Title: RANDOMIZED PHASE II TRIAL OF NEOADJUVANT CISPLATIN VS. DOXORUBICIN/CYCLOPHOSPHAMIDE ("AC") IN WOMEN WITH NEWLY DIAGNOSED BREAST CANCER AND GERMLINE BRCA MUTATIONS

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The INFORM: BRCA1/2 Study:
Investigation of Neoadjuvant Chemotherapy For Mutation Carriers: BRCA1/2

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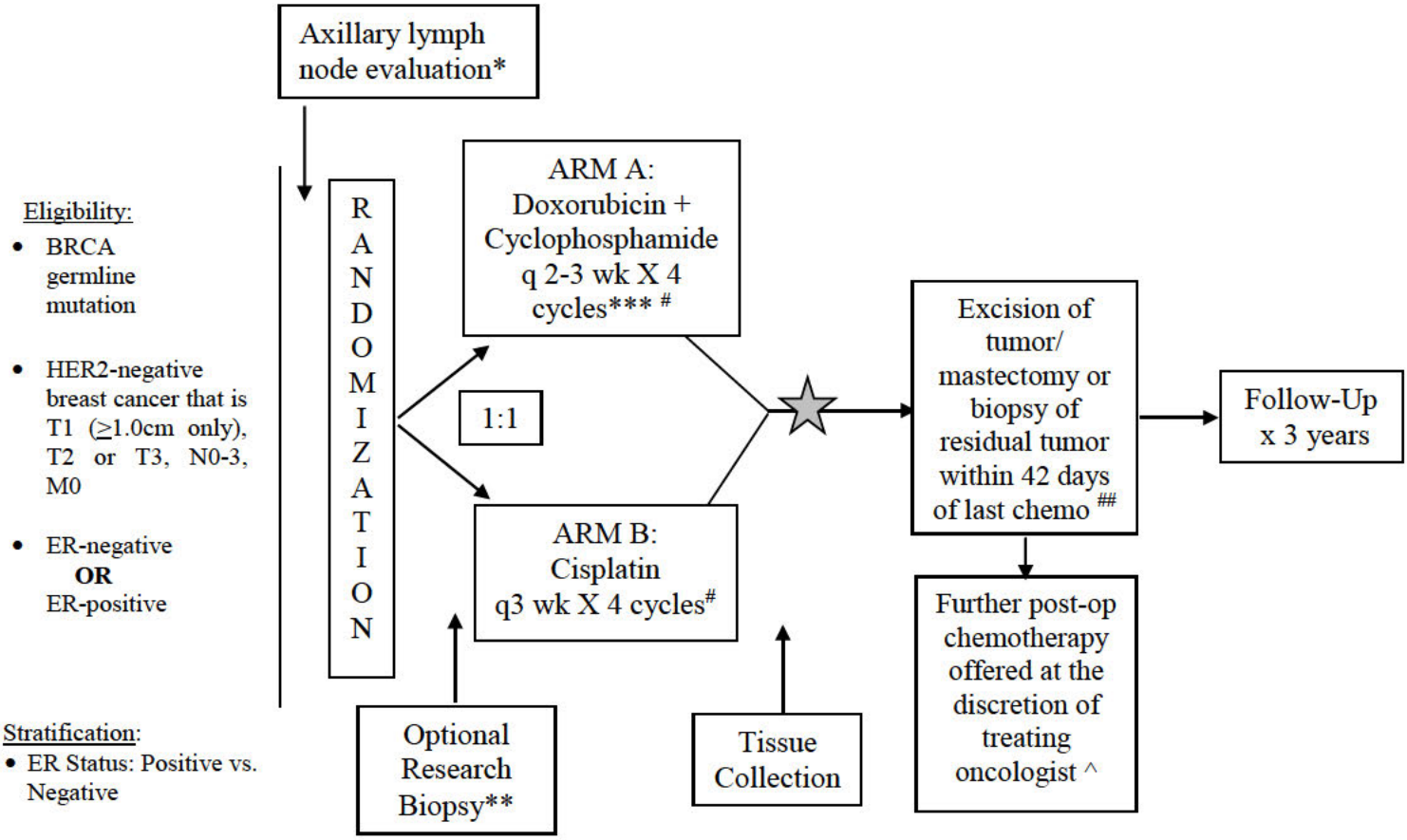
Agent(s):

Cisplatin- commercially available
Doxorubicin- commercially available
Cyclophosphamide- commercially available

SCHEMA



If clinically significant residual disease after 12 weeks of therapy patient may receive additional preoperative chemotherapy after biopsy.

* If there is no evidence of axillary involvement clinically (by exam and imaging) axillary node evaluation can occur before or after chemotherapy at the discretion of the treating physicians; if axillary nodes are clinically involved, FNA, core biopsy or axillary surgery should be performed prior to chemotherapy.

** Pre-treatment research core biopsies for correlative studies are optional and should be obtained prior to initiation of neoadjuvant chemotherapy (see section 5.3 and appendix E). If a clip was not placed previously, it should be placed at the time of the research biopsy.

*** ER-negative breast cancer must be treated with dose dense AC every 2 weeks with growth factor support; ER+ breast cancer may be treated every 2 or 3 weeks at the treating physician's discretion.

Growth factor support for Cisplatin and AC administered every 3 weeks is at the treating oncologist's discretion

While not mandatory, it is strongly recommended that a completion axillary dissection be performed at the time of definitive breast surgery if a positive node was detected prior to chemotherapy

^ Participants randomized to cisplatin should strongly consider anthracycline-based chemotherapy +/- taxane after surgery since long-term data using adjuvant cisplatin alone is not available.

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1. OBJECTIVES

1.1 Study Design

The study is a randomized phase II trial in women with breast cancer and a germline mutation in BRCA1 or BRCA2 comparing the pCR rate after doxorubicin (A) (60 mg/m2) and cyclophosphamide (C) (600 mg/m2) (AC) administered either every 2 or 3 weeks for 4 cycles (Arm A), to the pCR rate after neoadjuvant cisplatin (75 mg/m2) administered every 3 weeks for 4 cycles (Arm B). The goal is to enroll 170 women total, 85 to each arm of the study.

1.2 Primary Objective

The main goal of this study is to determine if the pathologic complete response (pCR) rate (determined by the Miller-Payne method [1]) [pCR in breast and nodes (i.e. RCB 0) or pCR in breast (i.e. Miller Payne 5) if nodes are not evaluable (i.e. positive nodes were removed surgically before chemo)] to neoadjuvant cisplatin is at least 20% greater than the pCR to doxorubicin/ cyclophosphamide (AC) in women with newly diagnosed breast cancer and a germline *BRCA* mutation.

1.3 Secondary Objectives

- To determine Residual Cancer Burden (RCB)[2] and compare the rates of RCB 0 as well as RCB 0 and RCB 1 (combined, with the inclusion of pCR in the breast when nodes are not evaluable, i.e. Miller-Payne 5 when positive nodes were removed prior to chemo) after neoadjuvant cisplatin or doxorubicin/ cyclophosphamide (AC) in women with newly diagnosed breast cancer and a germline *BRCA* mutation.
- To compare the rates of Miller Payne 4 (near pCR) and 5 (pCR) combined between those subjects who received neoadjuvant cisplatin and those who received neoadjuvant AC.[1]
- To determine the clinical response rate, defined as the number of partial and complete responses, after preoperative therapy with either cisplatin or AC in participants with a germline *BRCA* mutation and breast cancer.
- To compare the toxicities of cisplatin and AC preoperative chemotherapy in *BRCA* mutation carriers with newly diagnosed breast cancer.
- To collect pre-chemotherapy biopsies for future analyses of biomarkers that predict for response to cisplatin or AC chemotherapy in BRCA mutation carriers, including homologous recombination deficiency (HRD) using an assay developed by Myriad Genetic Laboratories.
- To determine if 3-year recurrence-free survival (RFS) is significantly better for germline BRCA mutation (gBRCAm) carriers with newly diagnosed HER2-negative breast cancer allocated to cisplatin vs.AC chemotherapy.
- To determine if 3-year RFS is significantly improved for gBRCAm carriers who achieved pCR compared with those who did not.

