy pre- or post-operatively at the discretion of the treating physician, after an end of treatment biopsy. Due to the infeasibility of evaluating pathologic response in participants who receive

non-protocol therapy prior to surgery, such patients will be counted as non-pathologic complete responses for the purpose of statistical analysis.

- Grade 3 neuropathy
- Other intolerable side effects
- Progressive disease
- Failure to achieve a pCR following treatment with docetaxel/carboplatin

6.4 General Concomitant Medication and Supportive Care Guidelines

Physicians will perform standard monitoring for toxicity to chemotherapy administration. Patients will be treated for toxicities at the discretion of the physician. Growth factor support with either filgrastim or pegfilgrastim is at the discretion of the investigator.

6.5 Women of Childbearing Potential

Women of childbearing potential (defined as women under the age of 55 with intact ovaries and uterus) are required to have a negative serum pregnancy test within 7 days prior to the first dose of docetaxel/carboplatin.

Female patients are required to use acceptable contraception during participation in the study.

If a patient is suspected to be pregnant, chemotherapy should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient becomes pregnant during therapy or within 6 months after the last dose of chemotherapy, the principal investigator must be notified in order to facilitate outcome follow-up.

6.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue for 6 cycles or until one of the following criteria applies:

- Documented and confirmed disease progression

- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol as determined by the principal investigator.
- Lost to follow-up
- Patient withdraws consent
- Principal investigator removes the patient from study
- Death
- Study closure.

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

6.7 Duration of Follow-up

Patients will be followed for survival on a yearly basis for 5 years. This can be achieved during routine clinical follow up visits, phone contact, or checking the electronic medical record. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

7.0 DOSE DELAYS/DOSE MODIFICATIONS

For patients who are unable to tolerate the protocol-specified dosing schedule, it is strongly recommended that dose modifications be made according to the dose adjustment schedule detailed in the table below. Deviations from the recommended modifications are permitted in the event that they are deemed medically necessary by the treating physician, with prior approval by the principal investigator. Medical necessity and PI approval must be documented in the patient's medical record. If carboplatin is to be delayed, administration of docetaxel shall be omitted as well. Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 4.0.

Table 1: Dose levels for docetaxel and carboplatin

	Dose Level 0 ***Starting Dose***	**Dose Level -1**	**Dose Level -2**	**Dose Level -3**
Docetaxel (mg/m^2)	75	60	50	Discontinue
Carboplatin (AUC)	6	5	4	Discontinue

Table 2: Dose modifications/delays for DOCETAXEL and CARBOPLATIN

Important table instructions: • Dose modifications must be based on AEs that occurred during the cycle (column 2) ***and*** AEs present on the scheduled cycle Day 1 (column 3). • **MODIFICATIONS APPLY TO BOTH CARBOPLATIN AND DOCETAXEL UNLESS SPECIFIED OTHERWISE.** • Dose modifications must be based on the AE requiring the greatest modification.		
NCI CTCAE v4.0 Category/Grade	Modifications for AEs that occurred during a cycle but RESOLVE PRIOR TO THE NEXT TREATMENT CYCLE (See footnote **a**)	Modifications for AEs that REQUIRE A DELAY IN ADMINISTRATION OF THE TREATMENT CYCLE (See footnote **b**)
Neutrophil count decreased: Grade 2 (1000-1199/mm^3), Grades 3, 4	Maintain dose	***ANC****: Hold until ≥ 1200/mm^3. If recovery takes:* 1-3 wks – maintain dose and add G-CSF *If receiving G-CSF and recovery takes:* 1 wk – maintain dose 2-3 wks – ↓ one dose level
Platelet count decreased: Grades 2, 3	Maintain dose	**Platelets**: *Hold until ≥ 75,000/mm^3. If recovery takes:* 1 wk – maintain dose; 2 to 3 wks - ↓ one dose level
Grade 4	↓ one dose level	*Hold until ≥ 75,000/mm^3.* ↓ one dose level
GI *(if related to chemotherapy)*: **Diarrhea** Grade 2	Maintain dose	↓ one dose level

Important table instructions:

- Dose modifications must be based on AEs that occurred during the cycle (column 2) ***and*** AEs present on the scheduled cycle Day 1 (column 3).
- **MODIFICATIONS APPLY TO BOTH CARBOPLATIN AND DOCETAXEL UNLESS SPECIFIED OTHERWISE.**
- Dose modifications must be based on the AE requiring the greatest modification.

NCI CTCAE v4.0 Category/Grade	Modifications for AEs that occurred during a cycle but RESOLVE PRIOR TO THE NEXT TREATMENT CYCLE (See footnote **a**)	Modifications for AEs that REQUIRE A DELAY IN ADMINISTRATION OF THE TREATMENT CYCLE (See footnote **b**)
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or discontinue	↓ two dose levels or discontinue
Mucositis - oral Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or discontinue	↓ two dose levels or discontinue
Vomiting *(despite antiemetics)* Grade 2	↓ one dose level (optional)	↓ one dose level
Grades 3, 4	↓ one dose level or discontinue	↓ two dose levels or discontinue
Investigations (hepatic): **Bilirubin, AST, alk phos** Grade 2	↓ one dose level	*Hold until bilirubin returns to the **baseline grade** and AST and alk phos have returned to ≤grade 1.* ↓ one dose level
Grade 3	↓ two dose levels	*Hold until bilirubin returns to the **baseline grade** and AST and alk phos have returned to ≤grade 1.* ↓ two dose levels

Important table instructions:

- Dose modifications must be based on AEs that occurred during the cycle (column 2) ***and*** AEs present on the scheduled cycle Day 1 (column 3).
- **MODIFICATIONS APPLY TO BOTH CARBOPLATIN AND DOCETAXEL UNLESS SPECIFIED OTHERWISE.**
- Dose modifications must be based on the AE requiring the greatest modification.

