Status Page

PROTOCOL 13-383

Permanent
Closed to New Accrual

Closure Effective Date: 07/24/2018
No new subjects may be enrolled in the study as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

Front Sheet

Report Generated: 03/29/2019 02:38 PM

Title: A Randomized Phase II Study of Preoperative Cisplatin Versus Paclitaxel in Patients with Triple Negative Breast Cancer Evaluating the Homologous Recombination Deficiency (HRD) Biomarker

Overall Institution: Dana-Farber Cancer Institute

Overall Principal Investigator	Email	Institution
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Sponsor Name	Sponsor Protocol No	Roles	Grant Number(s)
Myriad Genetics		Funding	
DF/HCC Investigator		Regulatory	
TBCRC		Funding	
Dana-Farber/Harvard Cancer Center		Funding	

Total Study-Wide Enrollment Goal: 165 **Total DF/HCC Estimated Enrollment Goal:** 165

Phase: II

Age: Adults

Age Ranges: >/= 18

Will all subjects be recruited from pediatric clinics?

CTEP Study: No

Management Group(s): BIDMC Breast Cancer
DF/HCC Breast Cancer
DFCI/BWH Breast Oncology
OTHER Registering Site

Primary Management Group: DF/HCC Breast Cancer

Investigational Drug? Yes

Drug(s), Biologic(s): CISPLATIN, PACLITAXEL

IND #: Exempt

IND Holder Type:

IND Holder Name: Exempt

Investigational Device? This study does not use an Investigational Device.

IRB of Record:

Risk Category: Greater Than Minimal Risk

Protocol Involves: Chemotherapy; Human Material Banking; Human Material Collection; Required Biopsy; Surgery

Date Range: (Medical Record Review and Specimen Collection studies)

Participating Sites under the DFCI IRB

Institution: Beth Israel Deaconess Medical Center
Brigham and Women's Hospital
Brigham and Women's Hospital at Faulkner Hospital
Dana-Farber Cancer Institute
Dana-Farber Cancer Institute at Milford
Dana-Farber Cancer Institute at South Shore

Participating Institutions Under Other IRB

Institution:	**Location:**
Baylor College of Medicine	HOUSTON, TX
Duke University Medical Center	DURHAM, NC
Indiana University/Purdue University Hospital	INDIANAPOLIS, IN
Memorial Sloan Kettering Cancer Center Monmouth	Middletown
Memorial Sloan-Kettering Cancer Center	NEW YORK, NY
Memorial Sloan-Kettering Cancer Center Basking Ridge	Basking Ridge
Memorial Sloan-Kettering Cancer Center Commack	Commack
Memorial Sloan-Kettering Cancer Center Rockville Centre	Rockville Centre
Memorial Sloan-Kettering Cancer Center Sleepy Hollow	Sleepy Hollow
Memorial Sloan-Kettering Cancer Center West Harrison	West Harrison
Seattle Cancer Alliance at Evergreen Health	Kirkland
The Johns Hopkins University School of Medicine	BALTIMORE, MD
University of Alabama at Birmingham	BIRMINGHAM, AL
University of North Carolina Chapel Hill	CHAPEL HILL, NC
University of Pittsburgh	PITTSBURGH, PA
University of Washington Medical Center	SEATTLE, WA
Vanderbilt University Medical Center	NASHVILLE, TN

Protocol Number: 13-383

Approval Date: 10/08/13 (IRB meeting date when protocol/consent approved or conditionally approved)

Activation Date: 01/29/14 (Date when protocol open to patient entry)

Approval signatures are on file in the Office for Human Research Studies, tel. 617-632-3029.

Date Posted	Revised Sections	IRB Approval Date	OHRS Version Date
01/29/14	Front Sheet and Consent Form revised due to Amendment #1	12/19/13	01/29/14
03/06/14	Delayed Activation Alert Page removed: BIDMC now ready for activation (note: previously activated at DFCI/BWH on 01/29/14)	10/08/13	N/A
04/10/14	Consent Form, Protocol and Front Sheet replaced due to Amendment #2	03/31/14	04/10/14
07/15/14	Front Sheet replaced due to Amendment #3	07/14/14	n/a
08/11/14	Front Sheet replaced due to Amendment #4	08/07/14	N/A
09/22/14	Study renewal / Consent Form footer replaced due to Continuing Review #1	09/18/14	N/A
09/25/14	Front Sheet replaced due to Amendment #6	09/25/14	N/A
10/20/14	Front Sheet replaced due to Amendment #7	10/08/14	N/A
10/21/14	Amendment #5: No change to online documents (Front Sheet already posted with Am 6 and Am 7)	09/23/14	N/A
11/07/14	Consent Form and Front Sheet replaced due to Amendment #8	10/29/14	11/05/14
01/12/15	Front Sheet replaced due to Amendment #9	01/07/15	N/A
01/16/15	Front Sheet replaced due to Amendment #10	01/14/15	N/A
02/09/15	Front Sheet replaced due to Amendment #11	02/04/15	N/A
04/30/15	Front Sheet replaced due to Amendment #14	04/28/15	N/A
05/08/15	Consent Form, Protocol and Front Sheet revised due to Amendment #12	04/28/15	N/A
05/08/15	Consent Form, Protocol and Front Sheet replaced due to Amendment #13 (updated Front Sheet already posted with Am #14)	04/20/15	05/08/15
05/15/15	Front Sheet replaced due to Amendment #15	05/15/15	N/A
Date Posted	**Revised Sections**	**IRB Approval Date**	**OnCore Version Date**
07/21/15	Correction Am #15: Front Sheet replaced	05/15/15	N/A
09/01/15	Front Sheet replaced due to Amendment #16	08/25/15	N/A
09/16/15	Study renewal/Consent Form footer replaced due to Continuing Review #2	08/20/15	09/16/15
03/29/16	Eligibility Checklist replaced due to Amendment #17	03/01/16	n/a
06/06/16	Consent Form, Protocol, Front Sheet and Eligibility Checklist replaced due to Amendment #18	05/19/16	06/03/16
06/09/16	Correction Amendment #18: Eligibility Checklist replaced	(05/19/16)	N/A
07/07/16	Front Sheet replaced due to Amendment #19	06/30/16	N/A
08/10/16	Study renewal/ Consent Form footer replaced due to Continuing Review #3	07/28/16	08/09/16
09/02/16	Protocol and Front Sheet replaced due to Amendment #20	08/22/16	N/A
Date Posted	**Revised Sections**	**Approved Date**	**Version Date (OnCore)**
03/06/17	Front Sheet replaced due to Amendment #21	03/03/17	N/a

05/15/17	Add Supplemental Biopsy Patient Pamphlet due to Amendment #22	05/11/17	N/a
06/26/2017	Front Sheet replaced per Amendment #23	06/01/2017	N/A
06/30/17	Study renewal/ Consent Form footer replaced due to Continuing Review # 4	06/22/17	06/26/17
07/26/17	Front Sheet replaced due to Amendment #24	07/20/17	N/A
10/02/2017	Front Sheet, eligibility checklist, and protocol replaced per Amendment #25	08/22/2017	N/A
12/14/2017	Front Sheet, Consent Form replaced per Amendment #26	12/14/2017	12/14/2017
02/05/2018	Temporary Closure to New Accrual; due to Funding (Effective Date: 01/26/2018; Amendment #27)	01/31/2018	n/a
06/06/2018	Study renewal/Consent Form footer replaced per Continuing Review #5	05/31/2018	06/06/2018
08/22/2018	Permanent Closure to New Accrual due to Funding; Amendment #28; (Effective 07/24/2018)	08/15/2018	N/A
03/29/2019	Front Sheet replaced per Amendment #29	03/26/2019	n/a
04/29/2019	Study renewal/Consent Form removed due to data analysis only per Continuing Review #6	04/24/2019	n/a

A RANDOMIZED PHASE II STUDY OF PREOPERATIVE CISPLATIN VERSUS PACLITAXEL IN PATIENTS WITH TRIPLE NEGATIVE BREAST CANCER: EVALUATING THE HOMOLOGOUS RECOMBINATION DEFICIENCY (HRD) BIOMARKER

Protocol Number

DFCI: 13-383
TBCRC: 030

Protocol Chair

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Coordinating Center	Dana-Farber Cancer Institute

RANDOMIZED PHASE II STUDY OF PREOPERATIVE CISPLATIN VERSUS PACLITAXEL IN PATIENTS WITH TRIPLE NEGATIVE BREAST CANCER: EVALUATING THE HOMOLOGOUS RECOMBINATION DEFICIENCY (HRD) BIOMARKER

- Protocol Revision Record -

Original Protocol:	**Version (1), 9/25/2013**
Amendment #1:	Version (1) 9/25/2013
Amendment #2:	Version (2), 2/24/2014
Amendment #3:	Version (3) 2/27/2015
Amendment #4:	Version (4) 4/10/2015
Amendment #5:	Version (5) 5/17/2016
Amendment #6:	Version (6) 8/14/2017

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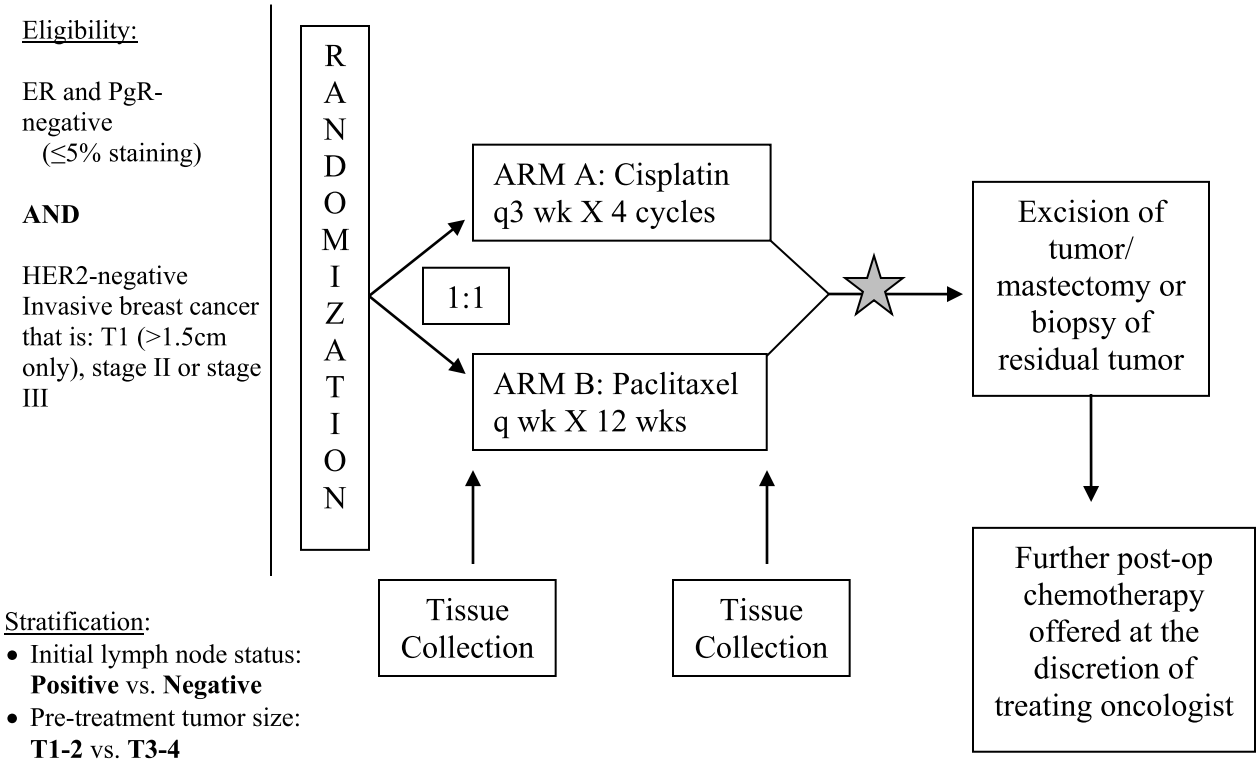
SCHEMA



★ If clinically significant residual disease after 12 weeks of therapy patient may receive crossover or alternative preoperative chemotherapy after biopsy.

1. STUDY DESIGN/SUMMARY

This is a phase II study randomizing patients with stage I with T1 > 1.5 cm, stage II or III triple negative breast cancer (TNBC) to preoperative cisplatin versus paclitaxel. The study is designed to evaluate the ability of the Homologous Recombination Deficiency (HRD) assay to predict pathologic response to preoperative chemotherapy. A total of up to 165 patients will be randomized to initiate treatment in up to 160 patients to one of the two preoperative therapies, cisplatin 75 mg/m2 every 3 weeks x 4 or weekly paclitaxel 80 mg/m2 x 12 weeks, and tissue collection for HRD and other correlative studies will be performed before and after treatment.

2. OBJECTIVES

2.1 Primary Objective

To compare the pathologic response to neoadjuvant platinum-based chemotherapy in TNBC with and without HR-deficiency, defined as a high HRD score or a *BRCA* mutation

To compare the pathologic response to neoadjuvant taxane-based chemotherapy in TNBC with and without HR-deficiency, defined as a high HRD score or a *BRCA* mutation

2.2 Secondary Objectives

- To evaluate whether the positive predictive value of HR-deficiency is greater for TNBC treated with cisplatin, as compared to TNBC treated with paclitaxel.
- To determine the association of HR-deficiency with pathologic complete response (pCR) to neoadjuvant platinum-based chemotherapy in TNBC.
- To determine the association of HR-deficiency with pCR to neoadjuvant taxane-based therapy in TNBC.
- To evaluate clinical and pathologic responses in TNBC treated with preoperative cisplatin and paclitaxel.
- To evaluate the performance of the HRD-LOH assay, the HRD-TAI assay, and the HRD-LST assay, to predict pathologic response to cisplatin or taxane therapy in TNBC.

2.3 Exploratory Objectives

- To explore the associations of gene expression signatures of taxane response and pathologic response to taxane chemotherapy.

- To determine the associations of the BRCA1/(avg BLM + FANCI) 3-gene mRNA signature to pathologic response to neoadjuvant platinum-based chemotherapy or neoadjuvant taxane-based therapy in TNBC.

- To determine the association of chromosome 15q26 copy number to pathologic response to neoadjuvant platinum-based chemotherapy in TNBC.

- To determine the associations of exome mutation number (Nmut) and proportion of mutational process D in exome mutation patterns to pathologic response to neoadjuvant platinum-based chemotherapy in TNBC.

- To evaluate the relationship between chromosome 5 LOH, loss of Rad17, as well as PAM50 subtypes including basal and claudin low to pathologic response to neoadjuvant chemotherapy in TNBC.

- To explore the associations between molecularly defined TNBC subtypes to pathologic response to neoadjuvant chemotherapy in TNBC.

- To explore the proportion of triple negative cancers negative for germline BRCA1/2 mutations that test positive for an alternative genetic anomaly contained in the 25-gene HCP profile.

- To evaluate the association of intratumoral and stromal lymphocytes[1,2] and pathologic response to taxane or cisplatin chemotherapy.

- To explore genomic alterations in circulating tumor DNA before and after protocol treatment and compare them with the alterations observed in the archival tumor sample.

All objectives will be repeated in the confirmed BRCA proficient TNBC population.

3. BACKGROUND

3.1 Study Disease

Globally, breast cancer is the most frequent female cancer and the leading cause of cancer death in women[3]. In the United States, ~230,000 new breast cancer cases are expected in 2012 and almost 40,000 deaths.[4,5] In women, the lifetime probability of developing invasive breast cancer is one in eight overall.[4-6] Breast cancer is a heterogeneous, genotypically and phenotypically diverse disease, composed of several subtypes.[7]TNBC is defined by absent expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) and it is itself an heterogeneous entity with subsets including basal-like subsets, an immunomodulatory, mesenchymal, stem-like, and luminal androgen subsets.[8]

Despite its high sensitivity to chemotherapy, TNBC is associated with decreased disease-free and overall survival.[9] Currently there are no effective target therapies in this subtype of breast cancer and chemotherapy remains the backbone of systemic treatment for TNBC. Thus, there remains an urgent need to better understand the biology of TNBC and to find predictors of treatment benefit.

<u>Homologous recombination deficiency and sensitivity to platinum based regimens</u>

Gene expression evaluation reveals several DNA repair genes that are either mutated or aberrantly expressed in TNBC. These defects include but are not exclusive to BRCA1/2 genes, and may explain TNBC high chemotherapy sensitivity to platinum-based regimens.[10]

Preclinical data have demonstrated that BRCA1 and BRCA2-deficient breast tumors exhibit differential chemosensitivity compared to BRCA1 and BRCA2-proficient cancers, with greater sensitivity to platinum and gemcitabine and less sensitivity to taxanes.[11-14] Currently, women with newly diagnosed, early-stage breast cancer arising in the setting of a BRCA1 or BRCA2 mutation continue to be treated systemically according to the same algorithm as is used in the treatment of sporadic breast cancer. This standard approach does not include treatment with a platinum chemotherapy agent despite strong preclinical and early clinical evidence suggesting the potential for high level activity in this population. A study of preoperative cisplatin in early-stage breast cancer patients with germline BRCA1 mutations documented high rates of pathologic complete response (72%) and other studies have documented high rates of pathologic complete response in mutation carriers including a neoadjuvant trial of gemcitabine, carboplatin and iniparib.[15-17]In this study, 17 women with germline BRCA1 or BRCA2 mutations have been enrolled and of these, 15/17 (88%) have achieved a complete pathologic response or have had minimal residual disease at the time of surgery (residual cancer burden,RCB, 0 or 1).[17]

In addition to BRCA1 and BRCA2, there are multiple additional homologous recombination-related genes that may be altered by mutation, rearrangement, DNA methylation or mRNA expression in TNBC which are hypothesized to result in impairment of the homologous recombination pathway and therefore could be markers of sensitivity to treatments that target defective DNA repair, as platinum agents.

Consistent with this fact, prior studies with cisplatin in TNBC without BRCA mutations have also showed promising results. Silver et al conducted a neoadjuvant trial of cisplatin in TNBC in which six (22%) of 28 patients achieved pathologic complete responses (pCR), including both patients with and without *BRCA1* germline mutations; 18 (64%) patients had a clinical complete or partial response. Factors associated with good cisplatin response include young age (P = 0.001), low BRCA1 mRNA expression (P = 0.03), BRCA1 promoter methylation (P = 0.04), p53 nonsense or frameshift mutations (P = 0.01), and a gene expression signature of E2F3 activation (P = 0.03), suggesting that, in TNBC, other biomarkers aside from BRCA may contribute to cisplatin response.[16]

Furthermore, Ryan et al conducted a neoadjuvant study of cisplatin and bevacizumab in women with TNBC. In this trial 15% of women without mutations achieved pCR.[17] Recently, data from the GepardSixto study also suggested a role for platinum agents in TNBC management. In this trial patients were treated with paclitaxel and non-pegylated-liposomal doxorubicin. Patients with HER2+ disease also received trastuzumab and lapatinib. Patients with TNBC also received bevacizumab. All patients were randomized 1:1 to receive concurrently carboplatin vs. not, stratified by subtype. Among patients with TNBC there was a statistical significant advantage for the group treated with carboplatin.[18] The results of the CALGB trial 40603 that examined the role of platinum agents in TNBC are pending. If consistent with the GepardSixto, it will be urgent to identify predictors of platin sensitivity.

