Neoadjuvant weekly paclitaxel and biomarkers of therapy response
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UW16106: Neoadjuvant weekly paclitaxel and biomarkers of therapy response

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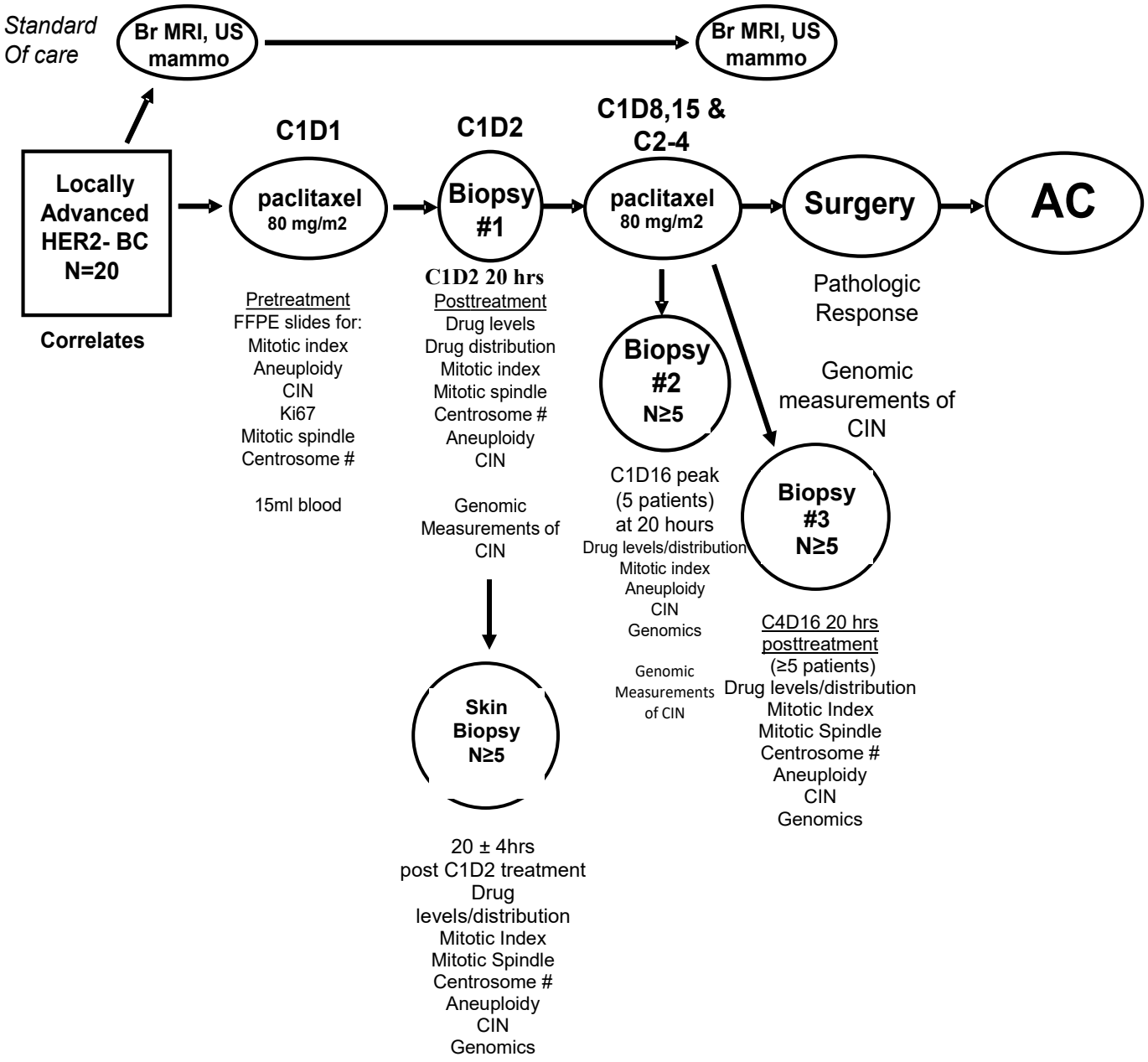
SCHEMA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Aneuploidy
CIN
Ki67
Mitotic spindle
Centrosome #
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C1D2
Biopsy #1
C1D2 20 hrs
Posttreatment
Drug levels
Drug distribution
Mitotic index
Mitotic spindle
Centrosome #
Aneuploidy
CIN
Genomic Measurements of CIN
Skin Biopsy N≥5
20 ± 4hrs post C1D2 treatment
Drug levels/distribution
Mitotic Index
Mitotic Spindle
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Aneuploidy
CIN
Genomics
C1D8,15 & C2-4
paclitaxel 80 mg/m2
Biopsy #2 N≥5
C1D16 peak (5 patients) at 20 hours
Drug levels/distribution
Mitotic index
Aneuploidy
CIN
Genomics
Genomic Measurements of CIN
Surgery
Pathologic Response
Genomic measurements of CIN
Biopsy #3 N≥5
C4D16 20 hrs posttreatment
(≥5 patients)
Drug levels/distribution
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Genomics
AC

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1. OBJECTIVES

1.1. Primary Objectives

1.1.1. To test if cancers with high chromosomal instability (CIN) respond to paclitaxel better than low CIN cancers.

1.2. Secondary Objectives

1.2.1. To identify patient-specific differences in tumor levels and distribution of paclitaxel at 20 hours after first dose and patient-specific differences in peripheral non-tumor tissue (skin or plasma) paclitaxel levels 20 ± 4 hours after first dose.

1.2.2. To determine if paclitaxel levels are higher at 20h after the 3rd dose than after the first dose, and if levels are higher at 20h after the 12th dose than the 1st and 3rd dose.