NCI CTCAE v4.0 Category/Grade	Modifications for AEs that occurred during a cycle but RESOLVE PRIOR TO THE NEXT TREATMENT CYCLE (See footnote **a**)	Modifications for AEs that REQUIRE A DELAY IN ADMINISTRATION OF THE TREATMENT CYCLE (See footnote **b**)
Grade 4	Discontinue	Discontinue
Infection or febrile neutropenia: Grade 2 (N/A for febrile neutropenia)	Maintain dose and add G-CSF prophylaxis for subsequent chemotherapy cycles if neutropenia was present.**c**	
Grade 3	Maintain dose and add G-CSF prophylaxis with subsequent chemotherapy cycles. If already receiving prophylactic G-CSF, ↓ one dose level.	
Grade 4	Maintain dose or ↓ one dose level and add G-CSF prophylaxis with subsequent chemotherapy cycles. If receiving prophylactic G-CSF, ↓ one dose level or discontinue.	
Investigations: **Creatinine increased** Grades 2, 3	Hold until serum creatinine is ≤ grade 1 ***and*** calculated creatinine clearance is ≥ 30 mL/min. – If creatinine clearance is > 50 mL/min, maintain dose. – If creatinine clearance is 30-50 mL/min, ↓ carboplatin one dose level.	

Important table instructions:

- Dose modifications must be based on AEs that occurred during the cycle (column 2) ***and*** AEs present on the scheduled cycle Day 1 (column 3).
- **MODIFICATIONS APPLY TO BOTH CARBOPLATIN AND DOCETAXEL UNLESS SPECIFIED OTHERWISE.**
- Dose modifications must be based on the AE requiring the greatest modification.

NCI CTCAE v4.0 Category/Grade	Modifications for AEs that occurred during a cycle but RESOLVE PRIOR TO THE NEXT TREATMENT CYCLE (See footnote **a**)	Modifications for AEs that REQUIRE A DELAY IN ADMINISTRATION OF THE TREATMENT CYCLE (See footnote **b**)
	If calculated creatinine clearance is < 30 mL/min but all other non-renal function AEs have resolved to ≤ grade 1 on the scheduled Day 1, carboplatin must be held and docetaxel may be administered. If calculated creatinine clearance subsequently improves to ≥ 30 mL/min by Day 1 of the next scheduled cycle, carboplatin may be resumed with docetaxel. The missed carboplatin dose will not be made up.	
Grade 4	Discontinue	
Other clinically significant AEs[d]: Grade 2	Maintain dose or ↓ one dose level	
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or discontinue	Discontinue

a ***Resolved means that all AEs are ≤ grade 1*** (except ANC/AGC [which must be ≥ 1200/mm^3] and bilirubin [which must be ≤ the baseline grade]) on Day 1 of the next scheduled cycle (i.e., treatment can be given without delay).

b ***Hold and check weekly. With exception of ANC/AGC and bilirubin, resume treatment when toxicity is ≤ grade 1. If toxicity has not resolved after 3 weeks of delay, discontinue docetaxel and carboplatin.***

Important table instructions: • Dose modifications must be based on AEs that occurred during the cycle (column 2) ***and*** AEs present on the scheduled cycle Day 1 (column 3). • **MODIFICATIONS APPLY TO BOTH CARBOPLATIN AND DOCETAXEL UNLESS SPECIFIED OTHERWISE.** • Dose modifications must be based on the AE requiring the greatest modification.		
NCI CTCAE v4.0 Category/Grade	Modifications for AEs that occurred during a cycle but RESOLVE PRIOR TO THE NEXT TREATMENT CYCLE (See footnote **a**)	Modifications for AEs that REQUIRE A DELAY IN ADMINISTRATION OF THE TREATMENT CYCLE (See footnote **b**)
c If grade 2 criteria for infection include topical antibiotics or other local treatment, use of G-CSF is at the investigator's discretion. **d** Determination of "clinically significant" AEs is at the discretion of the investigator.		

Table 3: Treatment management for docetaxel-related neuropathy

Nervous System Disorders Paresthesias Peripheral sensory neuropathy	**1-7 Days Duration**	**Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed**
Grade 1	Maintain docetaxel dose	
Grade 2	Maintain docetaxel dose[a]	Decrease docetaxel one dose level[b]
Grade 3	**First episode:** Decrease docetaxel one dose level[a] **Second episode:** Discontinue docetaxel	Discontinue docetaxel
Grade 4	Discontinue docetaxel	
a Must be resolved to ≤ grade 1 on Day 1 of the next cycle. **b** Hold chemotherapy (docetaxel and carboplatin) for ***persistent*** grade 2 neuropathy. When ≤ grade 1, resume treatment with dose modification for docetaxel (no dose reduction for carboplatin). If grade 2 toxicity persists after 3 weeks of delay, discontinue docetaxel.		

Table 4: Treatment management for docetaxel-related musculoskeletal pain

Musculoskeletal and Connective Tissue Disorders **Arthralgia** **Myalgia**	**1 – 7 Days Duration**	**Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed**
Grade 1 *(despite analgesics)*	Maintain docetaxel dose	
Grade 2 *(despite analgesics)*	Maintain docetaxel dose	Maintain docetaxel dose or Decrease docetaxel one dose level*
Grade 3 *(despite analgesics)*	First episode: Decrease docetaxel one dose level Second episode: Discontinue docetaxel	First episode: Decrease docetaxel one dose level* or Discontinue docetaxel Second episode: Discontinue docetaxel
* Hold docetaxel for *persistent* grade 2 or 3 musculoskeletal pain. When ≤ grade 1, resume treatment with dose modification for docetaxel. If grade 2 or grade 3 toxicity persists after 3 weeks of delay, discontinue docetaxel.		

8.0 REGULATORY AND REPORTING REQUIREMENTS

8.1 General

This study will use the descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) that is available at http://ctep.cancer.gov/reporting//ctc.html.

Information on all adverse events, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported as described in the following sections.

Adverse events experienced by participants will be collected and reported from initiation of study medication, throughout the study, and within 30 days of the last dose of study medication, as described in the Case Report Form Completion Guidelines. Participants who experience an ongoing adverse event related to a study procedure and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the participating investigator.

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. The investigator should notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

8.2 Definitions

8.2.1 Adverse Events (AEs)

An adverse event is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

8.2.2 Serious Adverse Event (SAE)

A serious adverse event is an undesirable sign, symptom, or medical condition which:

- is fatal or life-threatening;
- requires or prolongs inpatient hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or birth defect; or
- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above.
- Any other event, which, in the opinion of the investigator, could be considered serious.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

8.2.3 Expectedness

- Expected: Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the package insert or is included in the informed consent document as a potential risk.
- Unexpected: An adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the package insert or when it is not included in the informed consent document as a potential risk.