2. BACKGROUND

2.1 BRCA-related Breast Cancer

Breast cancers that develop in women with germline mutations in the genes *BRCA1* or *BRCA2* comprise approximately 5% of all breast cancers[3] and women with these mutations have up to a 90% chance of developing breast cancer.[4,5] *BRCA1* and *BRCA2* are essential to multiple aspects of DNA repair, including the repair of double strand (DS) DNA breaks through the process of homologous recombination (HR), and in preventing apoptosis.[6]

Approximately 80% of BRCA1-associated breast cancers lack estrogen receptor (ER), progesterone receptor (PR), and amplification of the growth factor receptor HER2, thus being known as "triple-negative" breast cancers (TNBC).[7,8] BRCA1-related breast cancers share several phenotypic and molecular features with sporadic TNBC, including low or absent expression of hormone receptors and HER2, high histologic and nuclear grade, high mitotic index and expression of basal cytokeratins and EGFR.[8-12] In addition, by gene expression profiling, the majority of BRCA1-related and sporadic TNBC cluster within the basal subgroup.[13] Furthermore, there seems to be a set of characteristic cytogenetic changes in *BRCA1*-associated breast cancers, a number of which have been described in sporadic basal-like cancers, including loss of 5q and frequent p53 mutations.[14-20]

BRCA1 is rarely mutated in sporadic breast cancers,[21] but the pathological and molecular parallels described above have led to speculation that BRCA1, or associated pathways, can become inactivated in triple-negative tumors via other mechanisms. In fact, recent studies have shown that a proportion of sporadic basal-like breast cancers may have a dysfunctional BRCA1 pathway due to gene promoter methylation or transcriptional inactivation. While there are conflicting reports regarding the function of BRCA1 and the methylation status of the corresponding gene promoter in relation to the phenotype of sporadic breast cancers,[22-24] one report did indicate that *BRCA1* mRNA expression was twofold lower in 37 basal-like sporadic breast cancers than in controls matched for age and grade ($p=0.008$).[25] Additionally, *ID4*, a negative regulator of BRCA1, was expressed at a 9-times higher level in basal-like breast cancers ($p<0.0001$).[25] These findings suggest that a BRCA1 deficiency might underlie the triple-negative phenotype in at least some sporadic cancers, but this deficiency might happen via several mechanisms.

Several studies have shown that basal breast cancers are more aggressive than other molecular subtypes of breast cancer and are associated with a shorter survival and increased chance of relapse. [9,26,27] However, basal-like breast cancers have also been found to be more responsive to doxorubicin containing preoperative chemotherapy than luminal and normal-like breast cancers.[28]

In contrast, there are no consistent pathologic features of *BRCA2*-associated breast cancers; like non-hereditary cancers, they are most often ER-positive (ER+). However, when compared to ER+ sporadic breast cancers, ER+ breast cancers in women with germline BRCA1 mutations are

more often of invasive ductal type with a high mitotic rate, pushing margins, and less tubule formation.[29,30]

2.2 Cisplatin in Breast Cancer

Platinum was studied in breast cancer in the 1970s and was shown to be active when given early in the course of the disease, but it was not adopted, perhaps because of the superior therapeutic index of other drugs then under development (the taxanes). The drug was initially tested in patients with advanced breast cancer, both as a single agent and in combination with other drugs. Small studies demonstrated objective response rates ranging from 42% to 54% with the use of cisplatin as a single agent, but response rates were lower in women who had received prior chemotherapy for metastatic disease.[31-33] When cisplatin was given after other chemotherapy, the response rate fell to 0-9%.[34-37]

Notably, these studies used cisplatin in patients regardless of ER, PR, and HER2 status. Several combination regimens were also explored, particularly cisplatin combined with taxanes, but there seemed little reason to continue these combinations when the taxanes were found to be so active and relatively nontoxic.[38] There was also interest in the evaluation of combined treatment with docetaxel and platinum. In several studies in unselected cases of metastatic disease, the overall response rate with this combination regimen was around 50%, even with prior adjuvant anthracycline therapy.[38]

There has recently been renewed interest in cisplatin for the treatment of breast cancer, in part because of improved strategies for managing its side effects, particularly nausea. A 2004 phase II study of preoperative paclitaxel and cisplatin demonstrated a 28% complete response rate and a 63% partial response rate in patients enrolled without regard to ER, PgR, and HER2 status[22].

Because of the many biologic similarities between BRCA1-associated breast cancers and sporadic (non-BRCA-associated) TNBC, and the rationale for cisplatin therapy in BRCA1 deficient breast cancers (see section 2.3 below), two prior DF/HCC studies have evaluated the pathologic complete response (pCR) rate of pre-operative cisplatin in TNBC. Silver et al demonstrated that six (22%) of 28 patients achieved pathologic complete responses, including both patients with *BRCA1* germline mutations; 18 (64%) patients had a clinical complete or partial response.[39] In the second trial, cisplatin and bevacizumab were administered pre-operatively to women with TNBC; both *BRCA1* and *BRCA2* mutation carriers achieved pCR, as did 15% of women without mutations.[40] Of note, in neither pre-operative cisplatin trial did women receive only cisplatin as their systemic therapy. All women received standard adjuvant chemotherapy after surgery, because there are no data that a pCR with cisplatin alone is sufficient to ensure good long-term outcome.

Several ongoing studies are evaluating the benefit of platinum agents in sporadic breast cancer. For example, the current CALGB neoadjuvant trial (CALGB 40603) in women with TNBC includes an assessment of the role of carboplatin in addition to standard AC/T chemotherapy with or without bevacizumab.

2.3 Neoadjuvant Chemotherapy

For patients with operable breast cancer, chemotherapy can be administered either after surgical resection of the cancer (i.e., adjuvant therapy) or prior to tumor excision, as neoadjuvant, or preoperative chemotherapy. While there is no survival advantage associated with use of preoperative compared to adjuvant chemotherapy[41], there are several theoretic advantages to neoadjuvant chemotherapy including: downstaging the cancer to allow breast preservation in a patient who would otherwise require mastectomy; the ability to assess response to chemotherapy; decreasing drug resistance by earlier exposure of the tumor (including micrometastases) to chemotherapy.[42] Several studies have shown that breast cancer patients, including those with basal-like breast cancers, who achieve a complete pathologic response (pCR) to preoperative chemotherapy have improved disease free and overall survival compared to those with residual disease at surgery. [42-46]

2.4 Cisplatin in BRCA-related Breast Cancer

As predicted by cell line, animal and human tumor data, and based on the recognition of the importance of BRCA1 and BRCA2 in DNA repair pathways, it has been hypothesized that BRCA-deficient cancers, including breast cancers, may be particularly vulnerable to chemotherapy, such as platinum agents, that create DS DNA breaks through interstrand cross-links.

Several groups have demonstrated that tumor cell lines (human breast and ovary) deficient in BRCA1 are unusually sensitive to the DNA cross-linking agents, including cisplatin and mitomycin, and that this sensitivity is reversed with either BRCA1 upregulation or restoration of BRCA1 function 4-8, 24.[47] In one study, treatment with cisplatin produced a dose-dependent reduction in cell growth in breast cell lines after 48 hours of treatment 8.[47-51] The BRCA1 defective cell line was 2-3 fold more sensitive to cisplatin compared with BRCA1 competent cell lines. This data suggests that cisplatin may be a good agent for BRCA1- mutated breast cancer.