1.2.3. Compare pre-existing versus post-treatment antimitotic effects at 20h after the 1st dose, 20h after the 3rd dose, and 20h after the 12th dose.

1.2.4. Correlate drug levels and distribution with biomarkers including mitotic index, aneuploidy, chromosomal instability, and Ki67.

1.2.5. Correlate pathologic response and clinical response with biomarkers including mitotic index, aneuploidy, CIN and Ki67.

1.2.6. To test if CIN increases in patient tumors in response to paclitaxel and to evaluate the feasibility of these measurements by genomic analysis.

2. BACKGROUND

2.1 Study Disease

Breast cancer is the most common cancer in women. According to the 2005 Surveillance Epidemiology and End Result (SEER) database, patients with locoregional spread have a 5-year survival rate of 83.6%[1]. In this patient population, chemotherapy remains a cornerstone of treatment for breast cancer, and taxanes remain one of the most effective agents. Several studies have demonstrated response rate to taxanes in the first line metastatic setting is 35-50%[2]. Based on these results, taxanes have been incorporated into adjuvant breast cancer regimens. For node positive cancer, addition of 4 cycles of paclitaxel 175mg/m2 q3 weeks after standard ACx4 (adriamycin and cyclophosphamide) improves survival[3]. When delivered every two weeks with GCSF support, there is a better outcome and lower risk of neutropenic fever[4]. Recently, investigators have suggested a potential benefit to sequential therapy with taxanes prior to AC. Either sequence is tolerable but the dose intensity of chemotherapy is higher when taxanes are delivered first[5]. Two studies of neoadjuvant taxane demonstrated an

increased rate of pathologic complete response (pCR) when taxane was given prior to an anthracycline-based chemotherapy rather than after[6].

Despite this success, a large number of patients do not respond to taxane therapy. For example, the pCR rate for neoadjuvant paclitaxel does not exceed 35% even when combined with radiotherapy[7]. Additionally, neuropathy and myelosuppression remain significant toxicities. If there were a manner to predict the response to taxane therapy, patients with low likelihood of response could undergo alternative treatment and potentially avoid these toxicities. Therefore, improved biomarkers of taxane response are needed for patient selection and personalization of optimal cancer therapy.

2.2 Rationale for Correlative Studies

2.2.1 **Taxanes effect on mitosis.** Taxanes are microtubule stabilizers first identified as natural products from *Taxus brevifolia*. Paclitaxel (Taxol®), the first member of this class, binds to β-tubulin and blocks microtubule depolymerization. In the classic model, stabilized microtubules interfere with kinetochore-microtubule attachments, thereby activating the mitotic checkpoint, leading to mitotic delay and cell death. Recent evidence suggests that paclitaxel can induce cell death through two distinct mechanisms, depending on the intracellular concentration of drug[8,9]. In one mechanism, long term mitotic delay leads to cell death directly from mitosis or after cells exit mitosis without division to form a single tetraploid cell. Tumors with a strong mitotic checkpoint are expected to be sensitive to this mechanism, while tumors in which the mitotic checkpoint is impaired are expected to be resistant. In the second mechanism, cells missegregate chromosomes as they exit mitosis, often generating more than two daughter cells. This mechanism is predicted to cause cell death due to massive chromosome loss. Aneuploid tumors are expected to be particularly sensitive to this mechanism. However, these cell culture findings have not yet been verified in patient samples. While patients are given a standard dose of paclitaxel based on previously established safety data from phase I trials, there is little available data on the concentration of paclitaxel within breast tumors following treatment. Paclitaxel levels have been previously assessed following treatment in brain tumors[10] but this is expected to differ significantly from extra-CNS tissue levels in part because of the blood-brain barrier. Given the importance of intracellular concentration on the effect of paclitaxel, identification of the relative concentration in vivo would assist in development of in vitro breast cancer models using this medication.

2.2.2 **The Mitotic Checkpoint.** Mitosis is the critical event in the cell cycle when duplicated chromosomes are accurately segregated into daughter cells. During mitosis, accurate segregation of sister pairs to daughter cells requires their attachment to opposite mitotic poles via microtubules. This is achieved via assembly of a protein-rich structure at the centromere, known as the kinetochore. Because accurate segregation is crucial to maintaining genomic integrity, cells have evolved a 'mitotic checkpoint' which ensures that each chromosome sister pair is attached to the spindle, and bi-oriented (i.e. each member of a pair is attached to opposite pole). If a single chromosome pair is unattached or is not bi-oriented, kinetochores generate a 'wait signal,' thereby activating the checkpoint. This wait

signal requires a number of protein components including Mad1, Mad2, Bub1, BubR1, Bub3, and CENP-E.

2.2.3 **Biomarkers of taxane response.** Several investigators have sought to identify predictors of response to taxane therapy. In an unbiased screen, gene expression arrays were performed from tumor samples prior to neoadjuvant chemotherapy with weekly paclitaxel followed by FAC (5-fluorouracil, doxorubicin and cyclophosphamide). The gene that correlated best with pCR was Tau, which had previously been identified as a microtubule stabilizer[11]. The authors sought to validate these findings through a retrospective analysis of 1,942 arrayed tissue specimens from NSABP B-28 (AC vs. AC followed by paclitaxel) and found that Tau expression by immunohistochemistry does not predict benefit from adding paclitaxel[11]. Recent work by this group has sought to integrate expression data with genes responsible for paclitaxel resistance identified through an RNAi screen, but this is not yet validated[12,13].