Recently, Birkbak et al. suggested that the number of subchromosomal regions with allelic imbalance extending to the telomere [N(tAI)] predicted cisplatin sensitivity in vitro and pathologic response to preoperative cisplatin treatment in patients with TNBC. Furthermore, they found an inverse relationship between BRCA1 expression and N(tAI) in sporadic TNBC, suggesting that N(tAI) could be a genomic measure of defective repaired DNA that may identify TBNC more likely to benefit from treatments targeting defective DNA repair, other than BRCA mutated cancers.[19]

Myriad Genetic Laboratories has developed a Homologous Recombination Deficiency (HRD) Assay that detects homologous recombination deficiency regardless of etiology or mechanism, as measured by levels of genomic instability and loss of heterozygosity. The assay is compatible with formalin-fixed paraffin-embedded (FFPE) tumor tissue. Statistically significant correlation with response to platinum in breast cancer in a neoadjuvant study of gemcitabine, carboplatin and iniparib was recently reported.[20]

The HRD Assay score was assessed in a cohort of 77 patients enrolled on PrECOG 0105 where pathologic response was assessed using the residual cancer burden (RCB) index.[21] Forty-four tumors were obtained from responders (RCB 0/I) and 33 tumors were from non-responders (RCB II/III). Genome-wide SNP data was generated from Affymetrix MIP arrays (n=15), a custom Agilent SureSelect XT capture followed by sequencing on an Illumina HiSeq 2500 (n=21), or using both assays (n=41). Sequencing scores were used for analysis when available and the correlation coefficient for samples analyzed using both assays was 0.76. Tumors were sequenced for BRCA1 and BRCA2 and variants classified as deleterious or suspected

deleterious. Read coverage across each exon was used to detect large rearrangements. The HRD score was derived by count of the number of LOH regions (>15 Mb and < whole chromosome) observed in the tumor genome. Germline and somatic BRCA1/2 mutation status was known in all patients and 19 tumors were classified as BRCA1/2 deficient: germline BRCA1 mutation (n=12), germline BRCA2 mutation (n=4), germline BRCA1&2 mutation (n=1), somatic BRCA1 mutation (1), and somatic BRCA2 (1) mutation. Three mutation carriers had ER+/PR+ (>5%) breast cancer. The average HRD score for responders was 16.2 and the average score for non-responders 11.2 (p=0.0003). 60 patients had HRD scores ≥10. No differences were noted between BRCA1/2 mutant vs. intact responders. If BRCA1/2 deficient samples were excluded (n=58), the association between response to treatment and HRD score remained significant (p=0.0006). In this group, out of 28 responders, 26 had HDR scores ≥ 10. Seventy-four percent of TNBC tumors had an HRD score of ≥ 10 (n=74). Overall, 70% of patients with an HRD score of ≥ 10 or BRCA1/2 mutation responded compared with 12% of patients with an HRD score of < 10 and intact BRCA1/2 (p=0.00002). Correlations between response and stage were not significant. [20]

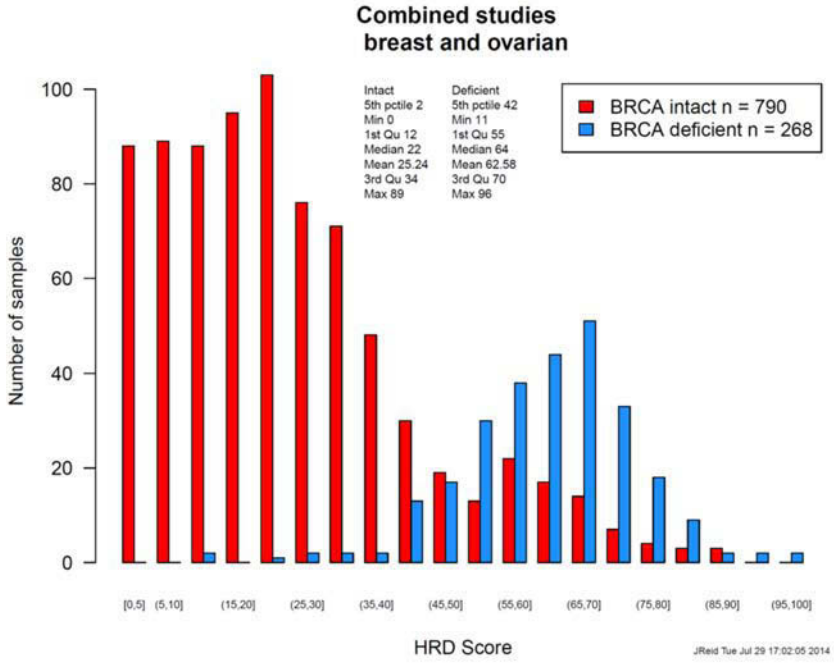
Among TNBC cases with BRCA intact (n = 56), the standard deviation (sd) for HRD score was 5.7, and in the subset that received neoadjuvant chemotherapy, scores did not vary between responders (n=10, sd = 5.4) and non-responders (n=17, sd = 5.7).

Three methods to evaluate HRD score have subsequently been developed: HRD-LOH, HRD-TAI, and HRD-LST. Each of the three individual HRD scores measures HRD in a slightly different way. Depending on the cohort analyzed (Stanford Platinum, Birkbak cisplatin, BBL BRCA subtypes), the individual methods perform slightly differently.

Rationale defining a threshold for HR deficiency

Ongoing efforts have investigated how to refine the intial version of the HRD . HRD score is the sum of the three individual scores (LOH+TAI+LST), and high HRD is now defined as HRD score ≥ 42. This threshold value was selected to have a high sensitivity for detecting HRD in breast and ovarian tumors. The training set was assembled from 4 different cohorts (497 breast and 561 ovarian cases) and consisted of 78 breast and 190 ovarian tumors that were lacking a functional copy of *BRCA1* or *BRCA2*, i.e., *BRCA*-deficient, based on mutation and methylation data (see Figure 1). We assumed that the distribution of HRD scores in *BRCA*-deficient samples would represent the distribution of scores in HR-deficient samples in general. In order to obtain a sensitivity of at least 95%, we set the threshold at the 5th percentile of the HRD scores in the *BRCA1/2* deficient tumors in the training set. Although the primary clinical application of the HRD score will be to predict response to neoadjuvant treatment, this was not the criteria for selecting the threshold. Response can be defined in various ways and is highly dependent on the etiology and chemotherapy regimens in an individual patient cohort. In contrast, a threshold based on the underlying biology of the tumor, i.e., HR deficiency, should be consistent across cohorts.

Figure 1: Threshold for HR deficiency



Validation of the Threshold: Cisplatin Neoadjuvant Cohorts 1 and 2

Archival tumor samples were obtained from 70 patients with TNBC from 2 clinical trials conducted at the Dana-Farber/Harvard Cancer Center under IRB approved protocols. One trial enrolled 28 patients who received neoadjuvant cisplatin therapy (Silver DP, et al 2010). The second trial enrolled 51 patients who received cisplatin and bevacizumab therapy (Ryan PD, et al 2009). HRD score and tumor *BRCA1/2* mutations were determined. Response was categorized by the residual cancer burden (RCB) class (Symmans WP, et al 2007) with pathologic response (PR) defined as RCB0 or I and pathologic complete response (pCR) as RCB 0. Logistic regression was used to assess HRD as a predictor of response to neoadjuvant therapy. All analysis was conducted according to a pre-specified statistical analysis plan. This study assesses the association of HRD, defined as HRD score ≥ 42 or *BRCA1/2* mutant, with response to cisplatin neoadjuvant chemotherapy in patients with TNBC.

Sixty-two tumors provided adequate tissue and passed DNA sequencing quality metrics. Thirty-one (50%) tumors were homologous recombination deficient, 22 (35%) were homologous recombination non-deficient, and 9 (15%) were undetermined. The association of HRD status with PR (RCB0/I) and pCR (RCB0) was tested by univariate logistic regression in 50 samples with complete HR status and clinical data. Homologous recombination status predicted both PR and pCR at the 5% level (Table 1). In a multivariate model of PR, homologous recombination status retained statistical significance when combined with clinical variables (p=0.0017, OR=12.08 (1.96, 74.4). When restricted to *BRCA1/2* non-mutated tumors (n=38), HRD (high HRD score) remained significantly associated with both PR (p=0.0039, OR=9.44 (1.69, 52.7) and pCR (p=0.018, OR=14.79 (1.48, 2001).[22]

Table 1: Association of HRD status with PR (RCB0/I) and pCR (RCB0)

Response	Homologous Recombination Deficient Number (% response)	Homologous Recombination Non-deficient Number (% response)	Odds Ratio (95% CI)	P-value
PR = no (RCBII, III)	14	19	Reference: Non-deficient	
PR = yes (RCB0, I)	15 (52%)	2 (9.5%)	10.18 (2.00, 51.89)	0.0011
pCR = no (RCBI, II, III)	21	21	Reference: Non-deficient	
pCR = yes (RCB0)	8 (28%)	0 (0%)	17.00 (1.91, 2249)	0.0066

3.2 BRCA1, BLM, and FANCI expression and response to platinum chemotherapy

In both neoadjuvant cisplatin trials (see above), BRCA1 transcript levels measured by qPCR were associated with cisplatin response (P = 0.007). In a combined analysis of data from both trials, lower BRCA1 transcript levels are associated with methylation of the BRCA1 promoter. BRCA1 mRNA levels are inversely associated with N_{tAI} in the two cisplatin trials (r_s = -0.50, P = 0.0053). This finding suggests that dysfunction of a BRCA1-dependent process or other abnormality causing low BRCA1 mRNA may be responsible for the high level of telomeric AI and also cisplatin sensitivity in many of these TN breast cancers.

Several specific sites of DNA copy number change were identified in each cisplatin trial, but only a short 15 megabase (MB) region on chromosome 15q26 was significantly higher in responders compared to non-responders in both trials. In addition gene expression array analysis identified only 3 genes that were consistently significantly different (higher in responders) in three platinum-based breast or ovarian cancer trials: MCM2, BLM, and FANCI. Interestingly, FANCI and BLM are both located on 15q26.1 and the proteins have been shown to localize to sites of DNA damage.

To investigate if higher expression of BLM and FANCI were specifically associated with platinum chemotherapy response, we analyzed the gene expression was analyzed in the single agent paclitaxel arm of an ovarian trial[23], and across the TN breast cancer subset of three neoadjuvant cohorts that received taxane-containing combination chemotherapy[24-26]. In the ovarian paclitaxel trial and in the TN subset of the taxane-containing multidrug trials there was no association between either BLM or FANCI and therapy response. These data suggests that high expression of BLM and FANCI are possibly specifically associated with sensitivity to DNA damaging agents like the platinum salts, but not with response to chemotherapeutics that alter microtubules such as taxanes. The mRNA results were combined into a predictive gene signature: (BRCA1 mRNA) / (average of FANCI and BLM mRNA). ROC analysis

demonstrates that this 3-gene mRNA signature is significantly associated with cisplatin response in TN cisplatin trials.[26]

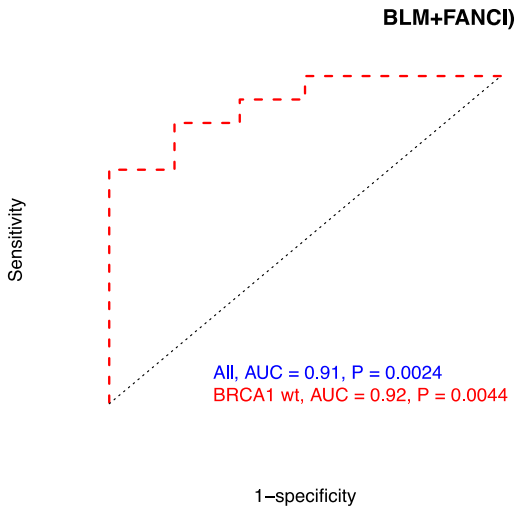


Figure 2: ROC analysis of 3-gene signature to determine cisplatin sensitivity in cisplatin trial. Blue: all cases; red; BRCA normal cases only

3.3 Number of somatic mutations (Nmut) and mutational process D and BRCAness

Whole genome sequencing studies of breast cancer have demonstrated that breast cancers have evidence of at least 5 different mutational processes that may operate to mutate the genome and give rise to cancer. [27] One of these mutational processes, process D, generates a high proportion of the somatic mutations observed in breast cancers arising in women with BRCA1 or BRCA2 mutation. This suggests that proportion of mutational process D may represent a readout of BRCAness in tumor somatic mutation spectra and may associate with greater sensitivity to platinum based chemotherapy. Methods have recently been reported that allow for deciphering of mutational processes extracted from whole exome sequence data.[28]

Through analysis of public TCGA somatic mutation data, the simple number of somatic sequence mutations (Nmut), including synonymous and non-synonymous changes, have been found to be associated with disease outcomes in serous ovarian cancers in both BRCA1 and 2-associated and BRCA-normal disease (Figure 3).

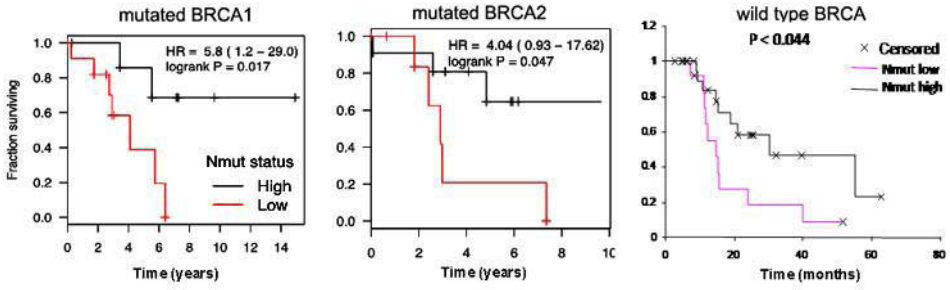


Figure 3: Kaplan Meier analysis of serous ovarian cancers according to the Nmut status high vs low defined by median number across all cases in the TCGA ovarian cancer cohort.

This finding suggests that the burden of somatic exome mutations may be associated with outcome after platinum-based chemotherapy independent of BRCA mutation status.

3.4 Gene expression signatures associated with taxane response

A number of different gene expression signatures have been reported for association with response to taxane-containing chemotherapy.[29,30]

In a study by Swanton C. et al, an RNA interference drug resistance screen in cell lines, including a triple negative breast cancer cell line, identified two distinct gene sets regulating sensitivity to paclitaxel.[31] The first set of genes is involved in mitotic spindle assembly checkpoint and the second set is involved in metabolism of the pro-apoptotic lipid, ceramide. A metagene combining these gene sets was associated with pCR in paclitaxel-treated cohorts (AUC 0.79 and 0.72) but not in non-paclitaxel treated cohorts (AUC 0.53, 0.59, 0.53, 0.64) of triple negative breast cancer.[29]

Of particular note is the genomic classifier that was published by Hatzis et al that was developed specifically for TNBC and included signatures trained on excellent pathologic response and on chemo-resistance following neoadjuvant taxane-anthracycline breast cancer.[30] The classifier was then tested on an independent validation cohort that had pathologic response rates of pCR 35% and pCR/RCB-I 50%, and median follow up of 3 years. Patients with TNBC cancer (N=74) who were predicted to be treatment-sensitive had a positive predictive value (PPV) for pathologic response (pCR/RCB-I) of 83% (95%CI: 36-100), as well as significantly improved 3-year DRFS (NPV) of 83% (95% CI 68 to 100) with absolute risk reduction (ARR) of 26% (95%CI: 4 to 48). Conversely, the PPV for 3-year relapse was 43% (95% CI: 28 to 55) if predicted treatment-insensitive. Finally, this test had a significant diagnostic likelihood ratio for predicted occurrence versus absence of 3-year distant relapse or death, if predicted to be treatment-sensitive, of 0.27 (95%CI: 0.01 to 0.94) for ER+/HER2-, and 0.35 (95%CI: 0.04 to 0.91) for TNBC. This genomic classifier is performed at MDACC under CLIA compliance using the Affymetrix gene expression platform, and is not currently available from RNA sequencing data.

In the same study by Hatzis et al, there was no predictive utility for pathologic response or 3-year DRFS using the PAM50 subtypes defined as basal versus non-basal. The claudin-low subtype was not evaluated in that analysis. The molecularly defined subtypes have also been evaluated using the MDACC gene expression data and were presented at ASCO 2013.[32] The authors noted that pCR rates were lower for the basal-like 2 and luminal AR-like subtypes (0 of 8 patients and 2 of 20 patients, respectively). The rate of pCR/RCB-I appeared lower in basal-like 2 subtype (1 of 8 patients), but was similar across all other subtypes. There was no relationship between subtype and DRFS in this analysis of 130 patients with TNBC. [30]

3.5 Tumor-associated lymphocytes as predictor of chemotherapy response

Several studies have shown the association between a lymphocytic infiltrates in breast cancer tissue and improved outcomes, including within TNBC subgroup.[33-35] A more recent study from the German Breast Group investigated the role of tumor infiltrating lymphocytes in predicting

response to neoadjuvant anthracycline and taxane chemotherapy.[1] They found that the percentage of intratumoral and stromal lymphocytes in pre-treatment tumor biopsies was a significant independent parameter for pathologic complete response in both a training (N=218) and validation (n=840) cohorts (P = 0.012; P=0.001 respectively). Thus this may be an important histological measure to predict which patients may benefit from neoadjuvant chemotherapy. It is unclear if the relationship between tumor-infiltrating lymphocytes and pathologic response is specific to a particular class of chemotherapy such as the taxanes, or may predict response to any type of chemotherapy.

3.6 Neoadjuvant therapy

For women with locally advanced TNBC who are not considered operable at presentation or who are not candidates for breast conservation at diagnosis, neoadjuvant chemotherapy is frequently considered. While there is no survival advantage associated with use of neoadjuvant compared to adjuvant chemotherapy,[36] neoadjuvant chemotherapy offers clinical downstaging to allow breast preservation, assessment of response to chemotherapy, and an ideal platform to evaluate tumor- or patient-specific biomarkers and better understand tumor biology and response to treatment.[37] Neoadjuvant therapy for TNBC may contribute long-term prognostic information as pathologic complete response (pCR) may be associated with improvement in disease-free survival for this subtype.[38,39]

As with most breast cancer, anthracycline and taxane-based chemotherapy regimens are commonly used in the treatment of Stage II-III TNBC. Given the survival equivalence of neoadjuvant versus adjuvant therapy for breast cancer, anthracyline and taxane therapy may be given together before surgery, together after surgery, or “sandwiched” around surgery, for example anthracycline or taxane before surgery, and the remainder of the treatment after surgery. Prior studies evaluating neoadjuvant antracycline-taxane combinations have reported pCR rates of 30-37.9% among patients with TNBC.[18,38,40] In the recently presented GeparSixto trial, combinations of liposomal doxorubicin and paclitaxel resulted in a pCR rate of 37.9% and with the addition of Carboplatin in a pCR rate of 58.7%.[18]

Taxanes, particularly paclitaxel, have shown activity in the treatment of TNBC. In a SOLTI preoperative study that randomizing patients to receive paclitaxel alone or in combination with iniparib, either on a once weekly or twice weekly schedule, 16% of patients in the paclitaxel alone arm had a pCR in the breast.[41]

Platinum-based chemotherapy agents have also demonstrated activity in the treatment of locally advanced TNBC.[9]As described above, small neoadjuvant trials evaluating platinum-based neoadjuvant chemotherapy for TNBC have resulted in promising pCR rates.[16,17,38] Ryan at al. examined the activity of Cisplatin plus bevacizumab in 51 patients with TNBC showing a 37% rate of Miller Payne 4/5 pathologic response.[17] Silver et al. examined the activity of Cisplatin in 28 patients with TNBC showing a 50% rate of Miller Payne 3/4/5 pathologic response and 22% rate of pCR. Only two patients had a BRCA germline mutation, and both patients had a pCR.[16]

Therefore, in studies focused on TNBC patients evaluating combination regimens, reported pCR rates range between 20%-58.7%. Anthracyclines and taxane combination therapy resulted in

pCR rates of 30-37.9% in this patient population. These data are consistent with pCR rates of 15-20% for single agent taxane, and suggest response rates including both complete response and near complete response (RCB 0/1) may be somewhat higher in a TNBC population.