A mouse model with knockout of p53 and BRCA1 in mammary tissue has been developed by Jonkers. These mice develop ER-negative, high grade invasive ductal cancers that stain for basal cytokeratins, similar to the BRCA1-associated breast cancers found in humans. The tumors that develop in these mice do not develop resistance to cisplatin, though resistance does develop to doxorubicin.[52,53]

Cisplatin has been shown to be effective in treating newly diagnosed breast cancer in *BRCA* mutation carriers in clinical trials, but data are limited. In a DF/HCC neoadjuvant trial using cisplatin in women with TNBC both women with *BRCA1* mutations in the trial achieved a pCR.[39] In a second trial using cisplatin and bevacizumab in women with TNBC, 2 of 7 *BRCA1* and 1 of 1 *BRCA2* mutation carriers achieved pCR.[40] These studies formed the basis of a trial of neoadjuvant cisplatin in women with *BRCA1* founder mutations in Poland. In that ongoing trial, 18 of 25 (72%) *BRCA1* carriers reported to date have had a pCR using cisplatin.[54] However, neither in the DF/HCC cisplatin trials nor in the Polish trial did women receive only cisplatin as their systemic therapy. All women received standard adjuvant chemotherapy after surgery, because there were and are no data that a pCR with cisplatin alone would be sufficient to ensure

good long-term outcome. Further, all three of these trials were single arm trials, so there have been no studies directly comparing response to cisplatin alone to response to more standard combination chemotherapy regimens.

Of note, almost no data exists regarding the efficacy of cisplatin in BRCA2-associated or BRCA-related ER-positive breast cancers. However, the previously mentioned Dana-Farber/Harvard Cancer Center (DF/HCC) study evaluating preoperative cisplatin in patients with TNBC included 3 patients with a BRCA2 mutation. Results showed that women with TNBC and a *BRCA2* mutation were responsive to cisplatin, but at least one progressed through treatment (unpublished data). The DF/HCC trial evaluating ciplatin in combination with bevacizumab in patients with TNBC also included one patient with a BRCA2 mutation; that patient achieved a pCR after cisplatin/bevacizumab therapy (manuscript pending).

Despite the lack of data addressing the efficacy of cisplatin in BRCA2 and/or BRCA-related ER-positive breast cancers, the same biologic defect thought to predict for the responsiveness of BRCA1-related breast cancers to cisplatin—a defect in homologous recombination (HR)—also exists in BRCA2-related breast cancers. In addition, poly(ADP-ribose) polymerase (PARP) inhibitors are novel agents that exploit a defect in HR through synthetic lethality. A phase 2 study evaluating olaparib, a PARP inhibitor, as monotherapy in women with germline BRCA mutations and advanced breast cancer resulted in a 41% response rate when used at the optimal dose.[55] Responses were achieved in both BRCA1 and BRCA2 breast cancers, underscoring the shared biologic defect in these cancers that also predicts for sensitivity to cisplatin. Likewise, responses were seen in both ER-negative and ER-positive BRCA-related breast cancers.

2.5 Tissue Biomarkers of Response to Cisplatin

In BRCA1 or BRCA2 germline mutation carriers, functionally relevant defects in homologous recombination are thought to occur only upon loss of the remaining wild type allele of either BRCA1 or BRCA2. Studies on carefully microdissected tumor samples and quantitative analysis of mutant vs. wild type allelic copy number have found that a majority (81-89%) of both ER+ and ER- breast cancers in BRCA1 mutation carriers have loss of the wild type allele of BRCA1 [56]. Thus, a sizable minority of tumors from BRCA mutation carriers does not have loss of the wild type BRCA1 and would not be expected to have functional defects in HR or be sensitive to drugs targeting defective HR such as cisplatin. Therefore, assays for loss of heterozygosity or loss of wt BRCA alleles may be predictive of response to cisplatin based therapy.

Loss of functional BRCA1 causes the spontaneous formation of triradial and quadriradial chromosome structures, which are cytologic indications of aberrant chromosome break repair and recombination [57]. The resolution of these chromosome rearrangements at mitosis can lead to loss of large regions of maternal or paternal chromosomes resulting in allelic imbalance (AI) extending from the breakpoint to the telomere [58,59]. In a recent study analyzing the pretreatment tumor tissue from two DF/HCC cisplatin-based neoadjuvant therapy trials in TNBC [39],[40], we report that the number of regions of telomeric allelic imbalance (NtAI) was significantly associated with cisplatin sensitivity as determined by pathologic response in both trials (Fig 1) [60].

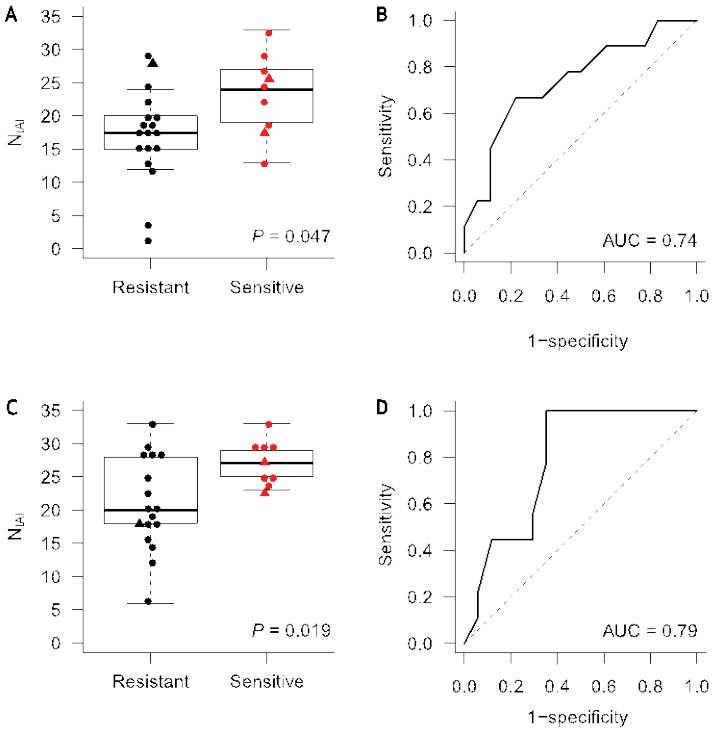

Figure 1. N_{tAI} and cisplatin response in breast cancer. In two clinical trials, TNBC patients were given preoperative cisplatin (Cisplatin-1, **Fig. 1 A-B**) or cisplatin and bevacizumab (Cisplatin-2, **Fig. 1 C-D**). Cisplatin resistant tumors are indicated in black, cisplatin sensitive tumors are indicated in red. Tumors with germline mutations in BRCA1/2 are indicated with triangles. **A and C**. Box plots showing N_{tAI} distribution in cisplatin resistant and sensitive tumors. **B and D.** Receiver operating characteristic curves showing the ability of N_{tAI} to predict for sensitivity to cisplatin.

Among the few tumors from BRCA1 or BRCA2 mutation carriers that were analyzed in this study, those that achieved a pCR also displayed a high NtAI in pretreatment tumor samples whereas tumors with low levels of NtAI were resistant to cisplatin. Although the case numbers are limited, the results suggest that NtAI may be also predictive of cisplatin response among BRCA mutation carriers. We performed a parallel analysis of the cisplatin trial samples to identify specific DNA copy number differences and gene expression (mRNA) differences associated with cisplatin sensitivity. We found that only copy number gain of chr 15q26 and overexpression of both Bloom helicase (BLM) and Fanconi Anemia complimentation group I (FANCI) from this chromosome region were consistently and significantly associated with increased sensitivity to cisplatin therapy in both trials (unpublished results).

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[61]

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.23 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 nonresponders 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[61].

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, HRDTAI, 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. A Mean HRD Score can be calculated using the arithmetic mean of the three individual HRD scores. In each cohort analyzed, the Mean HRD score provides superior performance, and as the Mean HRD score is not optimized to any particular cohort, it is may be more generalizable than any one testing method (Myriad, internal communication).