Other investigators have evaluated cell-based predictors of response to neoadjuvant paclitaxel and radiotherapy[7]. Surprisingly, pCR was not related to baseline Ki-67 staining, a marker of cell cycle and proliferation. However, response correlated well with a high fraction of mitotic cells per field at biopsy 24-72h after the first dose of paclitaxel. Interpretation of these data is clouded because all studies include additional chemotherapy or radiotherapy prior to response assessment, and the analysis of mitotic index occurred over a large window of time after paclitaxel administration (24-72h). In this study we would eliminate the variables of additional chemotherapy and variations based on time of analysis.

2.3 Hypothesis: Our hypothesis is that paclitaxel levels increase chromosomal instability (CIN) in tumors and that this is lethal to tumors that have pre-existing CIN.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Women with pathologically demonstrated breast cancer.

3.1.2 Patients must be candidates for neoadjuvant paclitaxel chemotherapy by their treating oncologist. No other investigational or commercial therapeutic agents may be given concurrently with the paclitaxel.

3.1.3 Patients must not have metastatic disease on staging work-up with CBC and liver function studies.

3.1.4 A formalin-fixed paraffin embedded tumor block (preferred) or unstained slides must be available from a prior biopsy of the primary tumor or lymph node. A minimum of 8 slides must be available.

3.1.5 The primary tumor or lymph node must be readily biopsied by surgery or radiology teams.

3.1.6 The primary tumor must be measurable by an imaging modality prior to treatment. This imaging modality is to be repeated after completion of 4 cycles of paclitaxel and prior to surgery. Such imaging modalities may include ultrasound, CT, mammography, or MRI. MRI will be the preferred imaging modality if available because it has the highest accuracy and positive predictive value for predicting pathologic complete response[14].All imaging will be performed per standard of care at the discretion of the treating physicians.

3.1.7 Subjects may not have had prior systemic chemotherapy regimens administered for treatment of their current breast cancer. However, studies (window studies, for example) that are deemed non-therapeutic, including those that utilize agents that are not FDA approved for the treatment of the patient's current breast cancer, are permitted.

3.1.8 Age $\geq$18 years. Breast cancer is rare in women < 18 years old. The safety of paclitaxel in pediatric population with breast cancer has not been established, therefore these patients are ineligible.

3.1.9 Patients must have adequate organ and marrow function as determined by the treating oncologist.

3.1.10 Patient must be willing to undergo additional biopsy of breast tumor or lymph node.

3.1.11 Patient must have the ability and willingness to sign a written informed consent document.

3.1.12 Women of childbearing potential (per UWCCC policy definition) must agree to use effective contraception as discussed with treating oncologist for the duration of the study.

3.2 Exclusion Criteria

3.2.1 History of allergic reactions attributed to compounds of similar chemical or biologic composition to paclitaxel including to other drugs formulated in Cremophor(R) EL (polyoxyethylated castor oil).

3.2.2 Patients with known HIV due to concern that chemotherapy may cause further immunosuppression and potential infectious complications.

3.2.3 Patients on non-aspirin anti-coagulation (Coumadin, heparins, or clopidogrel) or with documented bleeding disorders will be excluded due to risk of bleeding with biopsy.

3.2.4 Uncontrolled intercurrent illness including, but not limited to, ongoing or active severe infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, other malignancies requiring therapy or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.5 Pregnant women are excluded from this study because paclitaxel is a pregnancy category D drug and may cause deleterious effects to the fetus. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with paclitaxel, breastfeeding should be discontinued if the mother is enrolled in the trial.

3.3 Inclusion of Minorities

Women of all races and ethnic groups are eligible for this trial. Men will be excluded from this study because they may have different tumor biology than women and may metabolize paclitaxel differently than women.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be entered on study using the University of Wisconsin Carbone Cancer Center Oncore Database.

In accordance with institutional policies approved by the U.S. Department of Health and Human Services, each subject must acknowledge consent for treatment as a human subject in this study. Informed consent must be obtained prior to the initiation of protocol therapy.

Following registration, patients should begin protocol treatment within 14 days. Issues that would cause treatment delays must be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator must be notified of cancellations as soon as possible.

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and maintained in the subject study chart:

- Copy of required eligibility test results.
- Signed patient consent form.
- Signed HIPAA authorization form.

The research coordinator at the site will then register the subject into the Oncore database prior to starting study treatment. Oncore will assign the unique subject number.

There will not be access to the Oncore database registration until documented IRB approval is obtained and entered into the Oncore database.

5. TREATMENT PLAN

5.1 Administration

Treatment will be administered on an **outpatient** basis. Reported adverse events and potential risks are described in Section 8. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Paclitaxel (Taxol®) 80 mg/m^2 will be initiated as standard infusion on days 1, 8, 15 of a 21-day cycle. Patients will continue with paclitaxel 80 mg/m^2 for cycles 2-4 prior to surgery. Dose modifications, delays in therapy, and administration of growth factors for all treatment will be left to the discretion of the treating physician. Modifications to the treatment regimen and reason for changes will be recorded for potential correlation with drug levels.

5.2 Timing of surgery

We anticipate that definitive breast surgery will be performed after completion of the final cycle of paclitaxel. Patients will be allowed to receive additional neoadjuvant chemotherapy, such as AC, prior to surgery at the discretion of the treating physician, if deemed in the best interest of the patient. This chemotherapy must be initiated after completion of paclitaxel and imaging of the tumor to assess response to paclitaxel. Surgery is anticipated to be performed 3-6 weeks after completion of chemotherapy. However, surgery may be delayed or performed earlier at the discretion of the treating physicians. These modifications will not disqualify the patient from analysis in this protocol or result in removal of the patient from study. The timing of definitive surgery relative to treatment will be recorded.