3.7 Rationale

Chemotherapy remains the backbone of systemic treatment for TNBC. There is a lack of predictive markers of chemotherapy benefit in TNBC.[9] Stage II-III TNBC is commonly treated with anthracyline and taxane-based therapy, which can be given pre- or post-operatively. Previous data suggest that platinum-based therapies may have significant activity in TNBC, and it is conceivable that TNBC with homologous recombination deficiency may be particularly sensitive to these regimens. [5,16,17,20,38] This study was designed under the hypothesis that a biomarker that detects homologous recombination deficiency regardless of etiology or mechanism, the HRD assay, may predict pathologic response to cisplatin. Taxane chemotherapy, while a backbone of treatment for all subtypes of breast cancer, does not utilize homologous recombination as a mechanism of cytotoxicity, and the HRD assay may negatively predict benefit from taxane exposure. In this biomarker study, each arm evaluates a single agent in order to isolate the predictive value of the HRD assay for each agent. Acknowledging that anthracyclines are often used as therapy in breast cancer, the protocol allows the use of additional chemotherapy at the discretion of the treating physician, and patients will be eligible to receive anthracycline and alkylating agents post-protocol sequentially (as well as taxanes if not already received). Although the study structure is designed for research purposes and may not align with more commonly used non-research preoperative regimens, the research strategy should not compromise long term outcomes in these patients, as all patients are expected to receive both anthracycline and taxane-based therapy and multiple studies suggest that therapy administered in both the neoadjuvant and adjuvant settings lead to similar survival outcomes.[36,42,43]

This study aims to evaluate the performance of the HRD assay score to predict sensitivity to cisplatin or paclitaxel in TNBC. Additionally, further correlative analyses will evaluate other novel biomarkers of response to specific chemotherapy in TNBC.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study

4.1.1 Pathologic documentation of invasive breast cancer by biopsy (FNA alone is not adequate).

4.1.2 AJCC clinical stage I with T1 > 1.5 cm, stage II or III invasive breast cancer.

Participants with multicentric or bilateral disease are eligible if at least one lesion meets stage eligibility criteria for the study and no tumor is HER2-positive. In this circumstance, the investigator must determine which will represent the target lesion to be assessed for response. This should remain consistent throughout the study. The target lesion should be selected on the basis of its size (lesion with the longest diameter) and suitability for accurate repetitive measurements.

4.1.3 Tumors must be HER2 negative defined as HER2 0 or 1+ by immunohistochemistry (IHC) assays and /or lack of gene amplification by FISH defined as a ratio < 2 on invasive tumor by local review.

4.1.4 ER and PgR status by IHC must be known. Tumor must be ER and PR negative (≤5% staining) by local review.

4.1.5 Known BRCA1/2 status is not required for study entry. However patients known to have a germline deleterious BRCA1/2 mutation should be encouraged to consider a preoperative trial specifically designed for BRCA1/2 carriers, if available.

4.1.6 Breast imaging should include imaging of the ipsilateral axilla. For subjects with a clinically positive axilla by physical examination or clearly positive by imaging, axilary tissue acquisition is not required. For patients with a clinically negative axilla by examination and imaging, tissue acquisition is not required. For equivocal imaging findings, tissue acquisition (a needle aspiration, core biopsy) is required. SLN biopsy before neoadjuvant therapy is not allowed.

	Lymph Node Status by Physical Exam	
Lymph node Status by Imaging	Positive	Negative
Positive	LN Sampling **not** required but can be performed per physician discretion	LN Sampling **required**
Negative	LN Sampling **required**	LN Sampling **not** required

Participants with axillary adenopathy only are not eligible for this study.

4.1.7 Patients with a prior history of contra-lateral breast cancer are eligible if they have no evidence of recurrence of their initial primary breast cancer within the last 5 years

4.1.8 Women ≥ 18 years of age.

4.1.9 ECOG performance status ≤1 (see Appendix A).

4.1.10 Laboratory Evaluation

- Absolute neutrophil count (ANC) > 1,500 / mm3
- Platelet count > 100,000/ mm3
- Bilirubin < 1.5x upper limit of normal (ULN), for patients with Gilbert syndrome, direct bilirubin will be measured instead of total bilirubin
- ALT, AST ≤ 3.0 x ULN, ALK Phos < 2.5 x ULN
- Creatinine < 1.5 mg/dl or creatinine clearance > 60 cc/min
- Hemoglobin > 9 mg/dl

4.1.11 Use of an effective means of contraception is required in subjects of childbearing potential since study agents are known to be teratogenic. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Women of child-bearing potential must agree to use adequate contraception (barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.

4.1.12 Ability to understand and the willingness to sign a written informed consent document

4.1.13 Individuals with a history of other malignancies are eligible if they have been disease-free for at least 5 years and are deemed by the investigator to be at low risk for recurrence of that malignancy and did not receive prior chemotherapy. Individuals with the following cancers are eligible if diagnosed and treated within the past 5 years: cervical cancer *in situ*, and basal cell or squamous cell carcinoma of the skin.

4.1.14 Patient must be willing to undergo mandatory research biopsy and blood draw. Prior to biopsy procedures patients must be able to be off medications that could increase the risk of bleeding.

4.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

4.2.1 Prior chemotherapy: Prior non-taxane or platinum containing chemotherapy will be allowed if the prior exposure was at least 5 years ago and the exposure is thought not to potentially interact with the primary outcome of the trial or put the patient at undue risk, and should be reviewed with study PI on a case by case basis.

4.2.2 Any prior treatment for the current breast cancer, including chemotherapy, hormonal therapy, radiation or experimental therapy.

4.2.3 Ipsilateral breast recurrence, unless prior treatment consisted of excision alone for DCIS or breast conserving treatment (including radiation therapy) and hormonal therapy for DCIS or invasive breast cancer.

4.2.4 Ongoing use of any other investigational or study agents.

4.2.5 Peripheral neuropathy of any etiology > grade 1 (NCI CTCAE Version 4.0- Appendix B)

4.2.6 Significant hearing loss that would prevent cisplatin administration.

4.2.7 Renal dysfunction for which exposure to cisplatin would be unsafe or require cisplatin dose modification .

4.2.8 History of allergic reactions attributed to compounds of similar chemical or biologic composition to study drugs (e.g. cisplatin, paclitaxel).

4.2.9 Uncontrolled intercurrent illness including, but not limited to ongoing or active systemic infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, steroid dependent asthma, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.10 Any condition that would prohibit administration of corticosteroids

4.2.11 Currently pregnant or breast-feeding. All females must have a negative serum or urine pregnancy test at the Baseline visit (within 7 days of the first dose of study treatment). Females of childbearing potential must agree to use a medically acceptable method of contraception (e.g., abstinence, an intrauterine device, a double-barrier method such as condom + spermicidal or condom + diaphragm with spermicidal, or have a vasectomized partner with confirmed azoospermia) throughout the entire study period and for 30 days

after discontinuation of study treatment. The only subjects who will be exempt from this requirement are postmenopausal women (defined as women who have been amenorrheic for at least 12 consecutive months, in the appropriate age group, without other known or suspected primary cause) or subjects who have been sterilized surgically or who are otherwise proven sterile (i.e., bilateral tubal ligation with surgery at least 1 month before start of study treatment, hysterectomy, or bilateral oophorectomy with surgery at least 1 month before start of study treatment).

4.2.12 Uncontrolled diabetes (If random blood sugar > 200 mg/dL, perform fasting blood sugar to ensure < 200 mg/dL.)

4.2.13 Known HIV-positive individuals on combination antiretroviral therapy are ineligible because these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy.

4.3 Inclusion of Underrepresented Populations

Individuals of all races and ethnic groups are eligible for this trial. There is no bias towards age or race in the clinical trial outlined. This trial is open to the accrual of women only. Every effort will be made to include patients from minority populations.

5. REGISTRATION PROCEDURES

5.1 General Guidelines

Institutions will register eligible participants with the Dana-Farber /Harvard Cancer Center (DF/HCC) Office of Data Quality (ODQ) central registration system. Registration must occur prior to the initiation of therapy. Participants will be randomized to either preoperative cisplatin or paclitaxel in a ratio of 1:1 using block randomization by ODQ. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Overall Principal investigator (PI). If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the ODQ Registrar of participant status changes as soon as possible.

5.2 Registration Process for DF/HCC and DF/PCC Institutions

The ODQ registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical record and/or research chart. To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol and reflected on the eligibility checklist.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the ODQ at 617-632-2295.

4. The ODQ Registrar will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant when applicable.

5. An email confirmation of the registration and/or randomization will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration and/or randomization.

5.3 Guidelines for TBCRC Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute by the Project Manager.

Following registration, participants must begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study may be canceled. The Project Manager should be notified of cancellations as soon as possible.

5.4 Registration Process for TBCRC Sites

To register a participant, the following documents should be completed by the research nurse or data manager and emailed to CTOPM@dfci.harvard.edu or faxed to 617-632-5152:

- Clinic visit note documenting history and physical exam
- Copy of required laboratory tests including: Hematology (CBC with differential), serum chemistries (creatinine and/or creatinine clearance, bilirubin, ALT, and AST, Alkaline phosphatase,)
- Pathology report and documentation of ER/PR status and HER2 status.
- Mammogram, MRI, or Ultrasound report
- Signed participant consent form
- HIPAA authorization form (if separate from the informed consent document)

- Completed Eligibility checklist

To complete the registration process, the Project Manager will

- Register the participant on the protocol with the ODQ
- E-mail the confirmation of registration with the participant study number to the participating site
- Call the research nurse or data manager at the participating site and verbally confirm registration if needed

NOTE: Registration and randomization can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Standard Time Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Project Manager.

6. TREATMENT PLAN

6.1 Pre-treatment Criteria

6.1.1 Screening

Laboratory tests required for eligibility must be completed within 28 days prior to first dose of study treatment. Baseline tumor measurements must be documented from tests within 28 days (+/- 7 days) of study entry. Other non-laboratory tests must be performed within 28 days of study entry.

6.1.2 Clinical tumor evaluation (pre and post chemotherapy)

All patients will be required to have breast imaging (for example, ultrasound (US) or MRI) for accurate tumor measurement prior to initiation of study drugs and within 4 weeks after completion of protocol therapy. Prior to neoadjuvant chemotherapy,for subjects with a clinically positive axilla by physical examination or clearly positive by imaging, axilary tissue acquisition is not required. For patients with a clinically negative axilla by examination and imaging, tissue sampling is not required. For equivocal imaging findings, tissue acquisition (a needle aspiration, core biopsy) is required. SLN biopsy before neoadjuvant therapy is not allowed.

6.1.3 Pre- clinical diagnostic tissue

The following specimens from the pre-study clinical diagnostic core biopsy must be submitted:

- A representative H&E stained slide from each block
- ER, PR, and HER2 immunostained slides of the pre-study diagnostic core biopsy
- FFPE tumor block

If institutional policy prohibits the release of tumor blocks the following may be submitted (in addition to the H&E slides and ER, PR, HER2 immunostained slides):

- Minimum of 5 7-micron unstained tissue slides (charged slides are acceptable) from the clinical diagnostic pre-study core biopsy (for confirmation of pathologic response);
- One 4 um section on charged slide for H&E staining (for HRD analysis)
- 3-5 x 10um unstained sections on uncharged slides for DNA extraction (for HRD analysis). The tissue sections must be cut sequentially and in the same orientation. There should only be 1 section per slide.

Complete 13-383 SPECIMEN REQUISITION (Diagnostic and Surgery Blocks/Slides) form found in Appendix D and include in the shipment. These clinical tumor blocks and slides should be shipped at ambient temperature to:

Dana-Farber Cancer Institute
Attn: Eileen Wrabel
450 Brookline Ave., Dana 157
Boston, MA 02215

Please email Eileen Wrabel at ewrabel@partners.org with the sample information and tracking information the day before shipping specimens

The original H&E and immunostained slides will be returned to the originating pathology department after central pathology review has been completed. The pre-treatment core biopsy blocks will be returned to the originating pathology department after HRD assay is completed.

6.1.4 Pre-chemotherapy Research Core Biopsy

Research breast core biopsies of the target lesion will be obtained from all participants' prior to initiating protocol chemotherapy. These research biopsies are mandatory. It is strongly recommended that core biopsies be image-guided. If a clip was not placed at the time of diagnostic biopsy one should be placed at the time of the pre-chemotherapy research biopsy.

An acceptable research core biopsy is defined as a biopsy performed with a needle that is at least 14 gauge, preferably 11 gauge, with a minimum core length of 6 mm. If any of the required cores are less than 6 mm, additional core biopsies should be obtained. This information should be communicated to the operator performing the biopsy.

Ideally six (6) core biopsies will be obtained (in this order):

- Four cores with minimum length of 6 mm each should be frozen in Optimal Cutting Temperature (OCT); medium each core should be frozen in a separate cassette. The individual preparing the frozen cores should read Appendix C for optimal freezing instructions.

- Two core biopsies with minimum length of 6 mm each should be placed in a 10% neutral buffered formalin tube supplied by the study.

See Appendix C for Tissue labeling and documentation.

Complete the 13-383 SPECIMEN REQUISITION (Research Biopsy and Blood) form found in Appendix E and include in the shipment.

All biopsy samples should arrive during the week by Friday morning. Frozen specimens should be stored in a -80^{o} freezer until shipment; specimens in formalin should be stored at room temperature until shipment. The frozen cores should be shipped on dry ice. Cores in formalin should be shipped at ambient temperature. It is recommended that all research biopsies and research blood samples (see below) be shipped together in the mailing kit provided by the study. If so, the bottom of the shipping container should have dry ice for the frozen specimens; no dry ice should be put in the top section for the specimens in formalin.

All research biopsy cores should be shipped overnight in the kit provided to:

Dana Farber Cancer Institute
DF/HCC Core Blood and Tissue Bank
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

All samples will be de-identified and assigned a unique sample ID number on arrival.

Please email the DF/HCC Core Blood and Tissue Bank and Eileen Wrabel with the sample information and tracking information the day before shipping specimens.
DFCIBreastBank@partners.org
ewrabel@partners.org

All samples will be de-identified by assigning a unique sample ID number. Histopathologic review will be performed at DFCI on hematoxylin and eosin-stained frozen or FFPE tissue sections to document adequate tumor content in the specimens that we actually process.

Coded laboratory specimens will be stored in the Breast Tissue Repository of the DF/HCC Core Blood and Tissue Bank at DFCI. These specimens will become the property of DFCI. During the informed consent process participants will be asked if they will allow their specimens to be used for future research by DF/HCC and TBCRC investigators. See Section 10 for the TBCRC policy on specimen banking and future research. Shared specimens will be identified with a sample ID number; all patient identifying material will be removed.

The diagnostic core biopsy FFPE block or unstained slides and two FFPE research cores will be forwarded from DFCI to Myriad Genetic Laboratories for further processing and HRD assay analysis.

One frozen core will be held in reserve at in the DF/HCC Core Blood and Tissue Bank at DFCI until tissue adequacy for the HRD assay is confirmed by Myriad. If necessary, the reserve frozen core will be fixed and processed into an additional FFPE block and forwarded to Myriad Genetic Laboratories. Any remaining FFPE core tissue left over after the completion of the HRD assay will be returned to DFCI for tissue banking for future research.

6.1.5 Research Blood Sample Collection

The following research blood samples will be collected:

- Two 10 mL lavender top (EDTA Fisher #366643) tubes of whole blood at baseline

The samples will be banked in the DFCI Breast tissue repository of the DF/HCC Core Blood and Tissue Bank at DFCI in order to extract germline DNA to be used as normal DNA reference for tumor tissue-based studies and for future research purposes. These specimens will become the property of the DF/HCC. During the informed consent process participants will be asked if they will allow their specimens to be used for future research by DF/HCC and TBCRC investigators. See Section 10 for the TBCRC policy on specimen banking and future research. Shared specimens will be identified with a sample ID number; all patient identifying material will be removed.

This sample may be collected at any point during the research study; however it is recommended that the sample be collected on the same day as the research biopsy so that both the research tissue biopsy and research blood sample can be shipped at the same time in the container provided by the study.

Complete the 13-383 SPECIMEN REQUISITION (Research Biopsy and Blood) form found in Appendix E and include in the shipment.

The research blood samples, preferably with the research core biopsies, should be shipped on cold packs (or in the thermos provided with the core biopsy kit) overnight to:

Dana Farber Cancer Institute
DF/HCC Core Blood and Tissue Bank
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

Do not freeze whole blood tubes containing EDTA as this will destroy the samples. Whole blood can be shipped on cold packs.

All samples will be de-identified and assigned a linked sample ID number on arrival; all participant identification will be removed.

6.1.6 Cell-free DNA

The following research blood samples will be collected for all patients to assess cell-free circulating DNA.

- Two 10 mL Streck tubes will be collected at baseline, C2D1, and 12 weeks or end of protocol treatment (6 tubes total).

The 12-week Streck Tube may be collected at the pre-surgery or post treatment biopsy visit but must be collected prior to initiation of crossover or additional therapy. If patient discontinues protocol therapy early collect at the end of protocol treatment instead of 12 weeks.

The samples will be banked in the DFCI Breast tissue repository of the DF/HCC Core Blood and Tissue Bank in order to extract cell-free DNA to be used as normal DNA reference for tumor tissue-based studies and for future research purposes. These specimens will become the property of the DF/HCC. During the informed consent process participants will be asked if they will allow their specimens to be used for future research by DF/HCC and TBCRC investigators. See Section 10 for the TBCRC policy on specimen banking and future research. Shared specimens will be identified with a sample ID number; all patient identifying material will be removed.

Complete the 13-383 SPECIMEN REQUISITION (Research Biopsy and Blood) form found in Appendix E and include in the shipment.

The baseline sample can be included in the ambient temperature section of the shipping container. These research blood samples should be shipped within 24 hours of collection at ambient temperature overnight to:

Dana Farber Cancer Institute
DF/HCC Core Blood and Tissue Bank
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

DO NOT FREEZE OR REFRIGERATE STRECK TUBES. THIS WILL DESTROY THE SAMPLES.

All samples will be de-identified and assigned a linked sample ID number on arrival; all participant identification will be removed.

6.2 Agent Administration

This study includes preoperative therapy with either **cisplatin (ARM A) or paclitaxel (ARM B),** followed by surgery (or alternative chemotherapy if significant residual disease).

ARM A
Cisplatin: 75 mg/m^2 IV q 3 weeks x 4 cycles

OR

ARM B
Paclitaxel: 80mg/ m^2 IV q 1 week x 12 weeks (4 cycles)

One cycle will be 3 weeks. Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for cisplatin and paclitaxel are described in Section 7 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Preparation and administration of cisplatin and paclitaxel should follow institutional guidelines and may slightly deviate from the suggested preparation guidelines in the remainder of this section to accommodate institutional practice.