2.6 Doxorubicin and Cyclophosphamide "AC" in BRCA-related Breast Cancer

There is limited information regarding the effectiveness of combination alkylator and anthracycline-based chemotherapy in *BRCA*-associated breast cancers In a retrospective analysis of patient records conducted in Poland, Byrski et al found that 11 of 51 (22%) *BRCA1* mutation carriers treated for breast cancer with conventional neoadjuvant doxorubicin and cyclophosphamide ("AC") had a pCR.[62] Chappius et al reported that 4 (2 BRCA1 and 2 BRCA2) of 9 (44%) mutation carriers had a pCR to anthracycline-based neoadjuvant chemotherapy compared to 1 of 27 (4%) non-carriers (p=0.009), though the distribution of breast cancer subtype was not uniform between these groups.[63] In women with metastatic breast cancer, Kriege et al found no difference in the responsiveness of breast cancers to anthracycline chemotherapy between *BRCA1* mutation carriers and those without a mutation. *BRCA2* carriers, however, had a significantly increased response rate (89% vs 50%; *P*=.001), an unexpected result.[64]

Recently, Arun et al [65] reported a retrospective analysis of preoperative chemotherapy administered to 317 women who had undergone BRCA testing; 18% were *BRCA1* mutation carriers, 7% *BRCA2* carriers and 75% were non-mutation carriers. Ninety-five percent of the patients had received anthracycline-based chemotherapy. The pCR rate was significantly higher

in the BRCA1 carriers, 46%, compared to 13% in BRCA2 carriers and 22% in non-carriers (p = 0.0008). Interestingly, the pCR rate among triple negative breast cancers was not different between BRCA1 mutation carriers and non-carriers. In multivariate logistic regression, both ER-negativity and BRCA1 mutation were significant predictors of a pCR. The odds ratio for a pCR in BRCA1 carriers was 3.10 (p = 0.002).

The disparate results between these studies may be related to the retrospective nature of these studies as well as to the relatively small number of *BRCA* mutation carriers evaluated.

2.7 Tissue Biomarkers of Response to Anthracyclines

In 2010, we reported the identification of chr 8q22 amplification as a poor prognostic marker for ER-negative breast cancers despite adjuvant treatment with anthracycline-based chemotherapy. In vitro studies showed that siRNA knockdown of two genes from the 8q22 region, LAPTM4B and YWHAZ, resulted in increased sensitivity to anthracyclines but no change in sensitivity to taxanes or cisplatin. YWHAZ is a known anti-apoptotic protein possibly explaining its role in resistance to chemotherapy. Overexpression of LAPTM4B, a lysosomal protein, resulted in retention of the anthracycline, doxorubicin, in the lysosomal compartment, delaying its appearance in the nucleus. Finally, overexpression of these two 8q22 genes in pretreatment tumor biopsies was significantly associated with poor tumor response to neoadjuvant epirubicin monotherapy in women with primary ER-negative breast cancer (Li Y, Zou L.,Li Q, Haibe-Kains B, et al. Amplification of LAPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer. Nat Med. 2010 Feb;16(2):214-8.). The BRCA mutation status of the patients from this trial is unknown; however, this mechanism of anthracycline resistance is plausible for tumors arising in BRCA mutation carriers as well.

Tumor-associated Lymphocytes
Several studies have shown the association between lymphocytic infiltrates in breast cancer and improved outcomes, including within the TNBC subgroup[66,67].

Loi et al. demonstrated that after anthracyline and taxane -based adjuvant chemotherapy in the BIG 02-98 trial, those patients whose TNBC had a higher degree of lymphocytic infiltration had a significantly better disease-free and overall survival[66]. Likewise, a retrospective analysis of TNBC from ECOG studies 2197 and 1199 demonstrated that stromal lymphocytic infiltration was a strong and independent prognostic factor for improved DFS, DRFI and OS after anthracycline and taxane-based adjuvant therapy[67].

2.8 Rationale for Current Study

Due to the variable response rates reported with anthracycline-based chemotherapy in BRCA-associated breast cancers and the limited data using cisplatin in this population, it is important to directly compare cisplatin to a standard chemotherapy in women with *BRCA*-associated breast cancer. The fact that the small single-arm cisplatin trial [62,54] has led some to call for the addition or even the substitution of cisplatin or carboplatin to the adjuvant or neoadjuvant treatment of women with *BRCA* mutations seems premature and underscores the need for prospective, randomized data.

To date, germline BRCA testing in women with newly diagnosed breast cancer has provided information used primarily for decisions about surgical choices (i.e. prophylactic mastectomies and bilateral salpingo-oophorectomies) and surveillance practices (e.g., breast MRI). Thus far, BRCA status has not influenced the choice of chemotherapy and the same regimens are used to treat breast cancer in BRCA mutation carriers and non-carriers. Therefore, genetic testing is often performed during or after chemotherapy administration. If, however, a non-standard chemotherapy regimen such as cisplatin is demonstrated to be superior to current standard regimens, management of newly diagnosed breast cancer would change since earlier BRCA testing would be necessary in order to select the optimal chemotherapy.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Participant must have a confirmed germline **deleterious BRCA mutation.** Participants with a *BRCA1* or *BRCA2* classified as "variant, suspected deleterious" by Myriad Genetics are also eligible for the trial. Participants with only a *BRCA1* or *BRCA2* VUS (variant of uncertain significance) are not eligible for this study.

If a potential subject is considered high risk for carrying a BRCA1/BRCA2 mutation by NCCN criteria but does not have insurance coverage for testing or if results from available testing options will not be ready in time for enrollment in the study Myriad Genetic Laboratories may cover the cost of the test. Genetic testing does not have to be performed by Myriad Genetic Laboratories but a study-specific test request form is available for tests submitted to Myriad. This form may also be used for genetic testing which will be covered by the participant's insurance and may lead to more expedited testing.

3.1.2 **Histologic documentation**: Pathologic confirmation of invasive breast cancer by core or surgical biopsy (FNA alone is not adequate).

3.1.3 **Stage**: Clinical T1 $\geq$ 1.0 cm, T2 or T3, N0-3, M0.

Participants with multicentric or bilateral disease are eligible if at least one lesion meets stage eligibility criteria for the study (i.e., $\geq$1.0 cm operable breast cancer) and no tumor is HER2-positive (3+ by IHC or ISH amplified $\geq$ 2.0). 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.

3.1.4 **HER 2 status:** Tumors must be HER2 negative defined as HER2 0 or 1+ by immunohistochemical (IHC) assays and /or lack of gene amplification by FISH defined as a ratio < 2 on invasive tumor. A tumor is considered HER2+ if 3+ by IHC or ISH amplified $\geq$ 2.0. (For questions about HER2 status, investigators are advised to refer to the most recent ASCO guidelines.[68])

3.1.5 **ER and PgR** status by immunohistochemistry must be known. ER positive tumors are allowed in patients for whom the treating investigator has determined neoadjuvant chemotherapy is appropriate.

3.1.6 Breast imaging should include imaging of the ipsilateral axilla. For subjects with a clinically negative axilla, a sentinel lymph node biopsy will be performed either up front or after preoperative therapy at the discretion of the subject's physicians; for subjects with a clinically positive axilla, a needle aspiration, core biopsy or SLN procedure will be performed prior to registration to confirm the presence of metastatic disease in the lymph nodes. While not mandated by the protocol, it is strongly recommended that participants with positive lymph nodes undergo a level I and II lymph node dissection at the time of definitive surgery.