5.3 Data Acquisition and Management

Data elements will be extracted from subject medical records after informed consent and HIPAA authorization have been obtained. Data elements to be collected and maintained by the UWCCC OnCore database system and/or the researcher records include the following:

- Subject name and MRN (recorded in OnCore for subject registration purposes ONLY)
- Automated subject ID number (generated by OnCore)
- Verification of inclusion and exclusion criteria
- Demographic information of age, weight, height, race and ethnicity, history of prior cancer, family history of breast or ovarian cancer, menopausal status
- Mammographic breast density, specific mammographic or ultrasound abnormality, pathologic diagnosis including cancer type with estrogen, progesterone and HER-

2/neu status or particular benign diagnosis, tumor size, grade and stage, lymph node status, Ki67 score
- BRCA1/2 mutation status, if known
- Neoadjuvant therapy regimen received
- Response to neoadjuvant therapy

In order to maintain subject privacy, all research samples released from the BioBank will be coded with a unique identifier that can be linked to an individual only by a key maintained in the secure Oncore database. A link between the unique sample code to the MRN and subject name will be retained for the purposes of associating pathology data and subject outcomes with the functional effects of the subject-derived epithelial and stromal cells. All personal identifiers, including subject name, date of birth, address, and date of study clinic visit will be removed from researcher records to preserve individual confidentiality. This information will be kept by the principal investigator in a locked computer file and in the secure Oncore database. Coded data elements lacking identifiers will be stored separately on UWSMPH Department of Medicine workstations and researcher laptops. Study data elements will be shared exclusively with the investigators on this protocol; the key to the subject codes will not be shared.

5.4 **Sample Collection for Laboratory Analysis**

5.4.1 15 ml Blood will be drawn prior to chemotherapy to provide a sample of non-tumorous control cells for genomic, histological, and paclitaxel concentration analysis. This blood will be drawn into purple-top EDTA tubes and delivered to the University of Wisconsin 3P Lab for analysis.

5.4.2 Archived tissue from a prior biopsy of the primary tumor (or lymph node) will be obtained and used for biomarker and genomic analysis. One FFPE block, or 10 unstained slides will be obtained from archived tissue.

5.4.3 Tumor core biopsies will be obtained with goal timing as close as possible to 20 hours following initiation of the 1st, 3rd, and 12th paclitaxel infusions. The biopsy after the 1st dose of paclitaxel is required; the biopsy after the 3rd and 12th dose of paclitaxel (C1D16 and C4D16, respectively) are optional. Patients who have a biopsy after the 3rd dose of paclitaxel are not required to get a biopsy after the 12th dose, and similarly, patients who <u>do not</u> have the biopsy after the 3rd dose may still choose to undergo the biopsy after the 12th dose. . To achieve this, the UW Breast investigators will arrange to deliver the dose of chemotherapy on a Mon-Thursday between 12 and 1 PM, seeing an enrolled patient in urgent clinic if needed. Patients will have an appointment for breast biopsy at 8AM the following day so a breast biopsy will be able to be performed within 18-22h window, although we will actually attempt to perform this as close as possible to 20h post the start of paclitaxel administration. The breast biopsy may be performed with or without image guidance. The biopsy may be performed either by a coinvestigator or an interventional radiologist credentialed in breast biopsy. Time from initiation of paclitaxel to biopsy will be recorded. A total of 4 biopsy cores will be taken at

each biopsy time-point. Tissue (2 cores) will be flash frozen in liquid nitrogen and stored at -80°C or liquid nitrogen in the 3P laboratory until paclitaxel level measurements are performed. Additional samples (2 cores) will be fixed in formalin for analysis of biomarkers.

For subjects consenting to the optional tumor biopsies after the 3rd and 12th paclitaxel doses, the investigator will make the final determination as to which subjects will undergo the biopsy. The investigator may use the following criteria to make this determination: tumor size, tumor location and treatment received.

An optional skin biopsy (3-4 mm) will be obtained from consenting subjects as close as possible to 20 ± 4 hours following initiation of the 1st paclitaxel infusion. The skin biopsy may be performed by an appropriately credentialed medical provider on the protocol. Time from initiation of paclitaxel to skin biopsy will be recorded. Tissue will be flash frozen in liquid nitrogen and stored at -80°C or liquid nitrogen in the 3P laboratory until paclitaxel level measurements are performed.

5.4.4 If excess residual tumor is present at time of surgery, a sample will be obtained for biomarker and genomic analysis. One FFPE block, or 10 unstained slides, will be requested after pathologic review is complete for this analysis.

5.5 Laboratory analysis

5.5.1 Both tumor tissue and plasma samples taken at 20 hours will be assessed for paclitaxel levels by the 3P lab as previously described[9]. Tumor paclitaxel distribution will be measured by MALDI-TOF by the Li lab. A blood sample (15 mL drawn into purple-top EDTA tubes) will be drawn as close to the 20 hour timepoint as possible. Time of blood draw and tissue biopsies will be recorded for analysis purposes.

5.5.2 All tissue samples, including archival pretreatment biopsy (see 3.1.4) will be assessed for feasibility analysis of biomarkers including:

5.5.2.1 Markers of proliferation: Using H&E stained slides, the number of mitotic cells and interphase nuclei will be quantified. Typically, mitotic index is measured as the ratio of mitotic cells over total cells. Additional stains will be used to characterize the fraction and spindle morphology of mitotic cells. For example, tissue will be analyzed by immunofluorescence of cells stained with DAPI, phospho-H3, alpha-tubulin, and Mad2. Ki67 stain will be performed as a marker of proliferation.