Table 2: Treatment Summary

Agent	Pre-medications; Precautions	Dose	Route	Schedule	Supportive Therapy
Arm A: Cisplatin	Pretreatment hydration with at least 1 liter of sodium chloride 0.9% IV over 1-2 hours prior to cisplatin	75 mg/m^2	IV over approximately 60 minutes	q 3 weeks x 4 cycles	• Subject should receive 1 liter of sodium chloride 0.9% IV over 1-2 hours after cisplatin administration • Anti-emetics* • Mg and K supplementation* • Growth factor support*
Arm B: Paclitaxel	Hypersensitivity reaction prophylaxis	80mg/ m^2	IV over approximately 60 minutes	q 1 week x 12 weeks (4 cycles)	Growth factor support*

*Per physician discretion

6.3 Pre-treatment Criteria

On Day 1 of every cycle, vital signs, physical examination and laboratory tests will be performed per Section 11 Table 4. Pre-treatment criteria on Day 1 of every cycle will include:

- Absolute neutrophil count $\geq$ 1,000/mcL
- Platelets $\geq$ 100,000/mcL
- Creatinine $\leq$ 1.5 mg/dl or creatinine clearance $\geq$ 50 cc/min.
- Total bilirubin, $\leq$ 1.5X institutional ULN. For patients with Gilbert syndrome, direct bilirubin will be measured instead of total bilirubin.
- AST(SGOT)/ALT(SGPT) $\leq$ 3.0 X institutional ULN
- All non-hematologic toxicity $\leq$ grade 1

For patients receiving weekly paclitaxel, a complete blood count with differential should be performed prior to treatment administration. Criteria for treatment with weekly paclitaxel are outlinted in Section 7.2. Chemotherapy may be delayed up to 3 weeks. If chemotherapy cannot be delivered after a 3 week delay, because of toxicity, the participant must be removed from the study.

Note: Minor schedule changes owing to observed holidays, inclement weather, and so forth are permitted (+/- 3 days).

6.4 Treatment Regimens

6.4.1 ARM A: Cisplatin

Cisplatin will be given by IV over approximately 60 minutes at 75 mg/m^2 every 3 weeks, 4 cycles. Dosing calculation guidelines will follow each institution policies. Doses should be based on actual body weight. The participant should be weighed each cycle.

Hydration: Pretreatment hydration with at least 1 liter of sodium chloride 0.9% IV over 1-2 hours prior to cisplatin administration should be performed. Subject should receive 1 liter of sodium chloride 0.9% IV over 1-2 hours after cisplatin administration. It is important for the participant to remain well hydrated after cisplatin administration, especially for the next 24 hours. Oral hydration should be encouraged and nausea/vomiting must be well controlled.

Anti-emetics: Subjects should receive prophylactic anti-nausea medications according to ASCO guidelines for highly emetogeic chemotherapy regimens.

Supplementation: Magnesium supplementation: At the physician's discretion, participant may take 500mg Magnesium Gluconate QD for 7 days (or equivalent). Magnesium level may be checked between days 7-10 and if < 1.8mg/dl, then magnesium supplementation may be continued and adjusted at the physician's discretion.

Potassium supplementation*:* Patient may be given Potassium 30-40 mEq/day po for 7 days after each dose. Potassium level may be checked between days 7-10. If the measurement is < 3.5mmol/L, then potassium supplementation may be continued and adjusted at the physician's discretion.

Growth factor support: Use of growth factors is left to the treating physicians' discretion, according to ASCO guidelines

6.4.2 ARM B: Paclitaxel

Paclitaxel will be given as an IV infusion over approximately 60 minutes at a dose of 80mg/m^2 weekly x 12 weeks (4 cycles). Dosing calculation guidelines will follow each institution policies. Doses should be based on actual body weight. The participant should be weighed each cycle.

Hypersensitivity reactions prophylaxis: All patients should be pre-medicated prior paclitaxel administration in order to prevent severe hypersensitivity reactions. Such premedication may consist of 10 mg (orally) dexamethasone administered approximately 30 to 60 minutes before paclitaxel, 50 mg (IV) diphenhydramine (or its equivalent) administered 30 to 60 minutes prior to paclitaxel, and 300 mg cimetidine (IV) or 50 mg ranitidine (IV) administered 30 to 60 minutes before paclitaxel. If different institutional guidelines exist for administration or premedication for weekly paclitaxel, then the investigator should use their standard practice.

Growth factor support: Use of growth factors is left to the treating physicians' discretion, according to ASCO guidelines

6.5 Second core biopsy sample

For patients who are determined to have evidence of clinically significant residual disease by physical exam or imaging after completion of protocol treatment and who wish to cross over or continue with additional chemotherapy, an image-guided second biopsy is mandatory and required for tissue collection. This biopsy must be performed prior to the initiation of crossover or additional therapy. There is no size requirement for residual disease, and this determination is made by the treating provider. For patients who demonstrate progression after at least 6 weeks but no more than 12 weeks of preoperative chemotherapy, a tumor biopsy is strongly encouraged.

It is recommended that the post-therapy core biopsy be performed with image guidance. The following specimens must be submitted:

- Two core biopsies with minimum length of 6 mm each placed in a 10% neutral buffered formalin tube supplied by the study.

Complete 13-383 SPECIMEN REQUISITION (Research Biopsy and Blood) form found in Appendix E and include in the shipment. A collection and shipment kit will be provided by the study.

See section 6.1.4 and Appendix C for tissue labeling and documentation.

The research biopsy cores should be shipped at ambient temperature overnight to:

Dana Farber Cancer Institute
DF/HCC Core Blood and Tissue Bank
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

All samples will be de-identified and assigned a unique sample ID number on arrival.

Please email the DF/HCC Core Blood and Tissue Bank and Eileen Wrabel with the sample information and tracking information the day before shipping specimens.
DFCIBreastBank@partners.org
ewrabel@partners.org

6.6 Crossover or alternative pre-operative chemotherapy

If there is clinical or radiographic evidence of significant residual disease after completion of protocol treatment, after biopsy the patient may crossover or receive alternative preoperative chemotherapy. It is strongly encouraged that if crossover prior to surgery is being considered, that the case is discussed with the overall study primary investigator prior to biopsy. The selection of crossover therapy is determined by the treating provider, however it is strongly encouraged for patients who had been on the paclitaxel arm to cross over to receive cisplatin. The second tissue collection procedure marks the end of protocol mandated chemotherapy; however additional data will be collected to document crossover or alternative chemotherapy and response to the additional chemotherapy.

6.7 Definitive Breast Surgery

Definitive breast surgery must be performed no later than 42 days after administration of the last chemotherapy. Participants who did not undergo axillary node evaluation prior to chemotherapy must have ipsilateral axillary nodes evaluated at the time of surgery. It is strongly recommended that complete axillary dissection be performed at the time of surgery if a positive node was detected prior to chemotherapy. If contralateral mastectomy is performed concurrently, the pathology report from the contralateral breast must also be submitted for review.

Tissue must be collected under the supervision of a surgical pathologist. For all patients, including those who go directly to definitive surgery after preoperative chemotherapy or those who cross over before surgery, the following specimens must be submitted:

- A representative FFPE block of residual tumor (or 5 unstained slides containing residual tumor) identified in the surgical specimen for banking and future research studies.
- An H&E slide of every block from the post-treatment surgical breast excision and axillary surgery specimens will be required for central pathology review to determine the primary endpoint of pathologic response.
- A representative block of normal tissue (histologically normal breast, skin or negative lymph node) is requested for banking and future research studies

If a patient has a pCR, then there will be no tumor tissue available, however pathology will be centrally reviewed. Tissue block or slides of the tumor excision should be sent to DFCI within 60 days of surgery.

The H&E slides will be returned to the originating pathology department after central pathology review has been completed. Blocks will be returned upon request for diagnostic purposes.

Complete 13-383 SPECIMEN REQUISITION (Diagnostic and Surgery Blocks/Slides) form found in Appendix D and include in the shipment. These tumor blocks and slides should be shipped at ambient temperature to:

Dana-Farber Cancer Institute
Attn: Eileen Wrabel
450 Brookline Ave., Dana 157
Boston, MA 02215

Please email Eileen Wrabel at ewrabel@partners.org with the sample information and tracking information the day before shipping specimens.

6.8 Post-operative Chemotherapy

The second biopsy marks the end of protocol mandated therapy. Decisions regarding choice of post-biopsy additional chemotherapy will be made by the treating physician. However, it is recommended that participants randomized to cisplatin should receive anthracycline-based chemotherapy with or without a taxane after surgery as long-term data using single-agent cisplatin as neoadjuvant treatment of breast cancer is not available.

6.9 Concomitant Treatment and Supportive Care Guidelines

6.9.1 Prior and Concomitant Therapy

All diagnostic, therapeutic, or surgical procedures relating to malignancy should be recorded in the electronic case report form (eCRF), including the date, indication, description of the procedure(s), and any clinical findings. All previous treatments for breast cancer should be included.

All prior treatment or medication administered during the 30 days preceding the first dose of study treatment and any concomitant therapy administered to the subject throughout the study until 30 days after the final dose of study treatment should be recorded in the medical record. The generic name of the drug (or trade name for combination drugs) should be specified along with the duration of treatment and indication for use. If concomitant medication/therapy is administered for an AE, investigators should record that AE on the AE page of the eCRF.

Any medication that is considered necessary for the subject's welfare and that is not expected to interfere with the evaluation of study treatment may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

Any changes in documented, permitted concomitant treatment already being taken at the start of the clinical study should be recorded in the medical record, noting the type of medication, duration of use, and indication.

6.9.2 Permitted Concomitant Therapies and Medications

The following agents are permitted:

- Antiallergic measures such as corticosteroids and antihistamines
- Antiemetics in accordance with the ASCO guidelines
- Antidiarrheal therapy in accordance with the ASCO guidelines
- Growth factor support will be allowed at the discretion of the physician and should be in accordance with the ASCO guidelines
- Aspirin, nonsteroidal anti-inflammatory drugs, and anticoagulants are permissible but should be used with caution. However, they should be stopped appropriately prior to biopsy procedures due to risk of bleeding.

6.9.3 Prohibited Concomitant Therapies and Drugs

The use of investigational or other antitumor therapies, other than cisplatin and paclitaxel is prohibited during this study. If subjects receive additional antitumor therapies irrespective of the reason for which they are being given, this will be judged to represent evidence of treatment failure and study treatment will be discontinued. These subjects will complete all end-of-treatment assessments.

6.9.4 Supportive Care Guidelines

Prophylaxis of hypersensitivity reactions

Premedication should be used as prespecified previously or according to institutional guidelines.

Subjects should receive optimal supportive care throughout the study, including transfusions of blood and blood products, antibiotics, antiemetics, when appropriate. The reason(s) for treatment, dosage, and dates of treatment should be recorded.

Antiemetic Medications

Subjects should receive prophylactic anti-nausea medications according to ASCO guidelines for highly emetogeic chemotherapy regimens.

Intravenous Fluids
Subjects may receive intravenous fluids on study. Institutional guidelines should be followed for Paclitaxel and cisplatin. Specific guidelines for cisplatin (one possible regimen) are outlined in section 6.2.

Growth Factors
Growth factor support will be allowed at the discretion of the physician and should be in accordance with the ASCO guidelines

These guidelines should never replace sound clinical judgment.

6.10 Duration of Therapy

Patients assigned to ARM A will receive 4 cycles of cisplatin 75 mg/m2 every 3 weeks.

Patients assigned to ARM B will receive 12 weeks (4 cycles) of paclitaxel 80 mg /m2 every week.

Surgery should occur no later than 42 days after the last dose of protocol chemotherapy, or in case of significant clinically residual disease, after last dose of cross-over/alternative chemotherapy.

At the time of patient removal from protocol therapy and at off-study, the Treatment Completion/Off-study form should be completed.

6.11 Duration of Follow-Up

The patients will be followed until definitive breast cancer surgery is completed, or until change to cross-over/alternative neoadjuvant therapy in the case of significant residual disease after completion of neoadjuvant protocol therapy. For patients who change to cross-over/alternative neoadjuvant therapy, data on type of post-protocol treatment and response to therapy at definitive surgery will be collected.

Criteria for Removal from Study
Participants removed from study for unacceptable toxicity will be followed until resolution or stabilization of the toxicity. Participants will be removed from the protocol for any of the following:

- Any grade 4 non-hematologic toxicity
- Progression of tumor by physical exam or imaging study. In this situation confirmatory biopsy is strongly recommended prior to removal from study if not already performed for residual disease.
- Treatment delay of more than 3 weeks for any toxicity-related reason
- More than 2 dose reductions
- Receipt of additional antitumor therapies in addition to protocol therapy
- A toxicity develops which, in the opinion of the investigator, precludes further therapy

- For safety reasons the investigator considers it to be in the best interest of the participant that they be withdrawn
- Participant decides to withdraw consent from the study

The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant. In the event of unusual or life-threatening complications, participating investigators must immediately notify the PI:

Erica L. Mayer, MD, MPH
Dana-Farber Cancer Institute
450 Brookline Avenue
Yawkey 1259
Boston, MA 02215
Ph: 617-632-2335
Fax: 617-632-1930
email: emayer@partners.org

7. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Version 4.0 of the NCI CTCAE which is identified and located on the CTEP website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.). (Section 6.8)

All AEs experienced by participants will be collected from the time of the first dose of study treatment, through the study and until 30 days after the protocol treatment. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

7.1 Anticipated Toxicities

Refer to the FDA approved package insert for detailed information for cisplatin and paclitaxel.

7.1.1 AE list for cisplatin

Nephrotoxicity
The major dose-limiting toxicity of cisplatin is cumulative nephrotoxicity. Tubular necrosis of both proximal and distal renal tubules has been noted in 28% to 36% of subjects treated with a single dose of 50 mg/m2. It is first noted during the second week after a dose and is manifested by elevations in BUN and creatinine, serum uric acid and/or a decrease in creatinine clearance. Renal toxicity becomes more prolonged and severe with repeated courses of the drug. Renal function must return to normal before

another dose of cisplatin can be given. Nephrotoxicity can be reduced by IV hydration as well as avoidance of nephrotoxic drugs such as aminoglycoside antibiotics.

Nausea and Vomiting
Cisplatin causes moderate to severe nausea and vomiting in almost all subjects treated. Nausea and vomiting usually begin within 1 to 4 hours after treatment and last up to 24 hours. Various degrees of vomiting, nausea, and/or anorexia may persist for up to 1 week after the treatment. Delayed nausea and vomiting (beginning 24 hours or more after chemotherapy) has occurred with complete emetic control on the day of cisplatin therapy. The use of prophylactic and continuing antiemetic medication reduces these adverse effects.

Hypomagnesemia
Hypomagnesaemia have been reported in subjects treated with cisplatin and is probably related to renal tubular damage. It may become severe enough to cause tetany. Generally, serum electrolytes return to normal levels when cisplatin is discontinued and supplemental electrolytes are administered.

Ototoxicity
Ototoxicity has been observed in up to 31% of subjects treated with a single dose of cisplatin (50 mg/m2) and is manifested by tinnitus and/or hearing loss in the high frequency range (4000 to 8000 Hz). Decreased ability to hear normal conversational tones may occur occasionally. Ototoxicity can be more severe in children than in adults and more frequent and severe with repeated administration. Hearing loss can be unilateral or bilateral and is usually not reversible. During treatment with cisplatin, it is necessary to monitor hearing at each visit.

Myelosuppression
Myelosuppression occurs in 25% to 30% of subjects treated with cisplatin. The nadirs in circulating platelets and leukocytes occur between days 18 and 23, with most subjects recovering by day 39. Leukopenia and thrombocytopenia are more pronounced at higher doses (>50 mg/m2). Anemia (a decrease in hemoglobin of 2 g/100 ml) occurs at approximately the same frequency and with the same timing as leukopenia and thrombocytopenia.

Neurotoxicity
Neurotoxicity, usually characterized by peripheral neuropathies, has been reported. The neuropathies usually occur after prolonged therapy (4 to 7 months); however, neurologic symptoms have been reported to occur after a single dose. Although symptoms and signs of cisplatin neuropathy usually develop during treatment, symptoms of neuropathy may begin 3 to 8 weeks after the last dose of cisplatin. Cisplatin therapy should be discontinued when the symptoms are first observed. The neuropathy, however, may progress further even after stopping treatment. Preliminary evidence suggests peripheral neuropathy may be irreversible in some patients. Elderly patients may be more susceptible to peripheral neuropathy. Lhermitte's sign, dorsal column myelopathy, and autonomic neuropathy have also been reported. Loss of taste, seizures,

leukoencephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) have also been reported. Muscle cramps, defined as localized, painful, involuntary skeletal muscle contractions of sudden onset and short duration, have been reported and were usually associated in patients receiving a relatively high cumulative dose of cisplatin and with a relatively advanced symptomatic stage of peripheral neuropathy.

Ocular Toxicity

Optic neuritis, papilledema, and cerebral blindness have been reported in patients receiving standard recommended doses of cisplatin. Improvement and/or total recovery usually occurs after discontinuing cisplatin. Steroids with or without mannitol have been used; however, efficacy has not been established. Blurred vision and altered color perception have been reported after the use of regimens with higher doses of cisplatin or greater dose frequencies than recommended in the package insert. The altered color perception manifests as a loss of color discrimination, particularly in the blue-yellow axis. The only finding on funduscopic exam is irregular retinal pigmentation of the macular area.

Anaphylactic-Like Reactions

Anaphylactic-like reactions have been reported in patients previously exposed to cisplatin. The reactions consist of facial edema, wheezing, tachycardia, and hypotension within a few minutes of drug administration. Reactions may be controlled by intravenous epinephrine with corticosteroids and/or antihistamines as indicated. Patients receiving cisplatin should be observed carefully for possible anaphylactic-like reactions and supportive equipment and medication should be available to treat such a complication.

Hepatotoxicity

Transient elevations of liver enzymes, especially SGOT, as well as bilirubin, have been reported to be associated with cisplatin administration at the recommended doses.

Other

Vascular toxicities coincident with the use of cisplatin in combination with other antineoplastic agents have been reported. The events are clinically heterogeneous and may include myocardial infarction, cerebrovascular accident, thrombotic microangiopathy (hemolytic-uremic syndrome [HUS]), or cerebral arteritis. Various mechanisms have been proposed for these vascular complications. There are also reports of Raynaud's phenomenon occurring in patients treated with the combination of bleomycin, vinblastine with or without cisplatin. It has been suggested that hypomagnesemia developing coincident with the use of cisplatin may be an added, although not essential, factor associated with this event. However, it is currently unknown if the cause of Raynaud's phenomenon in these cases is the disease, underlying vascular compromise, bleomycin, vinblastine, hypomagnesemia, or a combination of any of these factors

7.1.2 AE list for paclitaxel

Myelosuppresion

Myelosuppression occurs in the majority of patients (neutropenia, leukopenia, thrombocytopenia, and anemia). Myelosuppression is dose related, schedule related, and infusion-rate dependent and, in general, rapidly reversible upon discontinuation.

Anaphylactic-Like Reactions

Hypersensitivity is thought to be caused by the Cremophor vehicle. Minor symptoms include hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, and tachycardia. More severe reactions include hypotension requiring treatment, dyspnea with bronchospasm, generalized urticaria, and angioedema. The majority (53%) of the reported reactions occurred within 2-3 minutes of initiation of treatment and 78% occurred within the first 10 minutes. Reactions usually occurred with the first and second doses.