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

3.1.7 Cardiac ejection fraction (LVEF) $\geq$ institutional lower limit of normal by MUGA/RVG or ECHO

3.1.8 Women or men $\geq$ 18 years of age

3.1.9 ECOG performance status 0 or 1

3.1.10 Laboratory Evaluation

• Absolute neutrophil count (ANC)	$\geq$ 1,500 / mm^3
• Platelet count	$\geq$ 100,000/ mm^3
• Bilirubin	$\leq$ 1.5 x institutional upper limits of normal (ULN); if patient has documented Gilbert's syndrome bilirubin must be < 3 X institutional ULN
• ALT, AST, ALK Phos	$\leq$ 2.5 x institutional ULN
• Glucose	< 200 mg/dl
• Hemoglobin	$\geq$ 9 g/dl
• Creatinine OR	$\leq$ 1.5 mg/dl
• Creatinine clearance (can be calculated by 24 hour urine or by the Cockroft-Gault formula in Appendix B)	$\geq$ 60 cc/min

3.1.11 Life expectancy of greater than six months

3.1.12 Use of an effective means of contraception is required in subjects of childbearing potential since cisplatin, doxorubicin and cyclophosphamide 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 and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation

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

3.1.14 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.

3.2 Exclusion Criteria

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

3.2.1 Any prior anthracycline or platinum based therapy at any time.

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

3.2.3 Ipsilateral breast recurrence, unless prior treatment consisted of excision alone for DCIS or breast-conserving treatment and hormonal therapy for DCIS or invasive cancer.

3.2.4 Peripheral neuropathy of any etiology that exceeds grade 1

3.2.5 Significant hearing loss that would prevent cisplatin administration.

3.2.6 Renal dysfunction for which exposure to cisplatin would be unsafe or require cisplatin dose modification (i.e., Cre > 1.5 mg/dl or GFR < 60 cc/min).

3.2.7 Use of any other investigational or study agents.

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

3.2.9 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.

3.2.10 Any condition that would prohibit administration of corticosteroids

3.2.11 Pregnancy or breast-feeding because the chemotherapy agents in this study have the potential for teratogenic or abortifacient effects as well as unknown but potential risks in nursing infants.

3.2.12 Uncontrolled diabetes (If non-fasting blood sugar > 200 mg/dl, perform a fasting blood sugar which must be ≤ 200mg/dl).

3.2.13 Any pre-existing medical condition that would represent toxicity in excess of grade 1 as measured by CTCAE (NCI Common Toxicity Criteria for Adverse Events version 4.03) unless the symptom is not considered medically significant by the treating investigator (e.g., alopecia)

3.2.14 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.

3.3 Inclusion of Women, Minorities and Other 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 men and women.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (ODQ) central registration system. Registration must occur prior to the initiation of therapy. 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 ODQ 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 registration on the study may be canceled. Notify the ODQ Registrar of registration cancellations as soon as possible.

4.2 Registration Process for Dana Farber/Harvard Cancer Center (DF/HCC) and Dana Farber/Partners Cancer Care (DF/PCC) Institutions

The ODQ registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the ODQ registration line at [redacted] and follow the instructions for registering participants after hours.

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 ODQ 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 [redacted]

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

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

4.3 Guidelines for External and 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 Study Coordinator should be notified of cancellations as soon as possible.

4.4 Registration Process for External and TBCRC Sites

To register a participant, the following documents should be completed by the research nurse or data manager and emailed to the Project Manager at [redacted] or faxed to [redacted]

- Confirmation of BRCA1 and/or BRCA2 mutation
- Copy of required laboratory tests including: Hematology (CBC w/differential), Serum Chemistries (potassium, BUN, creatinine, magnesium, total bilirubin, SGOT (AST), SGPT (ALT), and Alkaline Phosphatase, and pregnancy test (for women of child-bearing potential only).
- Signed informed consent form
- HIPAA authorization form (if separate from the informed consent document)
- Completed ODQ Eligibility Checklist
- Breast imaging documentation (MRI/US/Mammogram)
- MUGA or Echocardiogram report
- EKG report
- Pathology report and documentation of ER/PR and HER2+ status

- Clinic visit note documenting history and physical exam

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

NOTE: Registration and randomization with the ODQ 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.

5. TREATMENT PLAN

5.1 Randomization

Participants will be randomized to either preoperative cisplatin or doxorubicin plus cyclophosphamide (AC) in a ratio of 1:1 using block randomization. Randomization will be stratified by ER status (positive or negative).

5.2 Pre-chemotherapy Surgical Evaluation

Prior to neoadjuvant chemotherapy, some clinicians may choose to determine the pathologic axillary node status. This can be done by whatever means the surgeon feels is appropriate. This decision should be made in consultation with the treating surgeon and radiation therapist. For participants with clinically involved axillary nodes by exam or imaging, axillary metastases should be confirmed by FNA, core biopsy or axillary surgery prior to chemotherapy.

5.3 Pre-chemotherapy Research Core Biopsy (see Appendix E)

Research breast core biopsies of the target lesion for all participants prior to initiating protocol chemotherapy are desired, but are **optional**. **It is strongly recommended that core biopsies be image-guided.** A clip should be placed in the target lesion at the time of diagnostic biopsy. If it was not, a clip should be placed at the time of the research biopsy. If staging is to be done, research biopsy is to be performed after staging is completed.

Ideally six (6) core biopsies will be obtained:

- Two cores should be frozen in OCT; each core should be frozen in a separate cassette. The individual preparing the frozen cores should read appendix E and watch the teaching video which has been made available to participating sites. (see [redacted]

- Two core biopsies in an RNA Later specimen tube supplied by the study.

- Two additional core biopsies should be placed in a 10% neutral buffered formalin tube supplied by the study.

The order of specimen collection should be: the 1st core should be frozen and OCT embedded; the 2nd core should be placed into RNA Later; the 3rd core should be placed into formalin; the 4th core should be frozen and OCT embedded; the 5th core should be placed into RNA Later; and the 6th core into formalin. Each core frozen in OCT must be placed in its own cassette. The two cores for RNA Later may be placed in the same specimen container. The two cores to be placed in formalin may also be placed in the same formalin specimen container.

All biopsy cores should be shipped overnight to:

Attn: DF/HCC Core Blood and Tissue Bank
Dana Farber Cancer Institute
[redacted]

All samples should be labeled with the Participant initials, Participant Study ID number and the date of procedure. The DFCI laboratory staff will de-identify all samples and will assign each specimen a unique sample ID number.

Please email DF/HCC Core Blood and Tissue Bank and the DFCI BOC Inform Study Coordinator with the sample information and tracking information the day before shipping specimens.

[redacted]

Please include a copy of the 12-258 Specimen Requisition (Research Biopsy and Blood) form with your shipment which can be found in Appendix H.

**Please note that slides and paraffin blocks of the diagnostic biopsy and definitive tumor excision to be used for tumor response should be sent to a different address- see section 9.5. The address listed here is for fresh tissue research biopsies for correlative studies.

The coordinating center study coordinator will track biopsy specimens using CaTissue. Upon receipt of an email notification of sample shipment, the coordinating center study coordinator will log in the information into CaTissue. When the tissue sample is received by the staff in the DF/HCC Core Blood and Tissue Bank, the confirmation of receipt and specimen location information will be added to CaTissue. CaTissue will store a complete record of the biopsy samples that are collected and analyzed as part of this study.

All research biopsy samples should arrive during the week by Friday morning. If a biopsy must be performed at one of the non-Boston sites on Friday, the specimens should be stored over the weekend and shipped on Monday. Frozen specimens should be stored in a -80 freezer until shipment; specimens in RNA Later and formalin should be stored at room temperature until shipment.

The frozen cores should be shipped on dry ice. Cores in RNA Later and in formalin should be shipped at room temperature. It is recommended that all research biopsies 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 RNA Later and formalin.

All biopsy samples will be stored in the DF/HCC Core Blood and Tissue Bank indefinitely. Protocol defined assays will be performed during the course of this study. If any tissue remains after the protocol-defined assays are complete, it may be used for future correlative studies until all supplies are exhausted.