5.5.2.2 Aneuploidy: To assay for aneuploidy in a parsimonious manner, we will use multicentromeric single-color fluorescence in-situ hybridization (FISH). For example, using probes specific for centromeres of chromosomes 1, 5 and 19, we expect to see 6 signals per diploid cell. Aneuploidy is determined from

fixed specimens at pretreatment, ~20h post 1st, 3rd, and 12th treatments, and at the time of surgery if there is sufficient residual tumor.

5.5.2.3 Sequencing analysis: For patients who have multiple biopsies, assessments of fixed specimens (tumor, skin, blood) at pretreatment, ~20h post 1st, 3rd , and 12th treatments, and, at the time of surgery if there is sufficient residual tumor, will be performed using techniques such as SNP-CHIP and sequencing. Genome wide analysis will provide alternative measures for aneuploidy and CIN and will provide information about genetic alterations in paclitaxel-responsive and paclitaxel unresponsive tumors.

5.6 Future Studies

Additional blood and tissue specimens remaining will be stored for future studies related to cancer research. All future projects using the banked data or samples from this study will be submitted to the Institutional Review Board (IRB) for review and approval or exemption. Distribution of banked samples will be limited to researchers within UW-Madison. Request for samples would be directed to the study PI (Dr. Burkard) who will determine if the banked samples would be suitable for use in that study. Prior to distribution, scientific and IRB approval of the research study would need to be provided to the Dr. Burkard.

After study-related testing is complete, the blood and tissue samples from subjects will be stored in the Burkard Lab. These samples will be kept until they are exhausted or for a maximum of 10 years, whichever comes first. Subjects will be permitted to withdraw their samples from the study or the bank. They may withdraw their samples by informing the study PI.
To protect the confidentiality of subjects in this study, the blood and tissue samples will be coded. The key will be saved in computer files that are protected via passwords and access rights. The key will include: subject name, medical record number, procedure dates or pathology accession number (biopsy and surgery), and the sample identification codes (one per subject per sample). For subjects who are deemed eligible and are registered to the trial their subject ID number will also be listed on the key. The rest of the research data will also be saved in secure computer files or in the OnCore database which is only accessible via username, password, and access rights.

After subjects sign the informed consent form, the blood and tissue samples will be obtained by the research staff and coded prior to the samples being provided to the Burkard lab. The Burkard lab is only accessible to individuals involved with the research completed in that lab.

5.7 Duration of Follow Up

Patients will be followed until time of surgery at which time all tissue and plasma sample collection will have been collected and their response to neoadjuvant chemotherapy will be assessed.

Subjects may receive additional systemic therapy (following paclitaxel) prior to planned surgery, at the discretion of the treating physician. Adverse events that occur during this period, up until surgery, will be recorded.

5.8 Criteria for Removal from Study

Patients will be removed from the study if they withdraw consent. If the core biopsy on day 2 is not able to be obtained or the patient is not able to receive the 1st complete dose of paclitaxel, they will be removed from the study. If they are not able to complete the study due to any reason after day 2, their initial serum and tissue will still be analyzed.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dosing delays and dose modifications will be at the discretion of the treating physician. Such adjustments will be recorded for potential correlation to biomarkers.

7. PHARMACEUTICAL INFORMATION

7.1 **Adverse events and potential risks** (Micromedex®). The adverse events and potential risks associated with paclitaxel (Taxol®) include:

7.1.1 **Common**

- **Dermatologic:** Alopecia (55% to 96%)
- **Hematologic:** Anemia, any grade (47% to 96%), leucopenia (90%), neutropenia, any grade (78% to 100%), thrombocytopenia, any grade (4% to 68%)
- **Gastrointestinal:** Diarrhea (16% to 90%), inflammatory disease of mucous membrane (5% to 45%), nausea and vomiting, any grade (4% to 88%)
- **Immunologic:** Immune hypersensitivity reaction, any grade (2% to 45%)
- **Musculoskeletal:** Arthralgia, myalgia
- **Neurologic:** Peripheral neuropathy, any grade (42% to 79%)

7.1.1 **Serious**

- **Cardiovascular:** Atrial fibrillation (rare), Cardiac dysrhythmia (less than 1%), Cardiotoxicity, Congestive heart failure, Myocardial infarction (rare), Supraventricular tachycardia (rare)
- **Gastrointestinal:** Gastrointestinal perforation, Nausea and vomiting, Grade 3 or greater (10% to 29%)
- **Hematologic:** Anemia, Grade 3 or greater (2% to 34%), Deep venous thrombosis, Febrile neutropenia (2% to 55%), Neutropenia, Grade 4 (14% to 81%), Thrombocytopenia, Grade 3 or greater (1% to 17%
- **Immunologic:** Anaphylaxis, Immune hypersensitivity reaction, Grade 3 or greater (up to 4%), Opportunistic infection (up to 76%), Sepsis
- **Neurologic:** Grand mal seizure (less than 1%), Peripheral neuropathy, Grade

3 or greater (up to 10%), Seizure
- **Respiratory:** Pulmonary embolism

7.2 **Availability**

Paclitaxel (Taxol®) is an FDA approved medication for treatment of curable breast cancer and is available by prescription at pharmacies throughout the United States.

8 ADVERSE EVENTS

8.1 Only adverse events related to the research biopsies and research only blood draws will be collected. **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE assessment. A copy of the CTCAE version 4.03 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

8.2 **Attribution of the AE:**

Definite	The AE *is clearly related* to the study procedures.
Probable	The AE *is likely related* to the study procedures.
Possible	The AE *may be related* to the study procedures.
Unlikely	The AE *is doubtfully related* to the study procedures.
Unrelated	The AE *is clearly NOT related* to the study procedures.

8.3 Expected Adverse Events resulting from the administration of paclitaxel, any other systemic therapy and surgical intervention will not be reported. Unanticipated serious adverse events will be reported as outlined in sections 9 and 10.