8.2.4 Attribution

The investigator will determine the relationship of each adverse event, if any, to investigational treatment or study procedure. Causality should be assessed using the following categories: Unrelated, Unlikely, Possible, Probable, or Definite.

The degree of certainty with which an adverse experience is attributed to drug treatment (or alternative causes, e.g. natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the experience can be understood in terms of the following:

- Known pharmacology of the drug
- Reaction of similar nature being previously observed with this drug or class of drug
- The event having often been reported in literature for similar drugs as drug related (e.g. skin rashes, blood dyscrasia)
- The event being related by time to drug administration terminating with drug withdrawal (dechallenge) or reproduced on rechallenge.

The investigator may change his/her assessment of causality in light of follow-up information. All adverse events will be recorded, regardless of whether the event is thought to be related to investigational treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.

- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

8.3 Reporting Procedures

8.3.1 General

Adverse events will be captured in both the source documents and on the appropriate study-specific case report forms (CRFs). Given that each of the medications being utilized in this trial has a well-established safety profile, this protocol will utilize targeted AE reporting for all non-serious adverse events, as described below.

8.3.2 AE reporting requirements

- AE of any grade that requires a modification to treatment (e.g. dose modification, dose delay, or drug discontinuation)
- All grade 3 and 4 AEs, regardless of causality
- Any clinically significant laboratory abnormality, defined as an abnormality requiring medical intervention or further diagnostic work-up, as determined by the treating physician.
- All serious adverse events (SAEs).

8.3.3 Serious Adverse Events

Any serious adverse events that occur during the clinical study or within 30 days of receiving the last dose of study medication, whether or not related to the study drug, must be reported by the investigator. In addition, any SAEs which occur as a result of protocol specific diagnostic procedures or interventions must also be reported.

All serious adverse events must be reported to the FDA within 1 business day of becoming aware of the event. Events should be reported using a MedWatch form (FDA Form 3500A), which is available for download on the FDA website.

The SAE report should comprise a full, written summary detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports should be included. Follow-up information and follow-up reports should be forwarded to all appropriate entities within 24 hours. A copy of all documents submitted to the FDA should be sent to the Lead Site. Serious adverse events should be reported to the Institutional Review Board within 24 hours if they meet the reporting criteria set forth in the IRB Policy Manual.

9.0 PHARMACEUTICAL INFORMATION

9.1 Docetaxel (Taxotere)

9.1.1 Docetaxel Description

Docetaxel is an antineoplastic agent belonging to the taxoid family. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate. Docetaxel is a white to almost-white powder with an empirical formula of $C_{43}H_{53}NO_{14}\cdot 3H_2O$ and a molecular weight of 861.9. It is highly lipophilic and practically insoluble in water.

9.1.2 Clinical Pharmacology

Docetaxel is an antineoplastic agent that acts by disrupting the microtubular network in cells that is essential for mitotic and interphase cellular functions. Docetaxel binds to free tubulin and promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. This leads to the production of microtubule bundles without normal function and to the stabilization of microtubules, which results in the inhibition of mitosis in cells. Docetaxel's binding to microtubules does not alter the number of protofilaments in the bound microtubules, a feature which differs from most spindle poisons currently in clinical use.

9.1.3 Pharmacokinetics and Drug Metabolism

The pharmacokinetics of docetaxel have been evaluated in cancer patients after administration of 20 mg/m2 to 115 mg/m2 in phase 1 studies. The area under the curve (AUC) was dose proportional following doses of 70 mg/m2 to 115 mg/m2 with infusion times of 1 to 2 hours. Docetaxel's pharmacokinetic profile is consistent with a three-compartment pharmacokinetic model, with half-lives for the α, β, and γ phases of 4 min, 36 min, and 11.1 hr, respectively. Mean total body clearance was 21 L/h/m2. *In vitro* drug interaction studies revealed that docetaxel is metabolized by the CYP3A4 isoenzyme, and its metabolism may be modified by the concomitant administration of compounds that induce, inhibit, or are metabolized by cytochrome P450 3 A4.

9.1.4 Supplier(s)

Docetaxel is commercially available.

9.1.5 Administration

Docetaxel will be administered intravenously at a dose of 75mg/m^2 on Day 1 of each 21-day cycle. Premedication will be administered according to institutional guidelines but must include oral or intravenous steroids.

9.1.6 Expected Adverse Events

The most common adverse reactions across all docetaxel indications are infections, neutropenia, anemia, febrile neutropenia, hypersensitivity, thrombocytopenia, neuropathy, dysgeusia, dyspnea, constipation, anorexia, nail disorders, fluid retention, asthenia, pain, nausea, diarrhea, vomiting, mucositis, alopecia, skin reactions, and myalgia.

9.2 Carboplatin

9.2.1 Carboplatin Description

Carboplatin is supplied as a sterile, pyrogen-free, 10 mg/mL aqueous solution of carboplatin. Carboplatin is a platinum coordination compound. The chemical name for carboplatin is platinum, diamine [1,1-cyclobutane-dicarboxylato(2-)-0,0']-,(SP-4-2). It has a molecular formula of $C_6H_{12}N_2O_4Pt$ and a molecular weight of 371.25. It is soluble in water at a rate of approximately 14 mg/mL, and the pH of a 1% solution is 5 to 7. It is virtually insoluble in ethanol, acetone, and dimethylacetamide.

9.2.2 Supplier(s)

Carboplatin is commercially available.

9.2.1 Administration

Carboplatin dosing will be AUC6 on Day 1 of each 21-day cycle for 6 cycles. Premedication with antiemetics should be in accordance with institutional standards.

9.2.2 Expected Adverse Events

Myelosuppression, nausea and vomiting (moderately emetogenic), peripheral neuropathy (occurring in < 10% of patients, mild in severity), hepatotoxicity (mild, reversible elevations in liver function tests), and allergic reactions.

10.0 CORRELATIVE STUDIES

10.1 Tumor Biopsy

Research biopsies will be performed at baseline, Cycle 1 Day 7, and definitive surgery. The Cycle 1 Day 7 biopsy will be optional. For patients with multicentric disease, all core needle biopsy samples should be taken from the index lesion, for all biopsy timepoints. Collection should be performed according to the following guidelines.