Cardiovascular toxicity

Atrial arrhythmia (sinus bradycardia [usually transient and asymptomatic], sinus tachycardia, and premature beats); significant events include syncope, hypotension, other rhythm abnormalities (including ventricular tachycardia, bigeminy, and complete heart block requiring pacemaker placement), and myocardial infarction. Hypertension (possibly related to concomitant medication – Dexamethasone) may also occur.

Neurotoxicity

Sensory (taste changes); peripheral neuropathy; arthralgia and myalgia (dose-related, more common when colony-stimulating factors are also administered); seizures; mood

alterations; neuroencephalopathy; hepatic encephalopathy; motor neuropathy; and autonomic neuropathy (paralytic ileus and symptomatic hypotension).

Dermatologic toxicity
Alopecia (universal, complete and often sudden, between days 14- 21); injection site reactions (erythema, induration, tenderness, skin discoloration); infiltration (phlebitis, cellulitis, ulceration, and necrosis, rare); radiation recall; and rash.

Gastrointestinal toxicity
Nausea, vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis (neutropenic enterocolitis), ischemic colitis, and pancreatitis.
Hepatic: Increased AST, ALT, bilirubin, alkaline phosphatase; hepatic failure, and hepatic necrosis.

Other: Fatigue, headache, light-headedness, myopathy, elevated serum creatinine, elevated serum triglycerides, and visual abnormalities (sensation of flashing lights, blurred vision).

7.2 Toxicity Management/Dose delays/Dose modifications

Dose Delay
Chemotherapy must be delayed if the following criteria are not met on Day 1 of any cycle:

- ANC must be $\geq$ 1,000/mcL
- Platelets must be $\geq$ 100,000/mcL
- All non-hematologic toxicities must be $\leq$ grade 1
- Creatinine $\leq$ 1.5 mg/dl or creatinine clearance $\geq$ 50 cc/min.
- Total bilirubin, $\leq$ 1.5 X institutional ULN. For patients with Gilbert syndrome, direct bilirubin will be measured instead of total bilirubin.
- AST(SGOT)/ALT(SGPT) $\leq$ 3.0 X institutional ULN

All toxicities should be graded according to the Common Terminology Criteria for Adverse Events (Version 4.0) (Appendix B).

All dose reductions are maintained in subsequent cycles and should not be re-escalated. Any participant whose treatment is delayed must be evaluated on a weekly basis until adequate hematologic and non-hematologic parameters have been met. No more than two dose reductions are allowed. If further dose reduction is required, the participant should be removed from the study.

If chemotherapy is delayed > 3 weeks for any toxicity, the participant must be removed from the study

Dose modifications are to be made according to the system showing the greatest degree of toxicity. Dose adjustments for toxicity should be made according to the following table (Table 3) and guidelines:

Table 3: Dose reductions

Dose Level	Cisplatin
Starting dose	75mg/m^2
1st dose reduction	56mg/m^2
2nd dose reduction	37mg/m^2
3rd dose reduction	Discontinue

Dose Level	Paclitaxel
Starting dose	80mg/m^2
1st dose reduction	65mg/m^2
2nd dose reduction	50mg/m^2
3rd dose reduction	Discontinue

Allergic Reaction/Hypersensitivity:
Hypersensitivity reactions including anaphylaxis have been reported within minutes of administering cisplatin. **If a hypersensitivity reaction occurs during cisplatin infusion, the patient must be evaluated by the allergy service prior to any further cisplatin administration**. Subsequent cisplatin administration should be administered through a desensitization protocol under the supervision of the allergy service.

Hematologic Toxicity:
Neutropenia:

Dose modifications will be based on ANC not on total WBC.

Day 1 requirement: ANC must be $\geq$ 1,000/mm3 to administer day 1 chemotherapy for any cycle including weekly doses of paclitaxel. If ANC is $\leq$ 1,000/mm^3, hold chemotherapy and re-check CBC weekly (or more often at physician's discretion) until ANC $\geq$ 1,000.

If ANC < 1000 mm^3, hold cisplatin or paclitaxel until repeat measurement (at treating physician discretion). At that time:

- If ANC $\geq$ 1000 mm^3 upon repeat measure, reduce by one dose level and re-initiate chemotherapy. However, physicians have the option of administering growth factor support in between treatments instead of dose reduction.
- If ANC < 1000 mm^3 upon repeat measure, continue to hold until ANC $\geq$ 1000 mm^3
- If ANC takes > 21 days to recover, patients must discontinue study drug.

If platelets are < 100,000 mm^3, hold paclitaxel or cisplatin until repeat measurement (at treating physician discretion). At that time:

- If platelets are ≥ 100,000 mm^3 upon repeat measure, reduce by one dose level and re-initiate chemotherapy.
- If platelets are < 100,000 mm^3 upon repeat measure, continue to hold until platelets ≥ 100,000 mm^3.
- If platelets do not reach ≥ 100,000 mm^3 in 21 days, discontinue study drug.

Febrile Neutropenia:
Hold study drug until recovery from febrile/ infectious episode. Recovery will be defined as disappearance of fever and normalization of white cell blood count for more than 24 hours without need for anti-pyretics.

Upon recovery, cisplatin or paclitaxel will be started at the next reduced dose level. Antibiotic prophylaxis will be allowed at the investigator's discretion. However, for both febrile and non-febrile neutropenia, physicians have the option of administering cisplatin or paclitaxel at full dose with growth support factors if the treating physician thinks that the patient is benefiting from treatment and the risk/benefit ratio favors administration of full-dose chemotherapy.

For a second episode, the action taken is dependent upon the action taken for the first episode. For patients who have had a prior dose reduction without growth support factor administration, growth support factor should be added without subsequent dose reduction.

For third episode, the study PI should be contacted for discussion.
Treatment with cisplatin or paclitaxel may continue if the treating physician thinks that the patient is benefiting from treatment and the risk: benefit ratio favors continued chemotherapy. If the patient continues on treatment, a reduction by a second dose level is required.

Neurologic toxicity
For grade 2 neuropathy, motor or sensory, lasting > 7 days or any grade 3 neuropathy, the dose of cisplatin or paclitaxel should be held until resolution to ≤ grade 1 and then decreased by one dose level. Patients with grade 4 toxicity or who require more than two dose level reductions must discontinue study drug.

Diarrhea
For ≥ grade 3 diarrhea, chemotherapy should be delayed until ≤ grade 1. Cisplatin or paclitaxel should then be reduced by one level. No dose reduction will be made for grade 1 or 2 diarrhea.

Hepatic (Day 1 of each cycle only)
Grade 2, 3 or 4 elevation in bilirubin or AST/ALT: hold paclitaxel or cisplatin. If grade returns to ≤ 1 within 3 weeks, restart study treatment at a dose reduced by 1 level.
If, after a 3 week delay, toxicity has not returned to ≤ grade 1, discontinue study drug.

Any other Non-Hematologic Toxicity
For any ≥ grade 2 toxicity, hold chemotherapy and provide best supportive care.

7.3 Special Considerations

- For toxicities which are considered by the treating investigator unlikely to develop into serious or life–threatening events (e.g. alopecia, altered taste etc.), treatment may be continued at the same dose without reduction or interruption.
- The treating investigator may reduce a subject's dose for a toxicity of any grade/duration where s/he believes it to be in the best interests of the subject.
- Any consideration to modification of the above dose modification guidelines should be discussed with the Principal Investigator for approval or disapproval in advance.

8. DRUG FORMULATION/STORAGE/SUPPLY

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

As all agents are commercially available, each study site is responsible for prescribing and ordering study drugs for study participants enrolled at their site. Study drugs will the billed to patients as standard of care

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

8.1 Cisplatin

8.1.1 Description

For further information on cisplatin, please see the package insert

Other Names
Platinol ®-AQ

Classification
Organic platinum compound

Mode of Action
Cisplatin binds to DNA, thereby inhibiting DNA synthesis, in a cell cycle nonspecific manner.

Cisplatin injection is a clear, colorless, sterile aqueous solution, each mL containing 1 mg cisplatin and 9 mg Sodium Chloride, USP. HCl and/or Sodium Hydroxide is added to adjust pH of the solution. The active ingredient, cisplatin, is a yellow to orange crystalline powder with the molecular formula PtCl2H6N2, and a molecular weight of 300.1. Cisplatin is a heavy metal complex containing a central atom of platinum surrounded by two chloride atoms and two ammonia molecules in the cis position. It is

soluble in water or saline at 1 mg/mL and in dimethylformamide at 24 mg/mL. It has a melting point of 207°C.

8.1.2 Storage and Stability

Cisplatin is a sterile, multidose vial without preservatives. Store at 15°C-25°C. Do not refrigerate. Protect unopened container from light. The cisplatin remaining in the amber vial following initial entry is stable for 28 days protected from light or for 7 days under fluorescent room light. Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.1-7.There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

8.1.3 Compatibility

Plasma levels of anticonvulsant agents may become subtherapeutic during cisplatin therapy. In a randomized trial in advanced ovarian cancer, response duration was adversely affected when pyridoxine was used in combination with altretamine (hexamethylmelamine) and Cisplatin.

Prescription of concomitant drugs should address the Launch Lexi-Interact™ Drug Interactions Program.

8.1.4 Handling

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

8.1.5 Availability

Cisplatin is commercially available agent. Each institutional pharmacy should assure availability for the study.

8.1.6 Preparation

Preparation of cisplatin should follow each institutional guideline, and may slightly deviate from the suggested preparations guidelines in the remainder of this paragraph to accommodate different institutional guidelines. The active ingredient, cisplatin, is a yellow to orange crystalline powder with the molecular formula PtCl2H6N2, and a molecular weight of 300.1. Cisplatin is a heavy metal complex containing a central atom of platinum surrounded by two chloride atoms and two ammonia molecules in the cisposition. It is soluble in water or saline at 1 mg/mL and in dimethylformamide at 24 mg/mL. It has a melting point of 207°C.

8.1.7 Administration

Dose Specifics

For this protocol, 75 mg/m2 of cisplatin will be administered by IV infusion as an approximately 1- hour infusion every 3 weeks for 4 weeks.

Route of Administration

Administration of cisplatin should follow institutional guidelines, and may slightly deviate from the suggested administration guidelines in the remainder of this paragraph to accommodate different institutional practice. Cisplatin is administered as an IV infusion over approximately 60 minutes.

8.2 Paclitaxel

8.2.1 Description

Other Names
Taxol (NSC 125973)

Classification
Antimicrotubule agent.

Mode of Action
Promotes microtubule assembly and stabilizes tubulin polymers by preventing their depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell functions.

8.2.2 Storage and Stability

The intact vials are stored under refrigeration. Freezing does not adversely affect the product. Solutions diluted to a concentration of 0.3 to 1.2 mg/mL in normal saline, 5% dextrose, 5% dextrose and normal saline, or 5% dextrose in Ringer's solution are stable for up to 27 hours when stored at room temperature and normal room light.

8.2.3 Compatibility

Avoid the use of PVC bags and infusion sets due to leaching of DEHP (plasticizer).
Ketoconazole may inhibit paclitaxel metabolism, based on *in vitro* data.
Prescription of concomitant drugs should address the Launch Lexi-Interact™ Drug Interactions Program.

8.2.4 Handling

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

8.2.5 Availability

Paclitaxel is commercially available agent. Each institutional pharmacy should assure availability for the study.

8.2.6 Preparation

Preparation of paclitaxel should follow each institutional guideline, and may slightly deviate from the suggested preparations guidelines in the remainder of this paragraph to accommodate different institutional guidelines. Paclitaxel may be diluated in 0.9% sodium chloride injection, USP or 5% dextrose injection, USP. Paclitaxel must be prepared in glass, plypropylene or plyolefin containers and non-PVC containing (nitroglycerin) infusion sets. In-line filtration with a 0.22 micron filter is required.

8.2.7 Administration

Dose Specifics

For this protocol, 80 mg/m2 of paclitaxel will be administered by IV infusion as an approximately 1- hour infusion weekly for 12 weeks.

Route of Administration

Administration of paclitaxel should follow institutional guidelines, and may slightly deviate from the suggested administration guidelines in the remainder of this paragraph to accommodate different institutional practice. Paclitaxel is administered as an IV infusion over approximately 60 minutes.

8.3 Ordering

As all agents are commercially available, each study site is responsible for prescribing and ordering study drugs for study participants enrolled at their site. Study drugs will the billed to patients as standard of care.

8.4 Destruction

Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or that are prepared with unpreserved diluents (i.e., sterile water for injection, USP, or 0.9% sodium chloride for injection, USP) within 8 hours of vial entry to minimize the risk of bacterial contamination.

9. CORRELATIVE/SPECIAL STUDIES

Required tissue collection will be performed before and after protocol treatment as specified in Section 6. The following correlative studies are pre-specified:

- Homologous recombination deficiency (HRD) assay
- Hereditary cancer panel (HCP) assay
- Number of subchromosomal regions with allelic imbalance extending to the telomere (N(tAI)) and Long Segment Transition (LST) assays.
- Taxane Predictor Tests
- Whole exome sequencing and copy number findings to determine total number of mutations, proportion of mutational processes, and identify specific mutations or deletions in a panel of DNA repair genes.
- RNA-seq to evaluate predictive gene expression signatures including BRCA1/BLM+FANCI levels, TNBC subsets
- Chromossome 15q26 copy number, Chromossome LOH, loss of Rad17 and PAM 50subtypes
- Intratumoral and stromal lymphocytes

FFPE tissue blocks (or precut sections) will be sent to Myriad for further sample processing and DNA extraction for the HRD, NtAI, and LST assay(s). The HCP assay will be performed on clinical blood samples sent directly to Myriad. Frozen research cores will be histologically assessed for tumor content and nucleic acids (RNA, DNA) will be extracted at the Breast Tissue Repository at DFCI. Aliquots of nucleic acids will be sent to Myriad for whole exome sequencing and RNA-seq analysis. These data will be made available to all co-investigators. Remaining nucleic acids and tissues will be stored at the DFCI breast tissue repository for future or additional exploratory research applications such as gene expression array profiling, RT-PCR analyses, or proteomic analysis.

9.1 Risks of Research Biopsy and Procedures for Minimizing Risk

9.1.1 Potential risks for breast core biopsy are:

- Likely: local discomfort and minor bleeding.
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, pneumothorax, damage to adjacent organs.

Prior to the procedure, the physician performing the procedure will discuss the risks with each study participant, answer any questions, and obtain the standard clinical consent. Patients will be evaluated for comorbidities or concomitant medications that may increase the risk of potential complications.

9.1.2 Risks of Anesthesia

Local Anesthesia
All biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs. In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Patients will be queried if they have had previous allergic reactions to local anesthetics.

Intravenous Conscious Sedation
Certain biopsy procedures may require intravenous conscious sedation (IVCS). It is highly unlikely that is will be needed for a breast core biopsy. IVCS is a minimally depressed level of consciousness that retains the patient's ability to maintain a patent airway independently and continuously and respond appropriately to physical stimulation and verbal commands.

The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small but real; for example, in a prospective study of 14,149 patients undergoing IVCS during upper gastrointestinal endoscopies, the rate of immediate cardiopulmonary events was 2 in 1000.18 The 30-day mortality was 1 per 2,000 cases. In this study, there was a strong association between lack of monitoring and use of high-dose benzodiazepines with adverse outcomes. There was also an association between the use of local anesthetic sprays to the oropharynx and the development of pneumonia. In order to minimize the risk of intravenous conscious sedation, only qualified personnel will be responsible for conscious sedation. A minimum of two individuals will be involved in the care of patients undergoing conscious sedation—the physician performing the biopsy procedure, and the individual (M.D. or R.N.) who monitors the patients and his/her response to both the sedation and the procedure, and who is capable of assisting with any supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway should this be necessary. An emergency cart will also be immediately accessible to the room where the procedure is to take place, and emergency support services will be available on page. Patients will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Patients with active cardiac disease are excluded from this study. No local anesthetic spray to the oropharynx will be necessary, given that endoscopy is not a planned procedure. Following the procedure, patients will be observed closely in the recovery room for a minimum of 2 hours.

General Anesthesia
Because of the higher risk of general anesthesia compared with local anesthesia or intravenous conscious sedation, biopsies that would require general anesthesia in order to be performed are not permitted on this protocol, unless they are being done for clinical reasons, and excess tissue that otherwise would have been discarded is then banked for the purpose of this protocol.

9.2 Homologous recombination deficiency (HRD) assay

To perform the HRD assay, the following procedure will be done:

Extraction of DNA from FFPE tumors

A 5 micron H&E slide will be created and reviewed by a pathologist to facilitate enrichment of tumor derived DNA. Ten micron sections will be cut and regions of highest tumor cell density will be scraped from the slide.

For DNA extraction, the Promega Maxwell 16 FFPE Plus LEV DNA purification kit (Promega, Madison, WI) will be used. Tissue will be incubated overnight at 56 degrees Celsius with proteinase K in a shaking heat block. After the overnight incubation undigested material is spun out and the Maxwell cartridges are loaded. gDNA will be eluted in 60 ul of low TE.

For RNA extraction, deparaffinization will be performed using Qiagen deparaffinization solution, following by extraction on a Qiagen QIAcube instrument using reagents from the miRNeasy FFPE kit. These reagents are used according to the manufacurer's instructions.

Hybridization capture and sequencing
A custom SureSelect Target Enrichment (Agilent, Santa Clara, CA, USA) has been designed that included probes targeting ~55,000 SNPs distributed across the genome. The SNPs were selected from an initial set of 2.5 million SNPs. Selection criteria included the requirement that the SNPs have high heterozygosity across multiple ethnic groups, not be in linkage disequilibrium with one another, be evenly distributed across the genome, and be compatible to the probe design requirements of the target enrichment platform. In addition, ~600 probes were densely tiled across the exons of BRCA1 and BRCA2.

200ng – 1000ng of genomic DNA (gDNA) will be used for the SureSelect XT capture method. Briefly gDNA will be sheared on a Covaris E220 so that the peak size was between 150 and 200 nucleotides. Amplification of adapter-ligated library will precede an overnight hybridization at 65 degrees Celsius with the SureSelect biotinylated RNA library baits. Following hybridization between individual adapter-ligated libraries and the RNA library baits, index tags will be added by amplification so that pooled barcoded samples can be run on the Illumina HiSeq2500 sequencer (Illumina, San Diego, CA). 12 individual samples will be pooled together for sequencing runs in Rapid Run mode. Individual sample libraries will be combined such that each index-tagged sample will be present in equimolar amounts in the pool. For most purposes pools will be made so that each library will be at a final concentration of 10nM. From here the standard Illumina Sequencing protocol will be followed to denature and dilute the pooled libraries to 7pM for loading on Rapid and High Output flow cells.

BRCA1 and BRCA2 mutation screening
Sequence reads generated on the HiSeq2500 are trimmed at both the start and end to remove low quality bases that could generate spurious variant calls. Sequence trimming is largely performed according to the BWA programs' trimming algorithm (Burrows and Wheeler, 1994; Li and Durbin, 2009). For more detail see http://solexaqa.sourceforge.net/questions.htm. 20 is used as a threshold for trimming at the start of sequences and 30 for trimming at the end. These thresholds were derived empirically. It is expected that the sequence quality will deteriorate towards the end of a read, so we use a higher threshold at the end of sequences.