9 OVERSIGHT AND MONITORING PLAN

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for the regular review and monitoring of all ongoing clinical research in the UWCCC. A summary of DSMC activities are as follows:

- Reviews all clinical trials conducted at the UWCCC for subject safety, protocol compliance, and data integrity.
- Reviews all Serious Adverse Events (SAE) requiring expedited reporting, as defined in the protocol, for all clinical trials conducted at the UWCCC, and studies conducted at external sites for which UWCCC acts as an oversight body.
- Reviews all reports generated through the UWCCC DSMS elements (Internal Audits, Quality Assurance Reviews, Response Reviews, Compliance Reviews, and Protocol Summary Reports) described in Section 9.1 of this document.
- Notifies the protocol Principal Investigator of DSMC decisions and, if applicable, any requirements for corrective action related to data or safety issues.
- Notifies the CRC of DSMC decisions and any correspondence from the DSMC to the protocol Principal Investigator.

- Works in conjunction with the UW Health Sciences IRB in the review of relevant safety information as well as protocol deviations, non-compliance, and unanticipated problems reported by the UWCCC research staff.
- Ensures that notification is of SAEs requiring expedited reporting is provided to external sites participating in multi-institutional clinical trials coordinated by the UWCCC.

9.1 Monitoring and Reporting Guidelines

UWCCC quality assurance and monitoring activities are determined by study sponsorship and risk level of the protocol as determined by the PRMC. All protocols (including Intervention Trials, Non-Intervention Trials, Behavioral and Nutritional Studies, and trials conducted under a Training Grant) are evaluated by the PRMC at the time of committee review. UWCC monitoring requirements for trials without an acceptable external DSMB are as follows:

Intermediate Monitoring

Protocols subject to intermediate monitoring generally include UW Institutional Phase I/II and Phase II Trials. These protocols undergo review of subject safety at regularly scheduled DOWG meetings where the results of each subject's treatment are discussed and the discussion is documented in the DOWG meeting minutes. The discussion includes the number of subjects enrolled, significant toxicities, dose adjustments, and responses observed. Protocol Summary Reports are submitted on a semi-annual basis by the study team for review by the DSMC.

9.2 Review and Oversight Requirements

9.2.1 Serious Adverse Event – Reported Within 24 Hours

Serious Adverse Events requiring reporting within 24 hours (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu within one business day. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if immediate action is required. Within 5 calendar days, all available subsequent SAE documentation must be submitted electronically along with a 24 hour follow-up SAE Details Report and a completed UWCCC SAE Routing Form to saenotify@uwcarbone.wisc.edu. All information is entered and tracked in the UWCCC database.

The Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

For a multiple-institutional clinical trial the PI is responsible for ensuring SAEs are reported to the FDA as well as to all participating investigators.

See Section 10 for detailed instructions on SAE reporting.

9.2.2 Serious Adverse Event – Reported within 10 Days

Serious Adverse Events requiring reporting within 10 calendar days (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if further action is required. All information is entered and tracked in the UWCCC database.

The Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

For a multiple-institutional clinical trial the PI is responsible for ensuring SAEs are reported to the FDA as well as to all participating investigators.

See Section 10 for detailed instructions on SAE reporting.

9.2.3 Study Progress Review

Protocol Summary Reports (PSR) are required to be submitted to the DSMC in the timeframe determined by the risk level of the study (quarterly; semi-annually; or annually). The PSR provides a cumulative report of SAEs, as well as instances of non-compliance,

protocol deviations, and unanticipated problems, toxicities and responses that have occurred on the protocol in the timeframe specified. PSRs for those protocols scheduled for review are reviewed at each DSMC meeting.

Protocol Summary Reports enable DSMC committee members to assess whether significant benefits or risks are occurring that would warrant study suspension or closure. This information is evaluated by the DSMC in conjunction with other reports of quality assurance activities (e.g., reports from Internal Audits, Quality Assurance Reviews, etc.) occurring since the prior review of the protocol by the DSMC. Additionally, the DSMC requires the study team to submit external DSMB or DSMC reports, external monitoring findings for industry-sponsored studies, and any other pertinent study-related information.

In the event that there is significant risk warranting study suspension or closure, the DSMC will notify the PI of the DSMC findings and ensure the appropriate action is taken for the protocol (e.g., suspension or closure). The DSMC ensures that the PI reports any temporary or permanent suspension of a clinical trial to the sponsor (e.g., NCI Program Director, Industry Sponsor Medical Monitor, Cooperative Group Study Chair, etc.) and other appropriate agencies. DSMC findings and requirements for follow-up action are submitted to the CRC.

9.2.4 Expedited Reporting of Serious Adverse Events

Depending on the nature, severity, and attribution of the serious adverse event an SAE report will be phoned in, submitted in writing, or both according to Table 9.2.6 below. Serious adverse events must also be reported to the UW IRB according to their reporting guidelines.

Determine the reporting timeframe for the SAE in question by using the following table.