Based on previous experience, we estimate that not all single core needle biopsies will contain tumor tissue. In order to maximize the likelihood of sufficient tissue, approximately 4-6 cores should be taken at each biopsy time-point. Two cores will be frozen on dry ice in OCT blocks at bedside, two will be fixed in formalin, and two (at the baseline and end of treatment biopsies) will be collected in media and placed on wet ice. Biopsies will be performed under local anesthesia, using the same entry point, but reorienting the needle.

Breast tissue will be obtained by core needle biopsy, which will be performed on an outpatient basis. The procedure will be performed by either an experienced breast medical oncologist or a member of the breast imaging staff. The procedure will be described to the participant prior to each procedure, and the participant will be given the opportunity to ask questions. The procedure will be performed as follows. The skin will be cleaned with an antiseptic solution (Betadine). A local anesthetic (1% or 2% lidocaine) will be injected to the area that will be biopsied. A small incision will be made into the skin using a scalpel. Next, the biopsy needle will be inserted through the incision and tissue cores will be obtained.

Moderate pressure will be applied to the biopsy site for about 10 minutes. The incision site is Steri-Stripped and a 2x2 gauze pressure bandage is applied. No suture is required to close the small wound. The procedure is routinely done in the physician's office, and the patient is released home after the procedure. Potential complications include bleeding, pain, or infection. These risks are discussed with the patient prior to the beginning of each procedure.

Following the procedure, the collected tissue specimens will be delivered to the Lester & Sue Smith Breast Center Tissue Bank, where they will be processed and stored. In the event that baseline or surgical tissue samples are found to contain an insufficient amount of tumor for analysis, archival tissue may be requested.

10.2 Research Blood

Blood (50 mL total) will be drawn for research purposes at the following timepoints:

- Baseline
- Cycle 1 Day 7 (+/- 3 days)

- at end of treatment, as per the study calendar
- 2 months following definitive surgery, +/- 1 week,
- annually for five years or until disease recurrence, whichever comes first

Blood will be collected as follows at each of the above time points:

- 10 mL tiger top tube
- a 10 mL EDTA tube for plasma and DNA collection
- a 10 mL EDTA tube containing protease inhibitors (BD – P100 tube)
- a 10 mL lithium heparin (green top) tube for heparinized plasma
- a 10 mL Streck Cell-Free DNA BCT for plasma circulating DNA

Collected blood specimens will be processed as detailed in the laboratory manual. Specimens will be delivered to the Lester & Sue Smith Breast Center Tissue Bank, where they will be stored until further use.

10.3 Specimen Storage and Banking

Blood and tissue specimens obtained for this study will be delivered to the Lester & Sue Smith Breast Center Tissue Bank. The physical address for the Tissue Bank is:

Breast Center Tissue Bank
Alkek Building, N1110
One Baylor Plaza
Houston, TX 77030

Once received in the Tissue Bank, specimens will be processed and stored until utilized as per protocol. Samples will not contain any traditional patient identifiers; however, samples will be coded such that specimens are traceable back to the patient. The link between the specimen code and the patient will be maintained in a secure area, accessible only to the Tissue Bank Coordinator.

At the conclusion of the protocol-specified analyses, any leftover study blood and tissue samples may be stored indefinitely in the Tissue Bank for future research studies, provided that the participant has consented to future use. The subjects will be given the opportunity to consent to future use of samples in the consent form for the study. Samples will only be released for use in future studies after approval by the Principal Investigator and other regulatory bodies, as appropriate. Any specimens that remain after this protocol is closed with the IRB may be transferred to BCM protocol # H-12573, provided that the participant has consented to storage and future use of donated tissue.

10.4 Planned analyses

Collected tissue and blood specimens will be analyzed as described below. Additional analyses other than those listed below may be performed in the event of new information.

10.4.1 Patient-Derived Xenografts

Xenografts will be established from the biopsy specimens collected at baseline and end of treatment. Tissue collected from the Cycle 1 Day 7 biopsy will be used for genomic and proteomic analyses to study molecular changes and identify markers of response and resistance. Tissue collected from the Cycle 1 Day 7 biopsy will be used for genomic and proteomic analyses to study molecular changes and identify markers of response and resistance. Once obtained, specimens will be delivered to the Tumor Bank as quickly as is feasible following collection, but no later than 1 day after collection, as described in the laboratory manual. For fragment transplantation, samples will be minced into approximately 1 mm^3 fragments and transplanted directly into epithelium-free "cleared" fat pads of recipient SCID/Bg (Charles River Laboratories), or NSG mice (Jackson Laboratories) per patient. Mice will be palpated weekly and tumor growth measured using calipers.

Once established, xenografts will be characterized according to the methods described in Zhang, et al[43].

10.4.2 Genomic and proteomic analyses

Genomic and proteomic analyses will be performed on the established xenografts using the techniques available at the time analysis is conducted. Results obtained from these analyses will be compared to similar data from patient tumors. Genomic profiling (including gene expression profiling and aCGH) of tumor tissue will be performed to explore the relationship between genoproteomic factors and endpoints including response, etc as well as to explore the longitudinal changing patterns in profiling.

10.4.3 Whole genome sequencing

Both tumor and matched normal blood will be collected from each patient for the comparison of germline and tumor genotypes to verify that any detected mutations are of somatic origin. Strict requirements of quality, quantity, purity, and avoidance of necrotic tissue is essential, and only samples with greater than 50% tumor cellularity will be used to generate 5 mcg of genomic DNA from immediately flash frozen biopsies. Whole genome amplification (WGA) will be performed on smaller biopsies if required, by which the original DNA sample is amplified in a specific way from nanogram concentrations to microgram, while conserving the sequence representation of the template. At BCM-HGSC, the discovery phase uses support oligonucleotide ligation detection (SOLiD) sequencing instruments (Applied Biosystems, Inc.). A high-throughput SOLiD pipeline has been established at HGSC since 2008 for the purpose of supporting whole genome sequencing activities, and is now being implemented for identification of mutations in autosomal dominant ataxias, ovarian, breast, and pancreatic cancers and glioblastoma.