5.4 Overview of Preoperative Chemotherapy

This study includes a preoperative phase with participants randomized to either cisplatin or doxorubicin and cyclophosphamide (AC), followed by surgery. Treatment will be administered on an outpatient basis. Protocol treatment must begin with 7 days of randomization. Expected toxicities and potential risks as well as dose modifications for cisplatin, doxorubicin and cyclophosphamide are described in Section 6 (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.

Preoperative Chemotherapy

Arm A "AC":
Doxorubicin: 60 mg/m^2 IV q 2-3 weeks* x 4 cycles **plus**
Cyclophosphamide: 600 mg/m^2 q 2-3 weeks* x 4 cycles

OR

Arm B:
Cisplatin: 75 mg/m^2 IV q 3 weeks x 4 cycles

5.5 Pre-treatment Criteria

Requirements for Day 1 for any cycle for cisplatin or AC:

- ANC must be $\geq$ 1,000 /mm^3
- Platelets must be $\geq$ 100,000 /mm^3

- Creatinine must be ≤ 1.5 mg/dl or creatinine clearance ≥ 50 cc/min. (can be calculated by 24 hour urine or by the Cockroft-Gault formula in Appendix B)
- All non-hematologic toxicities must be ≤ grade 1 (unless considered medically insignificant by the treating investigator)

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.

5.6 Agent Administration

Note: Doses should be based on actual body weight. The participant should be weighed each cycle.

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

5.6.1 Arm A: Doxorubicin, Cyclophosphamide (AC)

Table 5.1: Arm A: Doxorubicin plus Cyclophosphamide (AC)

Agent	Pre-medications; Precautions	Dose	Route	Schedule	Supportive Therapy
Doxorubicin	N/A	60 mg/m^2	IV push or infuse according to local policy	q 2-3 weeks* x 4 cycles	• GCSF if receiving AC q 2 weeks** • Anti-emetic therapy at MD discretion
Cyclophos-phamide	N/A	600 mg/m^2	IV infusion according to local policy	q 2-3 weeks* x 4 cycles	

*Scheduling of AC: Participants with ER-negative breast cancers randomized to Arm A must be treated with AC on an every 2 week schedule. For ER-positive breast cancers, the choice of a two or three week schedule for AC is left to the treating physician's discretion.
** GCSF [i.e Filgrastim (5 µg/kg SQ) days 3-10, pegfilgrastim (6 mg SQ) day 2 of each cycle, filgrastim-sndz or other biosimilars per institutional guidelines] is mandatory if AC is administered every 2 weeks. If AC is administered every 3 weeks, growth factor may be administered at the physician's discretion.

- **Dose**: Doxorubicin 60 mg/m^2 IV bolus plus
  Cyclophosphamide 600 mg/m^2 IV

- **Scheduling of AC**: Participants with ER-negative breast cancers randomized to Arm A must be treated with AC on an every 2 week schedule. For ER-positive breast

cancers, the choice of a two or three week schedule for AC is left to the treating physician's discretion.

- AC chemotherapy may be administered per institutional guidelines.
- Supportive Care:
    - **GCSF** is mandatory if AC is administered every two weeks. If AC is administered every 3 weeks, growth factor use is optional. GCSF may include Filgrastim (5µg/kg) days 3-10, pegfilgrastim (6 mg SQ) day 2 of each cycle, filgrastim-sndz or other biosimilars per institutional guidelines.
    - **Anti-emetics:** subjects may be given **anti-emetics** per institutional guidelines.

5.6.2 Arm B: Cisplatin

Table 5.2: Arm B: Cisplatin

Agent	Pre-medications; Precautions	Dose	Route	Schedule	Supportive Therapy
Cisplatin	Hydration and magnesium supplementation are given per institutional guidelines	75 mg/m^2	IV bolus over 60 minutes	q 3 weeks for 4 cycles	• Post-treatment hydration • Growth factors at MD discretion • Magnesium and/or potassium supplementation at MD discretion • Antiemetic therapy

*See section 5.6.2 for an example Cisplatin treatment schedule and pre-medication details.
** Use of GCSF is left to the treating physicians' discretion.

- **Dose: Cisplat**in 75 mg/m^2 IVB q 3 weeks for 4 cycles
- **Cisplatin may be administered according to institutional guidelines** at participating sites. An example schedule for Cisplatin administration is available upon request.
- **Supportive Care:** 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.
    - **Magnesium supplementation**: At the physician's discretion, participant may take 500mg **Magnesium Gluconate QD f**or 7 days (or equivalent). Magnesium level will be checked on **day 7-10 and if < 1.8mg/**dl, then magnesium supplementation should be continued and **adjusted at the physicia**n's discretion. The schedule of supplementation is at the treating physician's discretion and is not mandatory.

- **Potassium supplementation**: Patient may be given Potassium 30-40 mEq/day PO for 7 days after each dose. Potassium level will be checked on day 7-10. If the measurement is < 3.5 mmol/L, then potassium supplementation should be continued and adjusted at the physician's discretion. The schedule of supplementation is at the treating physician's discretion and is not mandatory.
- **Antiemetic therapy for cisplatin:** Subjects should receive prophylactic anti-nausea medications according to ASCO guidelines for highly emetogenic chemotherapy regimens.
- **Growth factor support:** GCSF (i.e. Filagastrim, pegfilgrastim,filgrastim-sndz or other biosimilars per institutional guidelines) is not required after cisplatin; however, if it is indicated it can be administered at the physician's discretion.

5.7 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 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.
- Any clinical H&E slides from the biopsy will be reviewed by the study team in lieu of tumor excision. This is encouraged, but not required.

Complete 12-258 SPECIMEN REQUISITION (Research Biopsy and Blood) form found in Appendix H and include in the shipment. A collection and shipment kit will be provided by the study.

See section Appendix E for tissue labeling and documentation.

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

Attn: DF/HCC Core Blood and Tissue Bank
Dana Farber Cancer Institute
[redacted]

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

Please email DF/HCC Core Blood and Tissue Bank and DFCI BOC Inform Study Coordinator with the sample information and tracking information the day before shipping specimens.

[redacted]

5.8 Additional Pre-operative Chemotherapy

If there is clinical or radiographic evidence of significant residual disease after completion of protocol treatment, after biopsy the patient may receive additional preoperative chemotherapy. The selection of therapy is determined by the treating provider, however it is strongly encouraged for patients who had been on the doxorubicin + cyclophosphamide 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 additional chemotherapy and response to the additional chemotherapy.

5.9 Definitive Breast Surgery

Definitive breast surgery (excision and/or mastectomy) must be performed no later than 42 days from administration of the last chemotherapy. Participants who did not have axillary node evaluation prior to chemotherapy must have ipsilateral axillary nodes evaluated at the time of surgery. While not mandated by the protocol, it is strongly recommended that a completion 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.

5.10 Post-operative Chemotherapy

Definitive breast cancer surgery (excision or mastectomy) marks the end of protocol mandated therapy. Decisions regarding choice of post-surgical additional chemotherapy will be made by the treating oncologist. However, participants randomized to cisplatin should receive anthracycline-based chemotherapy with or without a taxane after surgery since long-term data using single-agent cisplatin as neoadjuvant treatment of breast cancer is not available.

5.11 General Concomitant Medication and Supportive Care Guidelines

5.11.1 Supportive Care 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.

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

5.11.3 Intravenous Fluids

Subjects may receive intravenous fluids on study. Institutional guidelines should be followed for AC and cisplatin. Specific guidelines for cisplatin (one possible regimen) are outlined in section 5.6.2.

5.11.4 Growth Factors

Growth factor support with GCSF (i.e. filgrastim, pegfilgrastim, filgrastim-sndz or other biosimilars per institutional guidelines) must be used during treatment with AC if administered every two weeks. Growth factor support may be used with cisplatin or AC administered every 3 weeks, at the treating physician's discretion.