For each read an in-house implementation of the Burrow Wheeler Transform algorithm is executed which performs a search of all exons in our database to determine the matching exon for each read.

To call variants each read is aligned with the expected wildtype sequence of the exon. This alignment is a pairwise alignment performed by JAligner (http://jaligner.sourceforge.net/). Any differences represent variants. Variant calls from all reads for a sample are compiled in order to calculate the frequencies of all identified variants.

For large rearrangement detection the number of reads that mapped back to each base was normalized using the total number of mapped back reads across all genes and SNP locations. For each run, or group of similar runs a median normalized read count value was determined for each base. Samples are then evaluated to see if their normalized read count average, across a given exon or partial exon, is 1.5 or 0.5 the median value. This evaluation is done qualitatively. If a 1.5 (or greater) value than the median is observed, the sample is determined to have an insertion. If a 0.5 (or lower) value than the median is observed, the sample is determined to have a deletion.

The CV of centered normalized read counts (copy number, where a value of 1 = 2 copies) for the exon 11 (largest exon) of both BRCA1 and BRCA2 will be determined. If this value is <0.09 the sample will be included for further analysis in all cases. If the CV is between 0.9-0.12, reviewers will evaluate the sample to see if calling is possible. If the value exceeded 0.12 the sample will be rejected as not being able to call.

CV will be calculated as follows:
Read count for a base =c
Sum of all c for a sample = C
c/C = N (normalized read count for a given base and sample)
N/(median N across all samples) = S, centered normalized read counts or copy number
SD(S for all bases of exon 11)/Mean(S for all bases of exon 11) = CV
Using CV instead of just SD is necessary for samples whose copy number value isn't centered at 2.

Mutations identified will only included in the analysis if classified as deleterious of suspected deleterious based on previously described criteria.[44]

9.3 Calculation of HRD LOH score

Allele specific copy number (ASCN) at each SNP location was determined using a previously described algorithm.[45]In that algorithm, HRD score was defined as the number of LOH regions longer than 15 Mb but shorter than the whole chromosome.

9.4 Calculation of HRD N(tAI) (Number of subchromosomal regions with allelic imbalance extending to the telomere) score

The N(tAI) will be similarly calculated from the reconstructed allele-specific copy number profiles of the genome as determined from the SNP sequence reads above.

Allele specific copy number (ASCN) at each SNP location were determined using algorithm described in Abkevitch et al.[45]

Homologous recombination deficiency (HRD) score is defined as the number of LOH regions longer than 15 Mb but shorter than the whole chromosome HRD score has been shown to be associated with *BRCA1*, *BRCA2*, and *RAD51C* deficiency in ovarian tumors.[45]

Telomeric allelic imbalance score (TAI) was introduced by Birkbak et al[19] and was shown to be associated with pathological complete response to cisplatin treatment in triple negative breast cancer. TAI score was defined as the number of regions with allelic imbalance that extend to one of the subtelomeres but do not cross the centromere. [19]A region was counted only if it encompassed a minimum number of SNPs (on average approximately 1.8 Mb). We tested for association of TAI score with *BRCA1*, *BRCA2*, and *RAD51C* deficiency in three datasets of ovarian tumors and found that the association is more significant if the cutoff for the size of TAI regions is increased to 11 Mb. Therefore, we defined a modified TAI score as the number of regions with allelic imbalance that (a) extend to one of the subtelomeres, (b) do not cross the centromere and (c)are longer than 11 Mb. [45]

9.5 Calculation of HRD LST Score

LST score is the number of break points between regions longer than 10 Mb after filtering out regions shorter than 3 Mb [46]. Different cutoffs for LST score were introduced for "near-diploid" and "near-tetraploid" tumors to separate BRCA1/2 intact and deficient samples. We have tested for association of LST score with BRCA1, BRCA2, and RAD51C deficiency in three datasets of 609 ovarian tumors (data not shown). We have observed that LST score increases with ploidy both within intact and deficient samples. Instead of using ploidy-specific cutoffs, the LST score was modified by adjusting it by ploidy:

$$LSTm = LST - kP$$

where P is ploidy and k is a constant. Based on multivariate logistic regression analysis with deficiency as an outcome and LST and P as predictors, k = 15.5 provided the best separation between intact and deficient samples.

9.6 Calculation of HRD combined score

A priori, the HRD combined score will be calculated as the sum of the three HRD component scores (HRD-LOH, HRD-TAI, and HRD-LST). This score ranges from 0 to 100, with higher scores indicating more HR deficiency. Scores of 42 or greater are defined as high HRD. HR deficiency is defined as either a high HRD combined score or a BRCA1/BRCA2 mutation.

9.7 Hereditary Cancer Profile

The Hereditary Cancer Profile (HCP) assesses germline mutational status of 25 clinically actionable genes related to familial risks of breast, colon, ovarian, endometrial, pancreatic cancers, as well as melanoma. Genes are analyzed both for single-base changes and large

rearrangements by next-generation sequencing. HCP profiling will be included as a secondary correlative analysis in this trial to explore the proportion of triple negative cancers that are negative for germline BRCA1/2 mutations yet may be explained by other genetic anomalies. Mutations identified in the target 25 genes will be confirmed using germline DNA from the baseline blood sample.

HCP (MyRisk) testing is not offered as part of the protocol, but is available commercially. Patients who pursue HCP testing will be asked to consent to release results to this trial for the exploratory analysis.

Assay Methodology
Ultra-deep targeted sequencing using Raindance ThunderStorm platform for DNA amplification and Illumina MiSeq or Hiseq next generation sequencing technology will be used for the study. Genomic DNA from blood or buccal swab will be isolated using Qiagen DNA isolation kit and Symphony automated robot. Purified DNA will be fragmented using Sonicman machine and concentrated by Ampure magnetic beads. Fragmented DNA is used as input into a Raindance merge process used to generate Raindance custom expanded content amplicon libraries with PCR reactions (5 amplicon per droplet PCR). After cycling, and clean-up the pooled PCR products from each sample are reamplified to incorporate sequencing adaptors and sample specific barcodes.Several samples will be pooled and sequenced on Illumina MiSeq (32 – 40 samples) or HiSeq platform (up to 960 samples).

PMS2 and CHEK2 HCP analysis:
PMS2 and CHEK2 present the unique challenge of having pseudogenes elsewhere in the genome that interfere with normal sequencing analysis. This can be alleviated with the amplification of surrogate sequencing targets in the form of a Long Range amplicons that do not carry any pseudogene sequences. Secondary tailed, barcoded amplicons targeting the exons will be generated using the long range amplicons as template.

For HCP analysis, these surrogate sequencing targets will be fed into an HCP pipeline separate from the handling of the other genes. In that separate pipeline, PMS2 and CHECK2 barcoded secondary amplicons will be run in groups of up to 1500 patient samples per MiSeq flow cell. Sequencing variants and gene conversion status will be assessable from that data.

Analysis of sequencing data:
Sequencing information generated by MiSeq / HiSeq run will be analyzed applying the following criteria:

1. Sequences are trimmed at the point where the quality generally drops below 30.
2. Sequences are identified by comparing to the list of known amplicon sequences.
3. For genes with pseudogenes, JAligner is used to determine which target the read better matches. Reads that better match a pseudogene are discarded.
4. To call point mutations and indels, sequences are aligned using JAligner and compared to the reference (wildtype) sequence.

To call Large-Rearrangements, each exon is internally normalized by total counts for all exons. Each count represents a sequence that is aligned to a particular amplicon. This normalized count

is compared with the expected value, for each exon. 50% of expected is a deletion, 150% is an insertion.

Gene List

1	BRCA1
2	BRCA2
3	MLH1
4	MSH2
5	MSH6
6	PMS2
7	TACSTD1 (aka EPCAM)
8	APC
9	MUTYH
10	PALB2
11	CDKN2A (aka p16 & p14ARF)
12	CDK4
13	TP53
14	PTEN
15	CDH1
16	STK11
17	SMAD4
18	BMPR1A
19	ATM
20	CHEK2
21	RAD51C
22	RAD51D
23	BRIP1
24	BARD1
25	NBN

9.8 Whole exome sequencing and copy number findings

Refer to 9.2. (Hybridization capture and sequencing)

9.9 RNA-seq

Whole Transcriptome Sequencing
Whole transcriptome libraries will be prepared from 500 ng of total RNA using an Illumina TruSeq Stranded Total RNA Kit. The isolated RNA is subjected to RiboZero depletion without additional fragmentation. Double stranded cDNA is synthesized from the fragmented RNA and the 3' ends are adenylated prior to adaptor ligation. The resulting library is then amplified, validated, normalized, and pooled prior to sequencing on an Illumina HiSeq2500.
Also refer to 9.2. (Hybridization capture and sequencing)

9.10 Intrinsic Subtype Analysis

TNBC is a known heterogeneous clinical entity, with numerous genomic signatures being able to split this group into distinct biologically-based classes. Perou and others have previously shown that within TNBC, exists all of the intrinsic subtypes of breast cancer. Thus, we propose to use the PAM50 algorithm[47] and the Claudin-low predictor[48] in order to determine each samples intrinsic subtype. Specifically, using the RNA-seq data from section 8.2, a platform to platform global normalization step will be performed to adjust the TNBC RNA-seq data obtained in this study relative to the TNBC subset of patients coming from the TCGA RNA-seq study. Once this normalization is complete, the PAM50 algorithm will be run exactly as described in Parker et al. 2009, and then the Claudin-low predictor run as described in Prat et al., thus classifying samples into 1 of 6 intrinsic subtypes.[36,47]

Other genomic signatures of relevance to be tested include an expression surrogate for loss of Chromosome 5q, which is based upon the expression levels of RAD17 and RAD50,[49] and average expression value for these two genes combined is determined, and then the patients are put into rank order expression levels based upon this calculated value. Next, a median split, and tertile splits of the patients are determined, and then this classification used to test for associations with RCB.

The intrinsic subtype classifications, and the RAD17/RAD50 expression values, will be tested for associations with RCB using univariate and multivariate testing, in models that include the standard clinical parameters and with models including other genomic predictors.

9.11 Vanderbilt TNBC Molecular Subsets

As TNBC has recently been shown to be molecularly heterogeneous,[8] we will determine whether TNBC molecular subtypes are independently associated with pCR and whether statistically enriched variants are associated sensitivity/resistance to cisplatin or paclitaxel. To determine the TNBC molecular subtype, gene level RNA-seq reads (RSEM or RPKM) for individual tumor samples will be correlated with each of six TNBC subtype centroids using Spearman correlation as previously described. [8] Candidate samples will be assigned to the TNBC subtype with the highest correlation, and those that had low correlation (correlation coefficient 0.1 or *P*-value 0.05) or are similar between subtypes (difference of two largest correlation coefficients, 0.05) will be considered unclassified.

In order to assess the association between TNBC subtype and pCR status we will construct a contingency table and perform Fisher's exact test. To determine the independent utility of TNBC subtype for predicting pCR status, we will fit a logistic regression model to our data and use age, clinical stage, treatment regimens, and nuclear grade as potential explanatory factors. A likelihood ratio test will then be performed to determine whether adding TNBC subtype provides a significant improvement in predictive value over a model already containing the other 4 explanatory factors.

To determine statistically enriched mutations acquired during treatment in each TNBC subtype, we will perform a paired t-test between variants (copy number alterations and somatic mutations)

identified by both WES (refer to 8.4) and RNA-seq (refer to 8.5) in pre-treatment biopsy material and in residual disease after cisplatin/paclitaxel treatment. Any variants statically enriched in residual disease will be validated and clonality assessed by high coverage (>200X) targeted DNA sequencing.

9.12 Tumor Infiltrating Lymphocyte Predictor

To evaluate the association of intratumoral and stromal lymphocytes[1,2] and pathologic response to taxane or cisplatin chemotherapy, the H&E slide of the pre-treatment core biopsy will be scanned and the virtual images will be assessed for both intratumoral and stromal lymphocytes by two pathologists (A.R. and L.C.). As described by Denkert et al., intratumoral lymphocytes are defined as intraepithelial mononuclear cells within tumor cell nests or in direct contact with tumor cells and will be reported as the percentage of the tumor epithelial nests that contain infiltrating lymphocytes. Stromal lymphocytes are defined as teh percentage of tumor stroma area that contains a lymphocytic infiltrate without direct contact to tumor cells. The percentages of intratumoral and stromal lymphocytes will be analyzed for association with RCB score and class and categorically for response (RCB 0/I) and non-response (RCB II/III), in all patients and in the two arms separately.

9.13 Taxane Predictive Tests

Taxane predictive tests will be performed. One assay will be performed within the clinical Molecular Diagnostics Laboratory (MDL) at M.D. Anderson Cancer Center (MDACC) under CLIA-compliance using the FDA-registered Affymetrix DX2 system for microarray-based gene expression profiling.[30] Therefore, the CLIA-compliant MDL's approved standard operating procedures for microarray-based gene expression profiles of breast cancer, quality control, and reporting of results will be followed. The assay requires high-quality RNA obtained from fresh tumor sample that was snap-frozen in OCT media or RNA preservative solution (e.g. RNAlater solution). A 1µg aliquot of RNA is reverse transcribed to cDNA, and subsequently transcribed from double-strand cDNA to labeled cRNA (cRNA yield is recorded), prior to labeling, fragmentation, and hybridization to an Affymetrix gene expression microarray. The array is scanned and the data are normalized using the MAS5.0 algorithm, and then scaled to our reference distribution of 1,322 breast cancer genes. Automated scripts (using R) within the clinical laboratory workstation then calculate the gene expression signature results for the predictive algorithm to classify the result as either sensitive or insensitive to taxane-based chemotherapy.
The second taxane signature analysis will be performed using RNAseq data as described in Juul et al.[29]

It must be noted that the taxane predictors have not been translated for use with RNAseq data, and to replace the assay procedures with an alternative technology that is clinically unproven at this time would depart from CLIA-compliance or clinical validation results for predicting pathologic response and distant relapse-free su rvival that underpin the relevance of this assay to this clinical trial.

9.14 Blood for circulating tumor DNA

Circulating-free DNA may be a source of tumor DNA where genetic analysis can be done. The analysis of this cf-DNA will be compared with the alterations observed in the archival tumor sample. Tumor-derived somatic mutation profiling in plasma, if feasible and reproducible, may represent an alternative to tumor-tissue in the future and ideally, will provide a more updated description of the tumor molecular profile vs an archival tumor sample from the same patient. This will be an exploratory assessement. Blood samples (3 samples of 10 mL) will be collected at baseline, at 3 weeks and at 12 weeks (end of protocol therapy). Patients who progress prior to completion of protocol therapy should have a sample collected at time of removal from protocol therapy. Detailed instructions for the collection, handling and shipment of samples are outlined in Section 6.1.6 Translational Science Review Committee

A Translational Science Review Committee (TSRC), composed of members of DFCI, study sponsor Myriad, and other participating correlative sites, will be formed at time of study initiation. The goal of the Committee will be to provide scientific oversight for the complex planned correlative analyses contained within this protocol. The Committee will cover topics including: evaluation of status of correlative work, address any concerns over shared tissues, and to evaluate any additional proposed correlative studies. The goal of the TSRC is to ensure transparency and lack of redundancy in the scientific work proposed in this trial.

10. SPECIMEN BANKING

Any leftover study blood and tissue samples may be stored for future research studies. The subjects will consent to the future use of samples in the consent form for the study. Any samples will only be released for use in future studies after approval by the Principal Investigator and other regulatory bodies, as appropriate.

The study PI and collaborators have approval by the TBCRC to use all research bio-specimens collected during the conduct of this trial to address the research questions described in the protocol document. All future use of residual or repository specimens collected in this trial for purposes not prospectively defined will require review and approval by the TBCRC according to its established policies, whether the specimens are stored in a central site or at a local institution in a virtual repository.

Secondary use of bio-specimens for new endpoints must be submitted to the TBCRC Central Office for possible review by the TBCRC Correlative Science Review Committee.

11. STUDY CALENDAR

Table 4 outlines the required data. Electronic Data Capture (eDC) will be completed for each participant and submitted to the DF/HCC ODQ.

After a participant has been determined to be eligible for this study and has provided informed consent, a pre-study baseline evaluation is required as indicated on Table 4 prior to initiation of cycle 1. This evaluation must be completed within 28 days prior to starting therapy, except as indicated.

Table 5 outlines the required specimens for this study.

Table 4: Study calendar

Study Procedure	**Pre-treatment**		**Treatment**		**Pre-surgery** (Within 4 weeks after last chemotherapy)	**Within 42 days of last chemotherapy**
	Screening (within 28 days of treatment start)	**Prior to Neoadjuvant therapy**	**Arm A: Cisplatin Day 1 each cycle**	**Arm B: Paclitaxel Day 1 each cycle**		
Informed consent	X					
Confirmation of invasive cancer on diagnostic biopsy [a]	X					
Sentinel node biopsy or needle aspiration if clinically suspicious axillary nodes [a]	X					
Randomization [b]		X				
Breast Imaging [c]	X (+/- 7 days)[c]				X	
Complete medical history	X					
Complete physical	X		X	X	X	
Weight, vital signs, height	X		X [d]	X [d]		
Performance status (ECOG)	X		X	X		
Adverse Event evaluation			X	X	X[i]	
Hematology (CBC, differential, platelets)	X		X	Weekly[e]		
Chemistries [f]	X		X	X		
Pregnancy test [g]	X					
Definitive Surgery[h]						X

a Pathology report is required for eligibility; 28 day window does not apply.

b Randomization will be performed using a stratified permuted block randomization scheme with a 1:1 ratio. Stratification will be based on initial lymph node status assessment (positive or negative).

c Affected breast imaging for tumor measurements must be performed within 28 days +/- 7 days prior to study start. Imaging modality may include ultrasound, mammogram, or MRI, at the discretion of the treating team. Imaging should be repeated at conclusion of study therapy, within 4 weeks after the last chemotherapy dose, to assess tumor response. The same imaging modality should be used for tumor measurements at each timepoint.

d Height is only required at baseline/pre-treatment

e For patients receiving weekly paclitaxel, a complete blood count with differential should be performed each week prior to drug administration.

f Chemistries must include chloride, potassium, sodium, BUN, serum creatinine, phosphorus, calcium, albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin, glucose, magnesium (NOTE: magnesium and phosphorus are optional and should be monitored at least intermittently)

g Women of childbearing potential only. All female participants must have a negative serum or urine pregnancy test at the Baseline visit (within 7 days of the first dose of study treatment).

h For patients who do not receive crossover or additional neoadjuvant therapy. If crossover therapy is being considered, this decision must be discussed with overall study primary investigator.

i Patient should be followed for AEs until 30 days after protocol chemotherapy.