Data generated from whole genome sequencing will be uploaded into a controlled-access database, maintained by Baylor College of Medicine. The BCM database will only be accessible by BCM personnel working on this project. All data downloaded by BCM personnel will reside behind a secure, firewall protected server. Sequencing data may eventually be uploaded into an online repository, maintained by the National Institute of Health (NIH). In this situation, the information in this database will be available only to researchers and institutions who have received approval from an NIH Data Access Committee after certifying their adherence to patient data protection policies for the project. Please note that traditionally-used identifying information about the patient, such as name, address, telephone number, or social security number, will NOT be put into any database used for this project.

10.4.4 Cell-free circulating tumor DNA

Cell-free circulating tumor DNA has been proposed as a surrogate biological material to define the genetic aberrations of a primary tumor and/or metastases in a given cancer patient, and to serve as a biomarker for diagnosis, prognostication, and monitoring of response to therapy. Recent studies have demonstrated that mutations in *PIK3CA* and/or *TP53* can be identified in the ctDNA from plasma samples of the vast majority of patients with advanced breast cancer and in approximately 50% of patients with early stage breast cancer.

Our hypotheses are that plasma ctDNA analysis may provide a means to predict relapse after primary breast cancer systemic therapy and to characterize the genetic composition of the resistant clones. Cell-free circulating tumor DNA analysis reflects disease burden and offers a substantial lead-time over radiologic detection for the detection of metastatic disease of up to 13.5 months. Furthermore, ctDNA analysis has been shown to constitute a source of DNA that allows for the investigation of the repertoire of somatic mutations found in micrometastatic and metastatic disease. ctDNA may also prove useful as an indicator of pCR before surgery. This could improve surgical decision making and allow for de-escalation of local therapy.

To address our hypotheses, mandatory plasma samples from enrolled patients will be collected at baseline prior to start of chemotherapy, when chemotherapy has been competed and then 2 months after surgery has been completed. Samples will also be taken yearly for a total of 5 years or until relapse. DNA will be extracted from the plasma samples following validated protocols. In brief, 10 mL blood samples collected in Streck Cell-Free DNA BCT® tubes and will be sent to the Breast Center Tissue Bank. Cell-free circulating tumor DNA (ctDNA) will be extracted and analyzed following established protocols.

In order to identify tumor DNA variants that will be used for personalized ultra-sensitive ctDNA assays for each patient in the study, DNA will be extracted from the primary tumor and subjected to targeted capture followed by massively parallel sequencing. We will focus the targeted capture approach on genes most frequently mutated in breast cancer. Based on a re-analysis of The Cancer Genome Atlas (TCGA) breast

cancer dataset, 100 most frequently mutated genes in breast cancer have been identified. A customized bait library based on these data could generate an informative ctDNA assay in > 96% of all breast cancers. The repertoire of somatic mutations obtained through targeted capture massively parallel sequencing of each primary cancer will be the basis for the development of a personalized ctDNA assay. This may be based on the digital droplet PCR approach; however, technology in this field is advancing rapidly. Since this field is rapidly evolving, the exact platform for analysis will be determined based on available technology. Since translocations and deletions provide the most specific analytes for ctDNA assays current efforts to search for recurrent translocations in breast cancer using combined DNA and RNA sequencing are very relevant to the development of the final assay that will be deployed. Changes in ctDNA will be correlated to outcomes.

11.0 STUDY CALENDAR

Baseline evaluations must be performed within 28 days prior to the start of treatment, unless otherwise noted:

	Screening / Baseline	C1D1[13]	C1D7 (+/- 3 days)	Cycles 2-6, Day 1[13]	Surgery[3]	EOT[12]	F/U[5]
Informed consent	X						
Medical history	X						
Physical exam, tumor measurement (2D)	X[2]	X		X		X	
Vital signs, weight, height	X	X		X		X	
Mammogram[10]	X					X	
Breast Ultrasound[11]	X					X	
Chest Imaging (optional) [7]	X						
Bone imaging[8]	X						
Hematology[1]	X	X		X		X	
Comprehensive metabolic panel	X	X		X		X	
Serum Pregnancy test[9]	X	X					
Docetaxel		X		X			
Carboplatin		X		X			
Research blood	X		X			X	X
Research tissue	X		X[4]		X		
Adverse events[6]	X	X		X		X	

1. WBC plus differential, hemoglobin, and platelets
2. Breast tumor measurements must be performed within 14 days prior to Cycle 1 Day 1.
3. To be performed between 3 and 5 weeks after completing neoadjuvant therapy
4. Optional.
5. Follow annually for survival for 5 years. Research blood draw should be collected 2 months (+/- 1 week) after the patient's definitive surgery, and then annually for five years or until disease recurrence, whichever comes first.
6. Collect AEs for 30 days following last day of chemotherapy.
7. Staging CT chest or CXR should be performed if clinically indicated, as determined by the treating physician. Optional unless clinically indicated. PET or PET/CT is permitted as an alternative to CXR or CT chest.
8. Bone imaging via PET/CT or nuclear medicine bone scan is required if one of the following criteria are met: a) Patient has unexplained bone pain; b) ALP > ULN.
9. Serum HCG only required for WOCBP. Required on C1D1 if it has been greater than 7 days since previous HCG
10. Bilateral mammogram required at baseline; unilateral mammogram may be performed at EOT. Bilateral breast MRI may be substituted for mammogram at the discretion of the treating physician. May be performed within 6 weeks prior to start of treatment.

11. Ultrasound required only of affected breast. May be performed within 6 weeks prior to treatment start.
12. EOT procedures must be performed within 2 weeks of completion of last cycle. In the event of early discontinuation, EOT procedures should be performed prior to the initiation of alternate treatment, if applicable. Breast imaging procedures are not required in the event of early discontinuation, but may be performed at the discretion of the treating physician.
13. May be perfomed 3 days prior to chemotherapy treatment.

12.0 MEASUREMENT OF EFFECT

12.1 Clinical Responses

Physical examination: The longest axis and the perpendicular axis of the measurable lesion should be measured and recorded in metric notation by tape, ruler or caliper technique in the source documents and on the case report forms. Breast tumor measurements should be taken at baseline (within 14 days prior to C1D1), on Day 1 of each subsequent cycle, and again following the completion of Cycle 6 (prior to surgery).