9.2.5 Expedited Reporting Table

Reporting Requirements for Serious Adverse Events
NOTE: Investigators MUST immediately report to the UWCCC DSMC and any other parties outlined in the protocol ANY Serious Adverse Events, that are related to the research procedure (post paclitaxel biopsy) (21 CFR 312.64).
An adverse event is considered serious if it results in ANY of the following outcomes:

1) Death.
2) A life-threatening adverse event.
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria* MUST be immediately reported to the UWCCC within the timeframes detailed in the table below:

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour; 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

** [research team: Only adverse events related to the research procedure (post paclitaxel biopsy) require expedited reporting.]*

Expedited AE reporting timelines are defined as:

- 24-Hour; 5 Calendar Days – The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- 10 Calendar Days – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE

10 SAE Reporting

10.1 SAE Requiring *[24]* Hour Reporting Occurs at UWCCC:

A. Report to the UWCCC:

Reference the **SAE SOP** (Standard Operating Procedure) and the **SAE Reporting Workflow for DOTs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for *[24]* hour initial and follow-up reports. **A follow-up report is required to be submitted within 5 calendar days of the initial *[24]* hour report.**

For this protocol, the following UWCCC entities are required to be notified:

a) saenotify@uwcarbone.wisc.edu
b) Study PI: Mark Burkard, MD, PhD
c) Disease Oriented Team Manager: Tammy Koehn
d) Any other appropriate parties listed on the SAE Routing Form (for follow-up reports only)

B. Report to the IRB:

Consult the UW-IRB website for reporting guidelines.

10.2 SAE Requiring *[10]* Day Reporting Occurs at UWCCC:

A. Report to the UWCCC:

Reference the **SAE SOP** and the **SAE Reporting Workflow for DOTs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for *[10]* day reports.

For this protocol, the following entities are required to be notified:

a) saenotify@uwcarbone.wisc.edu
b) Any appropriate parties listed on SAE Routing Form

B. Report to the IRB:

Consult the UW-IRB website for reporting guidelines.

C. Report to the FDA:

The treating physician and/or PI may report unexpected serious adverse events to the FDA using the voluntary reporting form (FDA Form 3500) at their discretion.

11 STUDY CALENDAR

Test/Assessment	Prior to Initiation of Paclitaxel	Cycle 1, day 2	Cycle 1 Day 16[2]	Cycle 2-4, day 1	After completion of paclitaxel[2]	Surgery
History and Physical	x			x		x
ECOG PS	x			x		
Pregnancy Test	Will be confirmed if ran by treating physician					
AST, ALT, CBC	Per oncologist			x		
Blood sample for genomics and paclitaxel levels	x					
Tumor/serum Paclitaxel Level and Distribution		x	x		x	
Optional skin biopsy[3]		x				
Toxicity Evaluation[1]	x			x		
Archived FFPE sample	x*					x
Fresh Biopsy or tumor sampling for analysis—4 cores (CIN, mitotic index, etc.)[2]		x	x		x	
Pathologic Evaluation for Response						x
Imaging Evaluation per 12.1	x				x	

* Slides obtained from prior biopsy of tumor may be obtained at any point after subject signs consent.

1. Only toxicities related to research only biopsies and blood draws will be collected and recorded.

2.C1D16 and C4D16 are optional and collections are only completed if patient consents.

3. Skin biopsy is optional and collection is only completed if patient consents.

12 MEASUREMENT OF EFFECT

12.1 **Methods for Evaluation**

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. Follow-up evaluations should be performed prior to surgery.

The same method of assessment and the same technique (MRI, ultrasound, CT, mammography, or clinical exam) should be used to characterize each identified and

reported lesion at baseline and prior to surgery. **MRI will be preferentially used for evaluation when medically reasonable. Imaging will be performed upon completion of paclitaxel chemotherapy, prior to any additional chemotherapy.**

12.2 Response Criteria- Pathologic

For the purposes of this study, patients should be evaluated for a pathologic response once at time of surgery. A pathologic complete response (pCR) will be defined as the absence of residual invasive cancer within both the breast and lymph nodes. In addition, pathologic evidence of significant tumor cell reduction will be noted.

12.3 Response Criteria- Clinical Response

The clinical response will be determined similar to RECIST 1.1 criteria,[15] modified to allow measurements of breast tumors by MRI, ultrasound, or mammogram as previously described[16]

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

We will treat CIN as a continuous variable by 6-chromosome FISH as the average % of cells that deviate from the modal number for each of the 6 chromosome FISH signals. Response to paclitaxel will also be considered as a continuous variable where response is determined by the % decrease in linear measurement of tumor size on the greatest dimension per RECIST-like criteria (as per 12.3). We will correlate Taxol response with CIN. Using alpha=0.05, 1-beta=80% and power our sample to distinguish the correlation coefficient of r = 0.6 from r=0 (null hypothesis). Thus, we estimate the study will require 19 patients. We anticipate dropout or other technical issue will affect data interpretation of at least one patient so we will enroll 20 subjects. Results will be interpreted in the context of other studies that evaluate the correlation of taxane sensitivity with CIN measured by FISH.

The standard normal deviate for $\alpha = Z_\alpha = 1.960$
The standard normal deviate for $\beta = Z_\beta = 0.842$
$C = 0.5 * \ln[(1+r)/(1-r)] = 0.693$

Total sample size = $N = [(Z_\alpha+Z_\beta)/C]^2 + 3 = 19$

13.2 Sample Size/Accrual Rate

The planned sample size is 20 patients, with accrual expected ~10 per year. The study is expected to accrue all patients within 2-3 years. The study will be terminated at the time when all patients have completed operative management and data collection using patient identifiers is complete. Subjects who are deemed unevaluable due to inadequate tissue or any other reason will be replaced.

13.4 **Analysis of Secondary Endpoints**

Secondary endpoints such as mitotic index, aneuploidy, and paclitaxel levels will be analyzed by fluorescence in situ hybridization, immunofluorescence, immunohistochemistry, and liquid chromatography as previously described[16-18].. More specifically for each secondary endpoint:

13.4.1 To identify patient-specific differences in tumor/non-tumor levels and tissue distribution of paclitaxel at 20 hours after first dose.
Tissue paclitaxel levels are measured by high-performance liquid chromatography (HPLC)of tumor and plasma samples and comparing variability between patients with descriptive statistics. Paclitaxel tissue distribution is measured by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) to compare variability in distribution patterns between patients with descriptive statistics.