Table 5: Required specimen submission

Specimen Type	Time Point					Kit Supply	Shipping Condition	Ship to
	Pre-study	Pre-treatment	C2D1 and 12 weeks	Definitive Surgery	Post-treatment core biopsy			
Representative H&E slide from each block from diagnostic biopsy[a]	X						Room temperature	DFCI Coordinator
FFPE tumor block from diagnostic biopsy[a]	X						Room temperature	DFCI Coordinator
Immunostained slides to confirm ER, PR and HER2 status[a]	X						Room temperature	DFCI Coordinator
4 Biopsy cores frozen in OCT[b]		X				CORE Prognostex[g]	Store in -80°C freezer; ship on dry ice	DF/HCC Core Blood and Tissue Bank
2 Biopsy cores in formalin		X[b]			X[e]	CORE Prognostex[g]	Room temperature	DF/HCC Core Blood and Tissue Bank
Two 10mL lavender top (EDTA) tubes of blood[c]	X					CORE Prognostex[g]	Use thermos provided in research kit or ship on cold packs	DF/HCC Core Blood and Tissue Bank
Two 10mL Streck tube of blood[d]		X	X[f]			CORE Prognostex[g]	Room temperature	DF/HCC Core Blood and Tissue Bank
H&E slides from definitive surgery[e]				X			Room temperature	DFCI Coordinator
H&E slides from axillary lymph node dissection[e]		X		X			Room temperature	DFCI Coordinator
FFPE tumor block from definitive surgery or post-treatment core biopsy[e]				X			Room temperature	DFCI Coordinator
Block of normal breast tissue, skin or uninvolved LN[e]				X			Room temperature	DFCI Coordinator

[a] See Section 6.1.3 for additional information.
[b] See Section 6.1.4 for additional information.
[c] See Section 6.1.5 for additional information.
[d] See Section 6.1.6 for additional information.
[e] See Section 6.6 for additional information.
[f] The 12-week Streck Tube may be collected at the pre-surgery or post treatment biopsy visit but must be collected prior to initiation of crossover or additional therapy.
[g] Kits may be ordered from CORE Prognostex at www.coreprognostex.com

12. MEASUREMENT OF EFFECT

The two major effects to be measured in this study are: tumor/pathological response to study drugs and HDR assay score.

12.1 Target Lesions

A baseline and presurgical imaging study of the breast is required; ultrasound, mammogram or MRI may be pursued at the discretion of the provider. The baseline imaging must be obtained within 28 days (+/- 7 days) of beginning therapy. The presurgical imaging should occur within 4 weeks after the last chemotherapy administration. If the participant demonstrates clinical progression at any time, repeat imaging is required. If there is discordance (clinical progression, but radiographic stable disease or response), study PI should be contacted to solve discordance.

In the event of multifocal or multicentric disease in the breast, the investigator must determine which will represent the target lesion. This should remain consistent throughout the study. The target lesion should be selected on the basis of its size (lesion with the longest diameter) and suitability for accurate repetitive measurements (either by imaging techniques or clinically).

Response criteria are based on the RECIST 1.1 criteria:

Radiographic Complete Response (CR):	Complete disappearance of the target lesion
Radiographic Partial Response (PR):	Greater than or equal to 30% decrease in the longest diameter (LD) of the target lesion taking as reference the baseline LD
Radiographic Progressive Disease (PD):	Greater than or equal to 20% increase in the LD of target lesion taking as reference the baseline LD or the appearance of one or more new lesions
Radiographic Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the baseline LD

12.2 Pathologic response

The MD Anderson RCB method[21] will be used to assess response. RCB 0/I will be considered good response; RCB II/III will be considered poor response. RCB0 will be considered pCR. Pathologic complete response in the lymph nodes is defined as no detectable invasive tumor by H&E. All patients with significant residual disease who proceed to alternative chemotherapy will be considered to belong to the poor response groupRCB is determined from: bidimensional diameters of the primary tumor bed in the resection specimen (d1 and d2), the proportion of the

primary tumor bed that contains invasive carcinoma (finv), the number of axillary lymph nodes containing metastatic carcinoma (LN), and the diameter of the largest metastasis in an axillary lymph node. Largest bidimensional measurements of the residual primary tumor bed are recorded from the macroscopic description in the pathology report and confirmed after review of corresponding slides. If multiple tumors are present, the dimensions of the largest are recorded. Bidimensional measurements of the primary tumor bed (millimeters) are combined as follows: The proportion of invasive carcinoma (finv) within the cross sectional area of the primary tumor bed is estimated from the overall percent area of carcinoma (%CA) and then corrected for the component of in situ carcinoma (%CIS): finv = (1 –(%CIS/100)) × (%CA/100).The transformed terms are then scaled to match the 95th percentiles of their respective distributions, and added to define the RCB index.[21] RCB score can be calculated using the following tool: http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

12.3 Radiographic assessment

Each participant will have a pre-therapy baseline imaging study. The longest diameter (LD) of the target lesion at the time of study initiation will be reported as the baseline LD. The baseline LD of the target lesion will be used as reference to further characterize the objective tumor response of the measureable dimension of the disease. There is no size requirement defining residual disease; the determination of the presence of clinically significant residual disease will be made by the treating provider. The radiologic response to treatment should be noted as complete response (no visible tumor present), partial response (tumor present but reduced in size from baseline), stable disease (no change in size of tumor) or progressive disease (tumor larger than baseline).

Both target and, in the event of multifocal or multicentric invasive cancer, nontarget lesions should be followed clinically and their clinical size recorded at baseline. Measurements thereafter are required; these lesions should be categorized at subsequent visits regarding whether there is evidence of progression. If progression occurs the study chair should be notified in order to determine whether the participant should come off protocol treatment.

12.4 Pathology Response Central Review

Central review of pathology slides of the pre-chemotherapy diagnostic breast biopsy and the post-chemotherapy tumor excision (or mastectomy) as well as pathology review of axillary nodes will be performed by Dr. Laura Collins, Beth Israel Deaconess Medical Center, Boston, MA. Dr. Collins will determine the Residual Cancer Burden (RCB) score and class that will serve as the primary pathologic response endpoint. Dr. Collins will be blinded to the treatment assignment of each participant and to the tumor HRD scores. Dr. Collins will also perform central review of ER, PR, and HER2 stained slides and will score the pre-treatment biopsies for tumor infiltrating lymphocytes (as described above).

12.5 HRD Assay score

HRD Assay score will be assessed by Myriad Genetic Laboratories. Refer to Sections 9.2 though 9.5.

13. ADVERSE EVENT REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. Lists of reported and/or potential AEs for cisplation and paclitaxel can be found in sections 7.1.1 and 7.1.2.

13.1 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

- **Attribution** of the AE:
  - 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.

13.2 Expedited Adverse Event Reporting

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form. Serious AEs will be followed until resolution or, if resolution is unlikely, until the event or sequelae stabilize.

Elective inpatient hospital admissions for non-toxicity related reasons will not be considered an SAE.

For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, unexpected grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Other investigative sites will report SAEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to the Overall PI within the timeframes detailed in the table below.

Table 6: DF/HCC Reportable AEs

Attribution	DF/HCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

The Overall PI will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

13.3 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

13.4 Routine Adverse Event Reporting

All adverse events, regardless of relationship to study treatment or grade, will be collected from the time the subject initiated therapy until 30 days after discontinuation of study treatment. All AEs must be followed until resolution or for 30 days after the subject's last study visit, whichever comes first. All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

14. DATA AND SAFETY MONITORING

14.1 Data Reporting

14.1.1 Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

14.1.2 Data Submission

Participating sites are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

14.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this trial. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed with the Principal Investigator, statistician and study team members. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the trial.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual, treatment regimen information, adverse events and serious adverse events reported by category, summary of any deaths on study, audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

14.3 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix G.

- The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

15. REGULATORY CONSIDERATIONS

15.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

15.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

15.3 Ethics

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
  - Title 21 Part 50 – Protection of Human Subjects
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
  - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
  - Title 21 Part 56 – Institutional Review Boards
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
- State laws
- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

15.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

15.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

15.6 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.

15.7 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.

16. MULTI-CENTER GUIDELINES

16.1 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 in the Data Safety Monitoring Plan in Appendix G.

16.2 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. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

16.3 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. The Primary Investigator will be the final arbiter of the manuscript content.

The outcome results of this trial will be made public within 24 months of the end of data collection. Interim accrual and toxicity results of this trial may also be periodically presented at meetings of the American Society of Clinical Oncology and the San Antonio Breast Cancer Symposium. A full report of the outcomes will be made public no later than two (2) years after the end of data collection.

17. STATISTICAL CONSIDERATIONS

This is a DF/HCC randomized phase II study of stage I-III triple negative breast cancer (TNBC) patients where patients receive as pre-operative therapy: Cisplatin (75 mg/m2 every 3 weeks x 4), or Paclitaxel (80 mg/m2 weekly x 12). All patients will be randomized in a 1:1 ratio stratified by initial lymph node status assessment (positive vs. negative) as well as by tumor size (pretreatment T1-2 vs T3-4). Required tissue collection will be performed before and after treatment.

This study is designed to evaluate the ability of the Homologous Recombination Deficiency (HRD) assay to predict pathologic response to neoadjuvant therapy in TNBC. Previous data suggests that the biomarker is associated to response to neoadjuvant therapy in TNBC.[20] We hypothesize that the HRD marker will be positively associated with pathologic response to

neoadjuvant platinum-based chemotherapy in TNBC and it will be negatively associated with pathologic response to neoadjuvant taxane-based therapy in this subset of tumors.

We anticipate that approximately 12.5% of tissue samples collected will be unevaluable for the HRD assay. Assessments of tissue quality will begin after the accrual of 50 patients to inform laboratory protocols. A single sample size re-estimation will be performed after 120 patients are enrolled based only on tissue failure rate and blinded to treatment assignment and clinical outcomes. Further, a single interim analysis of treatment efficacy will be conducted after 80 patients are evaluable for response.

17.1 Endpoints

The primary clinical endpoint will be pathologic response, and will be assessed using Symmans residual cancer burden (RCB) score patients with RCB-0/1 considered to have good response, and patients with RCB-2/3 considered to have poor response. Assessments of pathologic response will be conducted by central review and will be blinded to the treatment assignment of each participant and to the HRD assay.

The primary laboratory endpoint will be the HRD assay of pre-treatment tissue specimen, as defined in Sections 9.2 through 9.5. HR deficiency status (High score or a *BRCA* mutation) will be determined by Myriad Genetic Laboratories and be fully blinded to patient information, randomized treatment assignment, RCB scores, and other clinical endpoints.

The secondary clinical endpoints will also include pathologic complete response (pCR) (defined as RCB 0) and clinical radiologic response categorized as complete response, partial response, stable disease or progressive disease.

Exploratory laboratory endpoints will also include the NtAI assay, the BRCA1 3-gene mRNA signature, evaluation of chr 15q26, Nmut evaluation, and next generation sequencing of RNA and whole exome DNA.

17.2 Sample Size / Accrual

The initial target accrual is 160 patients (80 to Cisplatin and 80 to Paclitaxel). A total of up to 5 additional patients (for a cumulative total of 165 patients registered in the OnCore CTMS) will be allowed to to replace patients who were registered but never began treatment. Patients who register and start treatment may not be replaced. When 120 patients are enrolled on the study (75%), all available tissue specimens will be evaluated for HR-deficiency. The target sample-size will be re-calculated to the number expected to yield 140 evaluable specimens for the HRD assay (corresponding to 90% power for the primary objectives). A maximum of 187 patients would be enrolled to the study if there is a 25% or greater failure rate.

Observed failure rate at 120 patients	Final Sample Size
0%	140
5%	148

10%	156
12.5%	160
15%	165
20%	175
≥25%	187

We anticipate a constant accrual of 5 patients a month, resulting in 28 to 38 months of patient accession. Once target accrual is met, there will be an additional follow-up period of 4 months to observe pathologic response at the time of definitive surgery.

17.3 Analysis and Power of Primary Objectives

The consideration of sample size and power is based on the co-primary objectives to determine if there is a positive association between HR deficiency and pathologic response to cisplatin therapy, and to determine if there is a negative association between HR deficiency and pathologic response to taxane therapy. Each primary analysis will be conducted using a univariate logistic regression model and conducting a likelihood ratio test with a one-sided Type I error of alpha = 0.05.

Assumptions are based on previous data cited in the Background (Section 3.6). For the co-primary objectives, the response rates, defined as RCB 0/1, are assumed to be 40% to cisplatin therapy and 30% to taxane therapy. Under an assumption that the prevalence of HR deficiency will be 60% based on the findings presented by Richardson et al SABCS 2014. 70 evaluable patients per arm (140 total) will provide 90% power to detect a response rate of 55% in HR-deficient patients versus a response rate of 18% in HR-non deficient patients (odds ratio = 5.4). Power calculations for a two-sample difference in proportions are based on the method by Walter (Walter, D.E. “In Defence of the Arc Sine Approximation.” The Statistician, 28, 219-222 (1979).

The following table shows a sensitivity analysis of the effect sizes there will be 90% power to detect under varying true prevalences of HR deficiency in the target patient population

Table 7: Sensitivity analysis of the effect sizes for 90% power under varying true prevalences of HR deficiency.

	Response rates to cisplatin		
Prevalence High HRD	**High HRD Resp (%)**	**Low HRD Resp (%)**	**Odds Ratio**
0.5	0.58	0.22	4.99
0.55	0.56	0.20	5.14
0.6	0.55	0.18	5.44
0.65	0.53	0.16	5.98
0.7	0.51	0.13	6.92
0.75	0.50	0.10	8.84

Estimates of sensitivity, specificity, positive and negative predictive value will be reported with 95% binomial confidence intervals.

To test whether the predictive value of HR-deficient is treatment-specific, an interaction between HR deficiency and treatment arm will be evaluated in a logistic regression model using a likelihood ratio-test and one-sided alpha = 0.05. With 140 evaluable patients , there will be 82% power to detect an interaction corresponding to response rates:

- to platinum in HR-deficient = 52%
- to platinum in HR-non deficient = 16%
- to taxane in HR-deficient = 27%
- to taxane in HR-non deficient = 37%

which corresponds to odds ratios of 5.7 and 0.62 for response to ciplatin and taxane, respectively. Power for the test of interaction was calculated by simulation of binary data under the logistic regression model, and represents the proportion of p-values from the 10,000 simulated datasets that were less than 0.05.

As a secondary analysis of HR deficiency and RCB0/1 within each treatment arm, a multivariate logistic regression model will be used to estimate the odds of pathologic response after adjusting for stratification factors and other patient and tumor characteristics with a known or observed association with response to neoadjuvant therapy.

17.4 Interim Monitoring

Monitoring of safety will be conducted on a semiannual basis by the DF/HCC Data and Safety Monitoring Board (DSMB), and the exact timing of reporting will be determined by the meeting schedule of the DSMB.

In addition, a single interim analysis of efficacy will be conducted such that any ineffective treatment arms are closed to further accrual. The interim analysis will be timed to occur once 80 patients (50% of the target sample size) are evaluable for response. If either arm crosses the stopping boundary, then the study will continue as a single arm trial without adjustment of Type I alpha levels for the analysis corresponding to the remaining primary objective. Accrual will not stop for this interim analysis, and assessments of response and treatment efficacy will be made fully blinded to HRD score.

The threshold for declaring ineffective therapy is if 10% or fewer of patients have an RCB 0/1 response at surgery (e.g. ≤4 out of 40 patients). Under this rule, there is a >95% chance of stopping the study early if the true response rate is 0.05 and a 7.5% chance of stopping early if the response rate is 0.2 when exactly 40 patients are enrolled in a single arm. For the primary analysis, no adjustments will be made to the inferenctial methods describe in the above section. Stopping for ineffective treatments is noted to be strictly conservative in controlling the overall Type I error across the sequential hypothesis tests.

17.5 Analysis of Secondary Objectives

The association of HR deficiency to secondary clinical endpoints: pCR and clinical response, will be evaluated using the logistic regression model, and tests of interaction and main effects detailed above. The same analyses of HR deficiency and all clinical endpoints will be conducted in the subset of TNBC patients that are confirmed to have BRCA proficient disease.

Secondary objectives to evaluate individual components to the HRD assay and test the association of the LOH assay, the TAI assay, the LST assay, and a combined score of LOH, TAI and LST, will use receiver operating characteristic (ROC) analyses and inferences based on the Area Under the Curve (AUC) statistic and corresponding Wilcoxon rank sum test.

All statistical inferences for secondary objectives will use one-sided Type I errors of alpha = 0.05. Analysis plans for exploratory objectives are unspecified and hypothesis-generating. Any promising findings will be further tested in future studies.

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APPENDIX A: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B: NCI Common Toxicity Criteria for Adverse Events (CTCAE) v4.0

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

APPENDIX C: Tissue labeling and documentation

A. Instructions for Core Biopsies to be placed in 10% Formalin

1. 2. Ideally two core biopsies will be placed into 10% formalin. Both can be placed in the same specimen collection container. These biopsies should be at room temperature when stored and shipped.

B. Instructions for Core Biopsies to be Frozen

Prior to going to the radiology or procedure site, prepare at least 3-6 tissue molds with a thin layer of OCT frozen on a flat surface such as the inside shelf of a -80 freezer or a flat block of dry ice. **The bottom layer of OCT should be as flat as possible and frozen solid before the biopsy procedure begins.**

1. After biopsy is performed, the tissue mass is placed on a sterile gauze
2. Using forceps, separate the tumor tissue
3. Place 1 piece (core) of tumor tissue in each cassette; the last cassette will contain many small pieces of tumor tissue
4. Fill cassettes with OCT
   a. Completely cover tissue
   b. Limit the amount of bubbles
5. Place cassettes on dry ice and prepare for transport by limiting OCT leakage
6. Return samples to the lab and complete freezing of samples in OCT with dry ice (about 10 minutes freezing time)
7. Once samples are frozen, place in plastic bag; label bag with date, protocol number, participant number/study identifier
8. Store in –80C freezer until shipment

Research Biopsy Shipping Procedures

Please ship all specimens over-night. The frozen specimens must be shipped on dry ice. The specimens in 10% formalin should be shipped at room temperature. **All samples should arrive during the week by Friday morning**. If a biopsy must be performed at one of the non-Boston sites on a Friday, the specimens should be stored over the weekend and shipped on the following Monday. The frozen specimens should be stored in a -80 freezer until shipment; specimens in formalin should be stored at room temperature until shipment. All specimens should be shipped to:

Dana Farber Cancer Institute
DF/HCC Core Blood and Tissue Bank
Smith Building - SM 956
450 Brookline Ave
Boston, MA 02215

Tel: 617-582-8189

*** Please email the DF/HCC Core Blood and Tissue Bank and Eileen Wrabel with the sample information and tracking information the day before shipping specimens.**
DFCIBreastBank@partners.org
ewrabel@partners.org

APPENDIX D: 13-383 SPECIMEN REQUISITION (Diagnostic and Surgery Blocks/Slides)

Complete this form and include with the specimen shipment. Label ALL materials with participant initials, DFCI participant study ID, and the date the specimen was obtained. **Include a pathology report** with any archival tissue specimens being submitted.