Radiographic evaluation of tumor size: Mammogram and ultrasound imaging will be performed within 42 days of Cycle 1 Day 1, and again at the end of cycle 6 combination therapy.

RECIST 1.1 criteria will be used to assess clinical response

Complete Response (CR) is defined as the disappearance of all known disease based on a comparison between the pre-treatment measurements and the measurements taken at the completion of neo-adjuvant therapy. In addition there is no appearance of new lesions.

Partial Response (PR) is defined as a 30% or greater decrease in the longest diameter of the breast tumor between the pre-treatment measurements and the measurements taken at the completion of neo-adjuvant therapy. In addition there can be no appearance of new lesions or progression of any lesion.

Stable Disease (SD) Changes between a 30% decrease and a 20% increase in the longest diameter of the primary tumor, without appearance of new lesions..

Progressive Disease (PD): A 20% or greater increase in the total tumor size of the lesion from its pretreatment measurements or the appearance of new lesions at any point in study treatment.

12.2 Surgery

A pathologic complete response (pCR) is defined as no histology evidence of invasive tumor cells in the surgical breast specimen and sentinel or axillary lymph nodes.

All eligible women who have been treatment with combination therapy are included in the analysis of pCR. A patient is considered to not to have a pCR if any of the following are true:

1. There is histologic evidence of invasive tumor cells in the surgical breast specimen or the axillary lymph nodes.

2. The patient has discontinued neo-adjuvant treatment early due to refusal, toxicity, or radiographic or clinical evidence of progression and then goes straight to surgery where there is histologic evidence of invasive tumor cells in the surgical breast specimen and the axillary lymph nodes.

3. The patient has discontinued neo-adjuvant treatment early due to refusal, toxicity or radiographic or clinical evidence of progression and then receives alternative treatment.

4. The patient refuses surgery or is unable to undergo surgery due to a co-morbid condition.

12.3 Diagnosis of Breast Cancer Recurrence and Other Cancer Events

12.3.1 Local Recurrence

Local recurrence is defined as histologic evidence of ductal carcinoma in situ or invasive breast cancer in the ipsilateral breast or chest wall.

12.3.2 Regional Recurrence

Regional recurrence is defined as the cytologic or histologic evidence of disease in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral infraclavicular and/or ipsilateral axillary nodes or soft tissue of the ipsilateral axilla.

12.3.3 Distant Recurrence

Distant recurrence is defined as the cytologic, histologic, and/or radiographic evidence of disease in the skin, subcutaneous tissue, lymph nodes (other than local or regional metastasis), lung, bone narrow, central nervous system or histologic and/or radiographic evidence of skeletal or liver metastasis.

12.3.4 Second Primary Breast Cancer

Second primary breast cancer is defined histologic evidence of ductal carcinoma in situ or invasive breast cancer in the contralateral breast or chest wall.

12.3.5 Second Primary Cancer (Non-breast)

Any non-breast second primary cancer other than squamous or basal cell carcinoma of the skin, melanoma in situ, or carcinoma in situ of the cervix is to be reported and should be confirmed histologically whenever possible.

12.3.6 Death

Underlying cause of death is to be reported.

13.0 DATA AND SAFETY MONITORING

13.1 Data Management and Reporting

In compliance with the Dan L Duncan Cancer Center Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Dan L Duncan Cancer Center Data Review and Safety Committee (DRC) annually starting from the date the trial is activated. Adverse events reported on this study will also be reviewed on a biweekly basis at the Lester & Sue Smith Breast Center Clinical Research and Data Review meetings.

All patients who received any amount of study drug will be included in the safety analysis. Subjects will be monitored at each clinic visit and at any contact with the subject throughout the study for the occurrence of AEs and SAEs. The investigator or staff will inquire about the occurrence of AEs/SAEs at every clinic visit or contact during the study. Adverse events will be graded according to the NCI Common Terminology Criteria v. 4.0. All AEs that occur during active treatment will be recorded in subject source documents and on CRF, according to the guidelines specified in Section 8 of the protocol. Non-serious adverse events that occur within the 30 day follow-up period will only be recorded on the CRFs if the investigator believes there to be a possible relationship to study medication. Serious adverse events should be recorded, regardless of relationship to drug.

Safety analyses will include summaries of adverse event rates (both frequency and incidence tables), baseline laboratory parameters and changes from baseline, frequency of CTC toxicity grades for both laboratory and non-laboratory data. The investigators and others responsible for patient care should institute any supplementary investigations of major adverse events based on their clinical judgment of the likely causative factors.

13.2 Meetings

We will utilize the Data and Safety Monitoring Plan of the Dan L. Duncan Cancer Center at Baylor College of Medicine. During the clinical trial, clinical data on all participants enrolled on the study will be reviewed in our monthly breast center Data and Safety Monitoring Meeting.

We will have the trial reviewed at our Data Review Committee which will review accruals, serious adverse events, and cumulative toxicity data. The DRC will make recommendations about safety and tolerability.

13.3 Monitoring

As the lead site, Baylor College of Medicine will monitor all participating sites. Monitoring visits will occur at the following timepoints and can be conducted via phone or physically at the participating sites, at the discretion of the lead site:

1. After accrual of the first patient to each participating site.
2. After the last patient at each site completes study treatment.
3. At study close out.
4. At other intervals per discretion of the lead site.

14.0 REGULATORY CONSIDERATIONS

14.1 Protocol Review and Amendments

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards of each participating center.

Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB of each institution prior to implementation.

The Protocol Chair (or his designee) is responsible for the coordination and development of all protocol amendments, and will disseminate this information to the participating centers.

14.2 Informed Consent

The investigator (or his/her designee) will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary, that s/he may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment or relationship with the treating physician(s) or institution. The informed consent will be given by means of a standard written statement, written in non-technical language, which will be IRB approved. The subject should read and consider the statement before signing and dating it, and will be given a copy of the document. No subject will enter the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

14.3 Ethics and GCP

This study will be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

15.0 MULTI-CENTER GUIDELINES

15.1 Study Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Coordinating Center. The required documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures and recruitment material, etc.), IRB membership rosters, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Coordinating Center will provide each participating site with a comprehensive list of the necessary documents. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Coordinating Center.

The requirements for data management, submissions, and monitoring are outlined below.