Erythropoietin and related congeners may be administered for anemia associated with therapy or otherwise at the discretion of the treating physician.

5.12 Duration of Therapy

Preoperative therapy with cisplatin or AC is planned for 8-12 weeks (i.e., 8 weeks for dose dense AC and 12 weeks for cisplatin or AC administered every 3 weeks). Preoperative therapy will be followed by surgery no later than 42 days after the last dose of AC or cisplatin.

5.13 Criteria for Removal from Protocol Therapy

Participants will be removed from the protocol therapy for any of the following:

- Any grade 4 non-hematologic toxicity
- Progression of tumor by physical exam or imaging study
- Treatment delay of more than 3 weeks for any toxicity-related reason
- A toxicity develops which, in the opinion of the investigator, precludes further therapy, or
- For safety reasons the investigator considers it to be in the best interest of the participant that they be withdrawn, or
- Participant decides to withdraw consent from the study
- Patient non-compliance

The reason for treatment 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.

A ODQ Treatment Ended & Off Study Form should also be filled out when a participant completes study treatment and again when they come off study. Participants are considered off treatment either on the date of the last protocol chemotherapy treatment or the date a cross-over (alternative) treatment decision was made.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Nadine Tung MD at [redacted] and the Coordinating Center designee.

5.14 Duration of Follow Up

All patients will be followed for 3 years after surgery or until death, whichever occurs first. 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.

During follow up, information on survival, recurrence, additional treatment, surgery, and radiation will be collected from a participant's medical record via progress notes and results from any routine tests or procedures, when available. For participants that are no longer being seen at their treating institution, they may be contacted directly for survival assessment and to report any further anti-cancer treatment, annually for a total of 3 years. A participant's current treating physician or designee may also be contacted for this follow up information. The questionnaire in Appendix I outlines the specific information that should be captured by the study team.

Adverse events will be followed until 30 days after last dose of protocol treatment. Any treatment-related grade 3 or 4 AEs present 30 days after the last dose of protocol treatment should be followed until resolved to $\leq$ grade 2. This AE follow up can occur via phone call.

5.15 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Three year follow up completed
- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

For Centralized Subject Registrations, the research team submits a completed Off Treatment/Off Study form to ODQ when a participant comes off study. This form can be found on the ODQ website or obtained from the Coordinating Center.

6. 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 of the NCI Common Terminology Criteria for Adverse Events (CTCAE version 4.03) 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.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. 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. Participants may not come off study until all treatment related AEs have resolved to ≤ grade 2.

6.1 Anticipated Toxicities

6.1.1 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 and mannitol diuresis 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**.** An audiogram is recommended after a cumulative dose of 300 mg/m2. Subsequent doses of cisplatin should not be given until an audiometric analysis indicates that auditory acuity is within normal limits.

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 subject 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
Cisplatin neurotoxicity is characterized by peripheral neuropathies, which are sensory in nature but can also include motor difficulties such as reduced deep-tendon reflexes and leg weakness. The symptoms usually occur after prolonged therapy (4 to 7 months). Cisplatin therapy should be discontinued when serious neuropathy develops. The neuropathy, however, may progress further even after discontinuation of treatment.

Drug Interactions
Aminoglycosides: Cisplatin may enhance the nephrotoxic effect of Aminoglycosides. Loop Diuretics: May enhance the nephrotoxic effect of Cisplatin. Loop Diuretics may enhance the ototoxic effect of Cisplatin. Phenytoin: Cisplatin may decrease the serum concentration of Phenytoin.

6.1.2 Doxorubicin

Hematologic
Leucopenia (dose-limiting), thrombocytopenia, anemia nadir in 10-14 days with recovery usually in 21 days. In this study, doxorubicin is given every 14 days, as dose dense AC(ddAC). Growth factor support is required.

Dermatologic
Alopecia (usually complete; reversible); radiation recall reactions; increased sensitivity to sunlight.

Gastrointestinal
Nausea and vomiting (doxorubicin is generally considered moderately to highly emetogenic), anorexia, diarrhea, mucositis (stomatitis, esophagitis).

Cardiovascular
Cardiomyopathy may occur and is related to total cumulative lifetime dose. This risk for cardiomyopathy increases with total doses greater than 450 mg/m2.

ECG changes and less often, arrhythmias are seen. Rarely, sudden death has occurred.

Other
Red discoloration of urine for 24-48 hours after drug administration.
Doxorubicin is a vesicant and can cause tissue necrosis if extravasated.

6.1.3 Cyclophosphamide

Myelosuppression

Primarily leucopenia, but also thrombocytopenia.

Gastrointestinal
Nausea and vomiting (cyclophosphamide is considered moderately to highly emetogenic; onset of symptoms is somewhat delayed at 6-10 hours after administration).

Genitourinary
Sterile hemorrhagic cystitis (related to the accumulation of the acrolein metabolite in the bladder; minimize with hydration).

Endocrine
Amenorrhea and possible sterility

6.2 Toxicity Management

All toxicities should be graded according to the Common Terminology Criteria for Adverse Events (version 4.03).

The goal is to attempt to administer full doses of chemotherapy on schedule. All dose reductions are maintained in subsequent cycles. Any participant whose treatment is delayed must be evaluated on a weekly basis until adequate hematologic and non-hematologic parameters have been met.

Once a chemotherapy dose has been decreased for toxicity, that dose should be maintained through subsequent cycles and should not be re-escalated.

No more than two dose reductions are allowed. If further dose reduction is required, participant should be removed from the study.

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

6.2.1 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 /mm^3
- Platelets must be $\geq$ 100,000 /mm^3
- Creatinine $\leq$ 1.5 mg/dl or creatinine clearance $\geq$ 50 cc/min (can be calculated by 24 hour urine or by the Cockroft-Gault formula in Appendix B)
- All non-hematologic treatment-related toxicities must be $\leq$ grade 1 (unless considered medically insignificant by the treating investigator). See table 6.2.2.

6.2.2 Dose modifications for Hematologic toxicity (cisplatin and AC)

Toxicity	Grade	Guideline for management	Dose modification
HEMATOLOGIC (Occurring any time during previous cycle or on Day 1 of current cycle)			
Neutropenia (based	Grade 3 (which resulted in		Delay all treatment

Toxicity	Grade	Guideline for management	Dose modification
on ANC, not WBC)	a delay of ≤ 1 week)		until ANC ≥ 1000/mm^3 and treat at previous dose level.
	Grade 4* OR Any grade resulting in a delay of next cycle by > 1 week due to ANC* OR Febrile Neutropenia: 1st occurrence* ***without** use of growth factor in prior cycle	Begin growth factors	Delay all treatment until ANC ≥ 1000/mm^3 and treat at previous dose level.
	Grade 4* OR Any grade resulting in a delay of next cycle by > 1 week due to ANC* OR Febrile Neutropenia: 1st occurrence* ***with** use of growth factors		Delay all treatment until ANC ≥ 1000/mm^3 and reduce dose 25%.
	Grade 4* OR Any grade resulting in a delay of next cycle by > 1 week due to ANC* OR Febrile Neutropenia: 2nd occurrence* ***with** use of growth factors		Delay all treatment until ANC ≥ 1000/mm^3 and reduce dose 25%.
	Grade 4* OR Any grade resulting in a delay of next cycle by > 1 week due to ANC* OR Febrile Neutropenia: 3rd occurrence*		Discontinue protocol therapy.