Ship specimen(s) to: Dana-Farber Cancer Institute, Attn: Eileen Wrabel, 450 Brookline Ave Dana 157, Boston, MA 02215

Specimen Information

Participant Initials (FML): _____	DFCI Participant Study ID Number: _____	Date specimen(s) shipped: _____
Site: ☐ Right breast ☐ Left breast	Pathology reports included (Mark all that apply): ☐ Diagnostic (Pre-chemo)	☐Definitive Surgery (Post-chemo)

Specimen Type *(indicate inclusion in shipment by checking box)*	**Pathology Number(s) or Serial Coding**	**Quantity submitted**	**Date specimen obtained**
Diagnostic Biopsy (Pre-study) Requirements:			
☐ A representative H&E slide from each block of the diagnostic biopsy			
☐ ER, PR, and HER2 immunostained slides of the diagnostic biopsy			
☐ FFPE tumor block ***-Or if blocks cannot be released:*** ☐ one - 4µm section on charged slide from the diagnostic core biopsy **AND** ☐ Three to five - 10µm unstained sections on UNCHARGED slides from the diagnostic core biopsy. The tissue sections must be cut sequentially and in the same orientation. There should only be 1 section per slide. **AND** ☐ Five - 7µm unstained tissue slides (charged slides are acceptable) from the diagnostic core biopsy			
Definitive Surgery (Post-chemo) Requirements:			
☐ An H&E slide from each block of the definitive srugical breast excisiton and axillary surgery specimens			
☐ A representative FFPE block of residual tumor from the surgical specimen ***-Or if blocks cannot be released:*** ☐ Five unstained slides containing residual tumor from the surgical specimen			
☐ A representative FFPE block of normal tissue (normal breast, skin, or negative lymph node) from the surgical specimen ***-Or if blocks cannot be released:*** ☐ Five unstained slides containing (normal breast, skin, or negative lymph node) from the surgical specimen			

Responsible contact: _____

Email: _____

Phone number: _____

Site: _____

Mailing address (to return slides and blocks): _____

_____

_____

_____

APPENDIX E: 13-383 SPECIMEN REQUISITION (Research Biopsy and Blood)

Complete this form and include with the specimen shipment. Label ALL materials with participant initials, DFCI participant study ID, and the date the specimen was obtained.

Ship specimen(s) to: Dana Farber Cancer Institute, DF/HCC Core Blood and Tissue Bank
450 Brookline Ave, Smith Building SM-956, Boston, MA 02215

Specimen Information

Participant Initials (FML): _____ DFCI Participant Study ID Number: _____

Date specimen(s) shipped: _____

Time point: ☐ Pre-treatment research biopsy/Baseline ☐ 3weeks ☐ 12 weeks ☐ Post-treatment core biopsy

Specimen Type *(indicate inclusion in shipment by checking box)*	**Quantity submitted**	**Date specimen obtained**
☐ Biopsy core(s) frozen in OCT		
☐ Biopsy core(s) in formalin		
☐ Blood in lavender top (EDTA) tube		
☐ Blood in Streck tubes		
☐ Other, specify:		

Responsible Contact: _____

Email: _____

Phone number: _____

Site: _____

APPENDIX F: Cover Letter to Request Outside Blocks

Dear Pathologist,

We are writing to request archival tumor tissue on <<pt name or initials>>, DOB <<XX/XX/XXXX>>.
Specifically, we are requesting the following sample(s):

Institution: _____
Type of Procedure: _____
Date of Procedure: _____
Local Accession Number: _____

Institution: _____
Type of Procedure: _____
Date of Procedure: _____
Local Accession Number: _____

<<Pt name or initials>> has consented to participate in a **Randomized Phase II Neo-adjuvant study of cisplatin versus paclitaxel in patients with triple negative breast cancer: Evaluating a Homologous recombination deficiency (HRD) Biomarker**.

We would appreciate it if you would send the slides and tumor block of the diagnostic biopsy (and node evaluation if done) of this patient in order proceed to central pathological review of those, as pre-specified in the protocol.

Thank you very much for your cooperation. We recognize that tumor samples are a precious resource. If you should have any questions about this study, please do not hesitate to contact either me or the Clinical Research Coordinator.

Sincerely,

<<Institutional PI>>
<<Institutional PI Address>>

APPENDIX G: Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan

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1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: Dana-Farber Cancer Institute (DFCI) is responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA), etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (e.g. FDA, etc.). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution or Project Manager) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Office of Data Quality (ODQ): A group within DF/HCC responsible for registering human subjects for trials, ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. ODQ also coordinates quality assurance efforts related to multi-center clinical research.

2.0 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, **Erica Mayer, MD,** will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (e.g., FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with the FDA (investigator-held IND trials).
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.

2.2 Coordinating Center

The Coordinating Center will assume the following general responsibilities:

- Assist in protocol development
- Maintain FDA correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions with DF/HCC QACT.

- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to DF/HCC Sponsor for timely review.
- Distribute adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all participating investigators.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor Participating Institutions either by on-site or remote monitoring.
- Maintain Regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation all relevant communications.

2.3 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as needed (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.

- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior

to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to interventional trials (i.e. drug and/or device trials).

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution's IRB
- Participating IRB's approval for all amendments
- Annual approval letters by the Participaitng Institution's IRB.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPAA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an Authorization. This Authorization may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB and if applicable NCI/CTEP, will provide a consent template, which covered entities (Participating Institutions) must use.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per NCI requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned DF/HCC ODQ case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration and Randomization

See Protocol Section 5 for registration and randomization procedures.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC ODQ before receiving treatment. Treatment may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.7.3 Eligibility Exceptions

The DF/HCC ODQ will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC ODQ requires each institution to fully comply with this requirement.

3.8 DF/HCC Protocol Case Number

At the time of registration, ODQ requires the following identifiers for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.9 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe derivations from a protocol. All Participating Institutions must adhere to these

requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.9.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.9.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

3.10 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the

NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.10.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 13.

Participating Institutions must report the AEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB SAE Reporting Requirements.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Investigators will review any distributed AE reports, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

3.10.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. The Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

3.11 Data Management

The DF/HCC ODQ develops a set of electronic case report forms (eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC ODQ provides a web based training for eCRF users.

3.11.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

Incomplete or Questionable Data
If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC ODQ Data Analyst or study monitor. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC)

system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

Missing Forms
If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC ODQ and distributed a minimum of four times a year.

4.0 REQUISITIONING STUDY DRUG

All agents used on this study are commercially available. Check with the local Director of Pharmacy and/or the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB.

5.0 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. As the Coordinating Center, the DF/HCC Lead Institution or designee with the aid of the ODQ provides quality control oversight for the DF/HCC Multi-center Protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit subject source documents to the DF/HCC Lead Institution or designee for monitoring. Also, the Participating Institution may be subject to on-site monitoring conducted by the DF/HCC Lead Institution or designee.

The DF/HCC Lead Institution will implement on-site as well as virtual monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. The DF/HCC Lead Institute, or designee, will monitor each participating site regularly and on an ongoing basis while patients are receiving treatment. Should a Participating Institution be monitored once and then not accrue any additional patients or participant visits, then a second monitoring visit may not be necessary.
Monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration / treatment, regulatory records and site trial master files, protocol deviations, pharmacy records, response assessments, and data management. Additionally, regular and ongoing communication with Participating Institutions, will be accomplished by holding all site monthly teleconferences. The Lead Institution will keep in close touch with the Participating Institutions via email and phone. Source documents from Participating Institutions, will be collected at specific data points that support the primary and or secondary endpoints.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants' complete medical record and source documents for source documentation verification during the on-site visit. In addition,

upon request from a monitor or auditor, Participating Institutions should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the participating site. If there are concerns for protocol compliance, issues that impact subject safety or the integrity of the study are found, or trends identified based on areas of need, additional monitoring visits may be scheduled. On site monitoring visits can be supplemented with virtual monitoring assessments, provided that the minimum monitoring frequencies are adhered to.

Virtual Monitoring: The Coordinating Center will request source documentation from Participating Institutions as needed to complete monitoring activities. Participating Institutions will be asked to forward de-identified copies of participants' medical record and source documents to the Coordinating Center to aid in source documentation verification.

The Overall PI, or designee, will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participation in the study. The Coordinating Center may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the Coordinating Center.

5.2 Evaluation of Participating Institution Performance

5.2.1 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participating in the study. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

5.2.2 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination.

The following **minimum** accrual requirements are recommended:

- Phase II-III: 3 per site/annually. However, given the additional regulatory burden and cost of overseeing each site, a consideration of 5 per site/annually should be a minimum target for each site.

6.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 DF/HCC Sponsored Trials

One on-site audit will be scheduled by the ODQ, assuming at least three participants have been treated on protocol at the site. Approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Audit Notification

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Sub-Standard Performance

The DF/HCC Sponsor and DFCI IRB, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal regulations, will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation.

APPENDIX H: Plasma Processing for Circulating Free DNA (cfDNA)

The following instructions are for central laboratory processing at DFCI. Sites should process the samples as described in Section This protocol has been optimized for isolating plasma from blood for downstream analysis of cfDNA or specifically plasma DNA (pDNA). ***The key is to maximize removal of contaminating cells and genomic DNA from these cells as large genomic DNA fragments hinder plasma DNA analysis.***

Terminology: cfDNA = circulating free DNA, ctDNA = circulating tumor DNA, ptDNA = plasma tumor DNA
We like the term ptDNA to be more specific as ctDNA tends to be confused with CTCs (circulating tumors cells) and could also be used to describe tumor DNA found in any circulating bodily fluid (e.g. in the urine, or utDNA).

CRITICAL STEP
Time between blood draw and processing should be less than 2 hours. Gently invert tubes after blood draw. Do not shake or vortex tubes as cellular lysis could occur. This critical step can be dismissed if using special tubes designed to prevent cellular lysis from Streck Innovations, http://www.streck.com/product.aspx?p=Cell-Free%20DNA%20BCT

- In HOOD: transfer blood from EDTA tube to 15ml conical tube
- Centrifuge 15ml conical tubes for 10 minutes at 1500 ± 150g (=1500RCF)
- Transfer supernatant to a fresh 15 ml tube without disturbing the cellular layer using a disposable 10 ml serological pipette or a disposable bulb pipette IN HOOD.

CRITICAL STEP
Centrifugation separates plasma from leukocytes and erythrocytes. Leaving sufficient residual plasma in the tubes after the centrifugation and not disturbing the leukocyte layer when pipetting is a critical step in the sample preparation process. Be careful not to disturb the leukocyte layer in the tubes.

- Centrifuge the plasma in the 15 ml centrifuge tube for 10 min at 3000 ± 150g (=3000 RCF)

CRITICAL STEP
The 2nd centrifugation is necessary to remove any residual cells carried over from the first centrifugation step.
Cellular contamination can adversely affect downstream applications of cfDNA.

☐ Transfer supernatant to a fresh 15 ml centrifuge tube without disturbing the cellular layer using a
disposable 5 ml serological pipette or disposable bulb pipette IN HOOD.

CRITICAL STEP
Leave a residual volume of about 0.3 ml (~7 mm) on the bottom of the 15 ml tube to avoid cellular
contamination.

☐ After transferring the plasma to a new 15 ml centrifuge tube as described, gently mix plasma and record
total plasma volume (typically ~ 4 ml plasma per 10 ml blood) IN HOOD.

☐ Optional: Aliquot 1 ml plasma to 1.5 ml pre-labeled tubes with specimen ID.

☐ Store plasma in freezer at -80ºC

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**13-383**
Protocol Name:	**A Randomized Phase II Study of Preoperative Cisplatin Versus Paclitaxel in Patients with Triple Negative Breast Cancer without Germline BRCA Mutations: Evaluating the Homologous Recombination Deficiency (HRD) Biomarker**
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DFCI Research Nurse:	Peg Haldoupis, RN; Liz Kasparian, RN; Mary O'Driscoll, RN; Kathy Roche, RN; Myra St. Amand, RN, Beth Tiani, RN

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
***** Remember to check the ALERT PAGE*****

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	This study will evaluate the performance of the HRD assay score to predict sensitivity to cisplatin or paclitaxel in BRCA 1/2- proficient Triple Negative Breast Cancer. **Study Design**: Ph 2 study randomizing Participants to preoperative cisplatin versus paclitaxel – Section 1; **Study Rationale** – Section 3.7. **A cycle is 3 weeks** – Section 6.2.
Dose Calc.	• Cisplatin doses are calculated in mg/m^2 – Section 6.2 • Paditaxel doses are calculated in mg/m^2 – Section 6.2 • Dosing calculation guidelines will follow each institution policies – Section 6.3 • Doses should be based on actual body weight – Section 6.3
Study Drug Administration	**Cisplatin** – Sections 6.2, 6.3 and 8.1 • IV, to be administered over approximately 60 minutes every 3 weeks for 4 cycles – Section 6.3 • May be administered per Institutional policy – Sections 6.2 and 8.1.7 • ***Pre-treatment criteria*** – Section 6.3 • See Section 6.3 for pre and post hydration requirements, anti-emetic and supplementation recommendations **Paclitaxel** – Sections 6.2, 6.3 and 8.2 • IV, to be administered over approximately 60 minutes once per week for 12 weeks (4 cycles) – Section 6.3 • May be administered per Institutional policy – Sections 6.2 and 8.2.7 • ***Pre-treatment criteria*** – Section 6.3 • Premedication required and may be administered per Institutional practice – Section 6.3
Dose Modifications & Toxicity	*Dose Modifications/Dosing Delay for Toxicity* are outlined in Section 7 • This protocol uses NCI CTCAE criteria, version 4.0 – Section 7.2 • Anticipated toxicities are in Section 7.1 • Toxicity management, dose delays and dose modifications are in Section 7.2
Con Meds	*Concomitant Therapy* Guidelines are in Section 6.8 • Please review the cited sections for permitted, prohibited, and "use with caution" medications/therapies/foods
Required Data	*Study Calendar and Assessment Required data* are outlined in Section 11 • Participants on the paditaxel arm must have a CBC with differential each week prior to administration – Section 11, Table 2, footnote "g."
Charting Tips	**All study drugs require documentation of exact administration time.** Please be sure to DOCUMENT study medication **actual** UP/DOWN times in medical record (e.g. LMR, eMAR, nursing notes). **Edit eMAR as needed to match the exact time given**. • If there is a discrepancy in the infusion time, delay in administration, or infusion takes longer than is permitted by the guidelines of the protocol, please **document the reason for the discrepancy in the medical record.** Please also DOCUMENT any additional vital signs and routes of administration.

What have other patients who have participated and provided tissue samples for this type of research said?

"I realized that I am part of a larger community and that I benefit from the participation of others who have donated before me. Life is more than what I am going to get out of it. I want to make a difference for other patients who come after I do. If I can help other people with the disease, I am going to do it."

"It is active altruism – not just saying you want to help others but actively doing something that could change cancer treatments for future patients."

"I felt empowered by joining the trial. It was very satisfying to help other patients."

"I was a lot less apprehensive because the tissue sampling was done as part of a clinical trial where there is careful monitoring from my health care team and ongoing education and support from my research nurse."

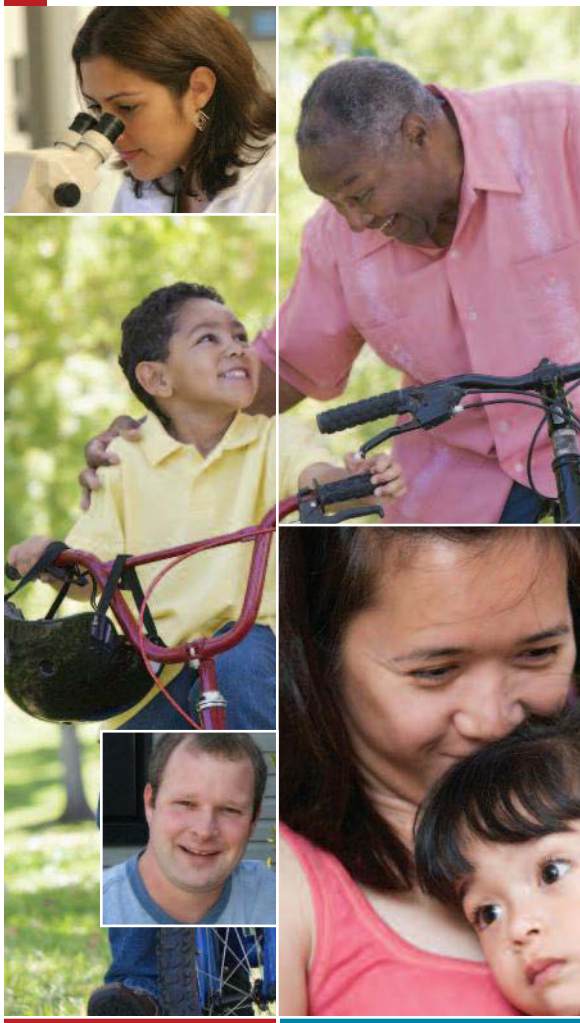
"The researchers are doing this trial so that, in the future, they can choose the right drug for the right person. The next person who is sitting where I am sitting today will get the treatment that works for him or her."

The development and distribution of this information was funded by Lilly Oncology.

For more information or to determine whether you are eligible to participate in a specific trial, please contact the following:

How You Can Help Adv Cancer Research

Providing Tissue Samp Part of a Clinical Trial



Research Advoca

Advancing Patient-Focu

Why might I want to provide tissue samples?

Your participation in a clinical trial with tissue samples could help improve the cancer treatments for future patients.

The drugs used to treat cancer today work for some patients but not for others. Patients and healthcare providers alike want improved and better-tailored cancer treatments for future generations. Doctors want to target the right treatment to the right person at the right time.

"I needed hope that the research might benefit me. I know now I am helping create tomorrow's medicines today. Clinical trials are how we get all of our drugs approved. The treatments I am offered today came from clinical trials where other patients like me participated and donated blood and tissue samples for research." — Patient

Before this can happen, however, researchers need to know more about how cancer cells respond to treatment. This is where you can help. Researchers critically need tissue samples from patients like you in order for cancer research to progress. They need to examine cancer cells in tissue samples provided before treatment, during treatment, and after treatment in order to understand how the cells change in response to therapy. Researchers can best determine this by sampling the cancerous tissue from actual patients several times over the course of a clinical trial.

Healthcare providers only ask patients to volunteer to provide tissue samples when it is considered safe and appropriate. However, it is important to discuss the risks with your doctor so that you can make an informed, voluntary decision that is right for you.

What does it mean to provide samples of my tissue for clinical research in cancer?

Tissue samples may be taken from a variety of different organs, such as lung, breast, bone, skin, liver, colon, bladder, or blood. Doctors use different types of procedures depending on the type of cancer, the site from which the tissue is to be obtained, the amount of tissue to be sampled, current levels of technology, and other factors.

Researchers then study the tissue samples to determine the answers to specific questions related to the treatment. In general, the results of the research studies conducted today will primarily benefit the patients of tomorrow. In a similar way, tissue samples provided by previous patients have resulted in advances in cancer treatment options that patients see today. This includes medications that target specific subtypes of cancer and tests that help determine how likely it is that cancer will recur. For example, researchers have developed certain tests for breast cancer that help determine a patient's risk that her breast cancer will recur and aid in her treatment decisions.

Definition:
Tissue is defined as a collection of similar cells that act together in doing a particular function in the body. When a portion of those cells is removed from the body for study, it is called a tissue sample or may be referred to as a biopsy.

What are some questions I may wa ask my doctor about providing tiss samples for research?

- What is the purpose of the research and you hope to learn?
- Am I likely to benefit from providing tiss ples?
- How will the tissue sampling procedure be performed?
- Will the tissue sampling procedure hurt?
- Is the tissue sampling procedure risky to me?
- How many tissue samples are you requesting of me? be taken at different times? If yes, how how often?
- What happens if I provide one tissue sa then decide I do not want to provide an Can I leave the study at any time?
- Do I have to make a special trip to the h clinic to provide the tissue sample or wi done as part of my regularly scheduled ments?
- Will these samples be used only in this stored for any other purpose in the futu
- How will my privacy (and my family's pr protected?
- Will I be able to find out the results of th conducted on my tissue samples?
- Will my insurance cover the costs or is t included in the study?

The answers to these questions depend o specifics of a trial and your situation and addressed by your healthcare team.