All data collected will be entered by the participating site into the Coordinating Center Electronic Database (eCRF), via ONCORE.

15.2 Records Retention

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. Records must be maintained for at least 5 years following the conclusion of the study, or in accordance with institutional policy, whichever is longer. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

15.3 Publication

It is understood that any manuscript or releases resulting from the collaborative research will be circulated to all participating sites prior to submission for publication or presentation.

16.0 STATISTICAL CONSIDERATIONS

16.1 Study Design

16.1.1 Purpose

This is a single arm open label phase II study in women with clinical stage 2 or 3 triple negative breast cancer to assess the anti-tumor activity (in terms of pathologic complete response rate) of neoadjuvant docetaxel in combination with carboplatin.

16.1.2 Primary Endpoint

The primary endpoint of this trial is pathological complete response (pCR), as defined in section 10.2. All eligible women who begin combination treatment will be included in the analyses of the primary study endpoint.

16.1.3 Sample Size and Trial Duration

In this trial, we will use an admissible Simon-like two-stage decision rule[44] in order to allow early stopping in the event of futility. We will enroll up to 44 pCR-evaluable participants. If there are fewer than 8 pCR's in the first 22 pCR-evaluable cases, the trial will stop. Otherwise, at the end of the trial, if there are 20 or more pCR's out of 44 participants, the new therapy will be deemed to be effective, while fewer than 20 will lead to a negative conclusion. The sample size is based on comparing the observed pCR rate to a rate of 35% that would be expected with standard chemotherapy, and the decision rules stated above will provide 90% power (alpha=10%, one-tailed) to detect a difference if the true pCR rate is 55% or better.

We anticipate that 3 patients per month will be screened for trial eligibility. Approximately 50% of the patients will be eligible and enroll. Thus, we anticipate screening about 90 patients to obtain 44 eligible and pCR-evaluable patients as calculated above. We expect accrual will be steady and will not be paused to wait for the last patient in the first stage to be evaluated for pCR.

The period encompassing enrollment, study treatment, and surgery will be 36 months [30 months to enroll 44 eligible patients and 5-6 months to await surgical results of last patient enrolled].

16.1.4 Statistical Analysis Plan

16.1.4.1 Primary outcome

pCR rate: The outcome of the trial will be evaluated using the decision rules decribed above. In the event that the realized pCR-evaluable sample size is different from planned, methods such as proposed by Green et al [45] will be used. The primary endpoint of pCR rate and the associated 95% confidence interval will be estimated from the number of pCR evaluable participants who are pCR's or not, using the method of Jung and Kim [46] to account for the two-stage nature of the design.

16.1.4.2 Secondary outcomes

Clinical response rate: The clinical response rate and associated 95% confidence interval will be estimated by the number of patients whose disease meets the RECIST 1.1 criteria of complete or partial response prior to surgery divided by the total number of eligible patients who began combination neoadjuvant treatment.

Radiological response rate: The radiological response rate and associated 95% confidence interval will be estimated by the number of patients whose disease meets with RECIST 1.1 criteria for complete or partial response at the evaluation prior to surgery divided by the total number of eligible patients who began combination neoadjuvant therapy.

Adverse Events: All eligible patients that received any chemotherapy will be considered evaluable for assessing adverse event rate(s). As described in Section 6.3.1 targeted reporting will be used. The maximum grade for each type of reportable adverse event will be recorded for each patient using the NCI-CTCAE v4.0 coding scheme. AE's by grade and attribution will be summarized descriptively as frequency tables.

16.1.4.3 Exploratory Studies

A number of exploratory or correlative studies are proposed for this study. Collection and establishment of patient derived xenograft lines is an important biologic outcome that will allow detailed examination of molecular markers of response and 'co-clinical trial' testing of other therapies in the future. Tumor engraftment rates will be estimated for tumor samples taken before and during chemotherapy. A paired analysis will compare rates. Successful xenografts will be fully characterized molecularly in order to allow exploratory analyses of potential predictive biomarkers.

17.0 REFERENCES

1. Swain, S. *Triple-Negative Breast Cancer: Metastatic Risk and Role of Platinum Agents*. in *ASCO Clinical Science Symposium*. 2008.

2. Huo, D., et al., *Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer.* J Clin Oncol, 2009. **27**(27): p. 4515-21.

3. Gonzalez-Angulo, A.M., et al., *Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer.* Clin Cancer Res, 2011. **17**(5): p. 1082-9.

4. Cleator, S., W. Heller, and R.C. Coombes, *Triple-negative breast cancer: therapeutic options.* Lancet Oncol, 2007. **8**(3): p. 235-44.

5. Liedtke, C., et al., *Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer.* J Clin Oncol, 2008. **26**(8): p. 1275-81.

6. Dent, R., et al., *Triple-negative breast cancer: clinical features and patterns of recurrence.* Clin Cancer Res, 2007. **13**(15 Pt 1): p. 4429-34.

7. Koplin, L.M. and T.X. O'Connell, *A new approach to the management of primary unresectable carcinoma of the breast: is radiation therapy necessary?* Am J Clin Oncol, 1983. **6**(5): p. 599-604.

8. Fisher, B., et al., *Effect of preoperative chemotherapy on the outcome of women with operable breast cancer.* J Clin Oncol, 1998. **16**(8): p. 2672-85.

9. Liu, S.V., et al., *Neoadjuvant therapy for breast cancer.* J Surg Oncol, 2010. **101**(4): p. 283-91.

10. Kennedy, C.R., F. Gao, and J.A. Margenthaler, *Neoadjuvant versus adjuvant chemotherapy for triple negative breast cancer.* J Surg Res, 2010. **163**(1): p. 52-7.

11. Bear, H.D., et al., *Bevacizumab added to neoadjuvant chemotherapy for breast cancer.* N Engl J Med, 2012. **366**(4): p. 310-20.

12. Huober, J., et al., *Effect of neoadjuvant anthracycline-taxane-based chemotherapy in different biological breast cancer phenotypes: overall results from the GeparTrio study.* Breast Cancer Res Treat, 2010. **124**(1): p. 133-40.

13. Von Minckwitz, G., et al., *A randomized phase II trial investigating the addition of carboplatin to neoadjuvant therapy for triple-negative and HER2-positive early breast cancer (GeparSixto).* Program and abstracts of the American Society of Clinical Oncology Annual Meeting May 31-June 4, 2013Chicago, Illinois. , 2013: p. Abstract 1004.