13.4.2 To determine if paclitaxel levels are higher at 20h after the 3rd dose than after the first dose, and if levels are higher at 20h after the 12^{th} dose than the 1^{st} and 3^{rd} dose.
This is measured by high-performance liquid chromatography of tumor and plasma samples and comparing difference at two time points within the same patient with paired statistics.

13.4.3 Compare pre-existing versus post-treatment antimitotic effects at 20h after the 1^{st} dose and 20h after the 3^{rd} and 12^{th} dose.
This is measured by tissue analysis of tumor samples as described previously [16] with phospho-histone H3 and stains for spindle morphology to quantify mitotic index and mitotic characteristics at two time points using paired statistical analysis.

13.4.4 Correlate drug levels and tissue distribution with biomarkers including mitotic index, aneuploidy, chromosomal instability, and Ki67.

13.4.5 Correlate pathologic response and clinical response with biomarkers including mitotic index, aneuploidy, CIN and Ki67.

Measurements of aneuploidy, CIN, and Ki67 will be performed as previously described [18]. These correlations will be considered preliminary and results will be interpreted in the context of other studies being performed in metastatic breast cancer.

13.4.6 To test if CIN increases in patient tumors in response to paclitaxel and to evaluate the feasibility of these measurements by genomic analysis.

Multiple genomic analyses have been proposed to measure CIN and aneuploidy in tumors. These include whole genome sequencing with high depth, SNP-array analysis, Comparative Genomic Hybridization and others. However, this is

rapidly changing field with new technologies and analytic methods emerging weekly. We anticipate performing high-depth whole genome sequencing from three independent core samples per biopsy as a pilot analysis. Based on our experience with initial samples, and on available technologies and analytic methods available at the time of analysis, we may select another genomic sequencing and/or hybridization methods to optimally determine the changes in CIN. Fluorescence-in-situ hybridization will be used as a companion method as described[18].

REFERENCES:

1. Surveillance Epidemiology and End Results (SEER). Available from: http://seer.cancer.gov/.
2. Gradishar WJ, Krasnojon D, Cheporov S, et al. Significantly longer progression-free survival with nab-paclitaxel compared with docetaxel as first-line therapy for metastatic breast cancer. *J Clin Oncol*. 2009;27(22):3611-3619.
3. Henderson IC, Berry DA, Demetri GD, 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):976-983.
4. Citron ML, Berry DA, Cirrincione C, et al. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. *J Clin Oncol*. 2003;21(8):1431-1439.
5. Puhalla S, Mrozek E, Young D, et al. Randomized phase II adjuvant trial of dose-dense docetaxel before or after doxorubicin plus cyclophosphamide in axillary node-positive breast cancer. *J Clin Oncol*. 2008;26(10):1691-1697.
6. Wildiers H, Forceville K, Paridaens R, Joensuu H. Taxanes and anthracyclines in early breast cancer: which first? *Lancet Oncol*;11(3):219-220.
7. Chakravarthy AB, Kelley MC, McLaren B, et al. Neoadjuvant concurrent paclitaxel and radiation in stage II/III breast cancer. *Clin Cancer Res*. 2006;12(5):1570-1576.
8. Brito DA, Rieder CL. The ability to survive mitosis in the presence of microtubule poisons differs significantly between human nontransformed (RPE-1) and cancer (U2OS, HeLa) cells. *Cell Motil Cytoskeleton*. 2009;66(8):437-447.
9. Yang Z, Kenny AE, Brito DA, Rieder CL. Cells satisfy the mitotic checkpoint in Taxol, and do so faster in concentrations that stabilize syntelic attachments. *J Cell Biol*. 2009;186(5):675-684.
10. Fine RL, Chen J, Balmaceda C, et al. Randomized study of paclitaxel and tamoxifen deposition into human brain tumors: implications for the treatment of metastatic brain tumors. *Clin Cancer Res*. 2006;12(19):5770-5776.
11. Pusztai L, Jeong JH, Gong Y, et al. Evaluation of microtubule-associated protein-Tau expression as a prognostic and predictive marker in the NSABP-B 28 randomized clinical trial. *J Clin Oncol*. 2009;27(26):4287-4292.
12. Juul N, Szallasi Z, Eklund AC, et al. Assessment of an RNA interference screen-derived mitotic and ceramide pathway metagene as a predictor of response to neoadjuvant paclitaxel for primary triple-negative breast cancer: a retrospective analysis of five clinical trials. *Lancet Oncol*.

13. Swanton C, Marani M, Pardo O, et al. Regulators of mitotic arrest and ceramide metabolism are determinants of sensitivity to paclitaxel and other chemotherapeutic drugs. *Cancer Cell*. 2007;11(6):498-512.
14. Croshaw R, Shapiro-Wright H, Svensson E, Erb K, Julian T. Accuracy of clinical examination, digital mammogram, ultrasound, and MRI in determining postneoadjuvant pathologic tumor response in operable breast cancer patients. *Annals of surgical oncology*. 2011;18(11):3160-3163.
15. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247.
16. Zasadil LM, Andersen KA, Yeum D, et al. Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Science translational medicine*. 2014;6(229):229ra243-229ra243.
17. Choudhary A, Zachek B, Lera RF, et al. Identification of selective lead compounds for treatment of high-ploidy breast cancer. *Molecular cancer therapeutics*. 2016;15(1):48-59.
18. Denu RA, Zasadil LM, Kanugh C, Laffin J, Weaver BA, Burkard ME. Centrosome amplification induces high grade features and is prognostic of worse outcomes in breast cancer. *BMC cancer*. 2016;16(1):1.