14. *Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. Early Breast Cancer Trialists' Collaborative Group.* N Engl J Med, 1988. **319**(26): p. 1681-92.

15. Early Breast Cancer Trialists' Collaborative, G., *Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials.* Lancet, 2005. **365**(9472): p. 1687-717.

16. Henderson, I.C., et al., *Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer.* J Clin Oncol, 2003. **21**(6): p. 976-83.

17. Mamounas, E.P., et al., *Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: results from NSABP B-28.* J Clin Oncol, 2005. **23**(16): p. 3686-96.

18. Bear, H.D., et al., *Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer:National Surgical Adjuvant Breast and Bowel Project Protocol B-27.* J Clin Oncol, 2006. **24**(13): p. 2019-27.

19. Sparano, J.A., et al., *Weekly paclitaxel in the adjuvant treatment of breast cancer.* N Engl J Med, 2008. **358**(16): p. 1663-71.

20. Vogel, C.L., et al., *First and subsequent cycle use of pegfilgrastim prevents febrile neutropenia in patients with breast cancer: a multicenter, double-blind, placebo-controlled phase III study.* J Clin Oncol, 2005. **23**(6): p. 1178-84.

21. Budd, G.T., et al. *First analysis of SWOG S0221: A phase III trial comparing chemotherapy schedules in high-risk early breast cancer.* in *ASCO*. 2013. Chicago, IL.

22. Alba, E., et al., *A randomized phase II trial of platinum salts in basal-like breast cancer patients in the neoadjuvant setting. Results from the GEICAM/2006-03, multicenter study.* Breast Cancer Res Treat, 2012. **136**(2): p. 487-93.

23. Silver, D.P., et al., *Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2010. **28**(7): p. 1145-53.

24. Sirohi, B., et al., *Platinum-based chemotherapy in triple-negative breast cancer.* Ann Oncol, 2008. **19**(11): p. 1847-52.

25. Carey, L.A., et al., *The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes.* Clinical cancer research : an official journal of the American Association for Cancer Research, 2007. **13**(8): p. 2329-34.

26. Sikov, W.M., et al., *Frequent pathologic complete responses in aggressive stages II to III breast cancers with every-4-week carboplatin and weekly paclitaxel with or without trastuzumab: a Brown University Oncology Group Study.* J Clin Oncol, 2009. **27**(28): p. 4693-700.

27. Chen, X.S., et al., *Weekly paclitaxel plus carboplatin is an effective nonanthracycline-containing regimen as neoadjuvant chemotherapy for breast cancer.* Ann Oncol, 2010. **21**(5): p. 961-7.

28. Kern, P., et al. *Pathologic response rate (pCR) and near-pathologic response rate (near-pCR) with docetaxel-carboplatin (TCarb) in early triple-negative breast cancer.* . in *Breast Cancer Symposium*. 2011.

29. Roy, V., et al., *A Phase II Trial of Docetaxel and Carboplatin Administered Every 2 Weeks as Preoperative Therapy for Stage II or III Breast Cancer: NCCTG Study N0338.* Am J Clin Oncol, 2012.

30. Chang, H.R., et al., *Differential response of triple-negative breast cancer to a docetaxel and carboplatin-based neoadjuvant treatment.* Cancer, 2010. **116**(18): p. 4227-37.

31. Baselga, J., et al., *Phase II genomics study of ixabepilone as neoadjuvant treatment for breast cancer.* J Clin Oncol, 2009. **27**(4): p. 526-34.

32. Tiley, S., et al. *Results of the East Carolina Breast Center phase II trial of neoadjuvant metronomic chemotherapy in triple-negative breast cancer (NCT00542191).* in *ASCO Annual Meeting*. 2012. Chicago, IL: ASCO.

33. Ryan, P.D., et al. *Neoadjuvant cisplatin and bevacizumab in triple negative breast cancer (TNBC): Safety and efficacy.* in *ASCO Annual Meeting*. 2009. Chicago, IL: ASCO.

34. Hait, W.N., *Anticancer drug development: the grand challenges.* Nat Rev Drug Discov, 2010. **9**(4): p. 253-4.

35. Clarke, R., *The role of preclinical animal models in breast cancer drug development.* Breast Cancer Res, 2009. **11 Suppl 3**: p. S22.

36. Voskoglou-Nomikos, T., J.L. Pater, and L. Seymour, *Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models.* Clin Cancer Res, 2003. **9**(11): p. 4227-39.

37. Landis, M.D., et al., *Patient-derived breast tumor xenografts facilitating personalized cancer therapy.* Breast Cancer Res, 2013. **15**(1): p. 201.

38. Marangoni, E., et al., *A new model of patient tumor-derived breast cancer xenografts for preclinical assays.* Clin Cancer Res, 2007. **13**(13): p. 3989-98.

39. Bergamaschi, A., et al., *Molecular profiling and characterization of luminal-like and basal-like in vivo breast cancer xenograft models.* Mol Oncol, 2009. **3**(5-6): p. 469-82.

40. Zhang, X., et al., *P5-21-01: A Renewable Tissue Resource of Phenotypically Stable Human Breast Cancer Xenografts for Preclinical Studies.* Cancer Res, 2011. **71**(24 Suppl): p. Abstract nr PS-21-01.

41. Petrillo, L.A., et al., *Xenografts faithfully recapitulate breast cancer-specific gene expression patterns of parent primary breast tumors.* Breast Cancer Res Treat, 2012. **135**(3): p. 913-22.

42. Li, S., et al., *Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts.* Cell Rep, 2013. **4**(6): p. 1116-30.

43. Zhang, X., et al., *A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models.* Cancer research, 2013. **73**(15): p. 4885-97.

44. Jung, S.H., et al., *Admissible two-stage designs for phase II cancer clinical trials.* Stat Med, 2004. **23**(4): p. 561-9.

45. Green, S.J. and S. Dahlberg, *Planned versus attained design in phase II clinical trials.* Stat Med, 1992. **11**(7): p. 853-62.

46. Jung, S.H. and K.M. Kim, *On the estimation of the binomial probability in multistage clinical trials.* Stat Med, 2004. **23**(6): p. 881-96.