Toxicity	Grade	Guideline for management	Dose modification
	***with** use of growth factors		
Platelets	Grade 1-2	Re-check CBC weekly (or more often at physician's discretion)	Delay all treatment until Platelets ≥ 100,000/mm^3 and treat at previous dose level.
	Any grade resulting in a delay of > 1 week)		Delay all treatment until Platelets ≥ 100,000/mm^3 and reduce dose by 25%.
	Grade 3 or 4: 1st occurrence		Delay all treatment until Platelets ≥ 100,000/mm^3 and reduce dose 25%.
	Grade 3 or 4: 2nd occurrence		Delay all treatment until Platelets ≥ 100,000/mm^3 and reduce dose 25%.
	Grade 3 or 4: 3rd occurrence		Discontinue protocol therapy.
NON-HEMATOLOGIC			
Diarrhea (on Day 1 of new cycle or anytime during previous cycle)	Grade 1		No dose modifications
	Grade 2		Delay all treatment until diarrhea has resolved to Grade 1.
	Grade 3 or 4		Delay all treatment until diarrhea has resolved to Grade 1. Reduce all treatment doses by 25%.
Bilirubin (on Day 1 of a new cycle)	Grade 2 (> 1.5-3.0 x ULN)		Decrease doxorubicin dose by 50%
	Grade 3 (> 3.0-5.0 x ULN)		Decrease doxorubicin dose by 75%

Toxicity	Grade	Guideline for management	Dose modification
	Grade 3 or 4 (> 5.0 x ULN)		Delay doxorubicin and cyclophosphamide until bilirubin ≤ 5.0 x ULN and then reduce dose by 75%.
Neurosensory Toxicity (on Day 1 of a new cycle)	Grade 0-1		No dose modification
	Grade 2		Delay cisplatin until neuropathy is ≤ grade 1. Reduce cisplatin dose by 50%.
	≥ Grade 3		Discontinue protocol therapy.
Tinnitus/ Significant Hearing Loss	≥Grade 2		Delay cisplatin until tinnitus or hearing loss has resolved to ≤ grade 1. Dose reductions are at the discretion of the treating physician.
Mucositis	≥ Grade 3		Delay treatment until mucositis ≤ grade 1. Reduce cisplatin dose by 25%; reduce doxorubicin and cyclophosphamide doses by 20%.
Nephrotoxicity (creatinine)	See section 6.2.3		

Toxicity	Grade	Guideline for management	Dose modification
Cardiac: If the participant has any of the following: ▪ Symptoms of CHF and a diagnosis of CHF is confirmed ▪ Myocardial infarction ▪ > 15% decline in LVEF from baseline, or > 10% decline from baseline to below lower limits of normal			Discontinue protocol therapy
Cardiac: PACs or PVCs without cardiac dysfunction during and shortly after doxorubicin infusion			No dose modification
Fatigue			No dose modification
Alopecia			No dose modification
Transaminase elevations			No dose modification
Nausea and/or vomiting			No dose modification
Other non-hematologic	Grade 1		No dose modification
	Grade 2		All non-hematologic treatment-related toxicities must be ≤ grade 1 (unless considered medically insignificant by the treating investigator)**
	≥ Grade 3		Delay treatment until toxicity improves to ≤ grade 1. Reduce cisplatin dose by 25%; reduce doxorubicin and cyclophosphamide

Toxicity	Grade	Guideline for management	Dose modification
			doses by 20%. **

**Veno-thrombotic events are excluded from dose modifications; dose modifications are per investigator discretion

6.2.3 Nephrotoxicity (Cisplatin only; no dose modification for AC)

Cisplatin may be associated with renal toxicity. The likelihood of this toxicity may be decreased by adequate hydration and prevention of nausea and vomiting.

In the event that Creatinine is > 1.5 mg/dl, measure or calculate creatinine clearance. If creatinine clearance ≥ 50 cc/min, chemotherapy may be administered. If creatinine clearance < 50 cc/min the creatinine (+/or creatinine clearance) should be checked weekly until the creatinine is ≤ 1.5 mg/dl and/or creatinine clearance is ≥ 50 cc/min.

If chemotherapy is delayed > 1 week because of decreased renal function, the cisplatin dose should be reduced in the following manner:

- 1st dose reduction: Reduce Cisplatin by 25% (56 mg/m^2)
- 2nd dose reduction: Reduce Cisplatin by 50% (of initial dose) (37 mg/m^2)
- If 3rd dose reduction is required, participant must be removed from study.

If the Creatinine is still > 1.5 mg/dl but ≤ 2.0 mg/dl and creatinine clearance is < 50 cc/min but ≥ 40 cc/min after treatment has been held for two weeks, the treating physician may consult the PI to discuss proceeding with cisplatin dose reduction of 50%. No cisplatin will be administered if Creatinine is > 2.0 or creatinine clearance is < 40 cc/min.

6.2.4 Hypersensitivity Reaction

Hypersensitivity reactions including anaphylaxis have been reported within minutes of administering cisplatin. **If a hypersensitivity reaction occurs, the patient must be evaluated by appropriate clinicians per institutional standards prior to any further cisplatin administration**. Subsequent cisplatin administration should be administered through a desensitization protocol under the supervision of appropriate clinicians.

7. DRUG FORMULATION AND ADMINISTRATION

- 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.

- 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.

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

7.1 Availability and Preparation

Cisplatin
Commercially available cisplatin injection (1 mg/ml): Each ml of sterile, unpreserved solution contains: cisplatin 1.0 mg of cisplatin with 9 mg of sodium chloride and 1 mg of mannitol in water for injection. Hydrochloric acid is added to adjust the pH. Single-dose glass vials of 10, 50, and 100 ml contain 10, 50, and 100 mg of cisplatin, respectively. None of the injection components, such as IV needles, syringes, and sets, should have aluminum components, because aluminum is incompatible with cisplatin. Please refer to the FDA-approved package insert for complete product information.

Preparation of cisplatin is per institutional standards.

Doxorubicin
Doxorubicin is commercially available as a lyophilized powder for reconstitution in 10, 20, and 50mg vials. Also available as 2 mg/ml solution for injection in 10, 20, 50, 75 and 200 mg vials. Please refer to the FDA-approved package insert for complete product information.

Reconstitute the vials of doxorubicin powder with 5, 10, 25, 50 or 75 ml, respectively, of sodium chloride for injection, USP, resulting in a concentration of 2 mg/ml.

Preparation of doxorubicin is per institutional standards.

Cyclophosphamide
Cyclophosphamide is commercially available as powder for reconstitution in 500mg, 1g and 2g vials. Please refer to the package insert for complete product information.

Reconstitute 500mg, 1g and 2g vials with 25, 50, or 100ml of sterile water for injection for a final concentration of 20mg/ml. Vigorous shaking and/or gentle warming may be necessary.

Preparation of cyclophosphamide is per institutional standards.

7.2 Stability and Storage

Cisplatin

Vials of cisplatin injection, USP, are stored at room temperature between 4°C - 25°C. **Do not refrigerate or freeze cisplatin solutions, since a precipitate will form**. IV needles, syringes, or sets having aluminum components should not be used in preparing or administering cisplatin solutions. An interaction will occur between aluminum and platinum from cisplatin, causing a black precipitate that is visible in the cisplatin solution. Diluted solution of carboplatin is physically and chemically stable for up to 8 hours at ambient room temperature. The solution should be discarded after 8 hours as no antibacterial preservative is contained in the formulation.

Doxorubicin

Intact vials of doxorubicin solution should be stored in the refrigerator. Intact vials of powder for reconstitution should be stored at room temperature. Reconstituted solutions are stable for 7 days at room temperature and 15 days under refrigeration when protected from light. Commercially available solutions labeled as such are intended to be multi-dose vials.

Cyclophosphamide

Store intact vials of powder at room temperature (15-30°C). Reconstituted solutions are stable for 24 hours at room temperature or for 6 days in the refrigerator (2-8°C). Solutions further diluted for infusion in 0.9% NaCl or D5W are stable for 24 hours at room temperature and 6 days under refrigeration.

8. STUDY CALENDAR AND BIOSPECIMEN COLLECTION

8.1 Required Data

Table 8.1 outlines the required data. Case Report Forms or 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 signed and written informed consent, a pre-study baseline evaluation is required as indicated on Table 8.1 prior to initiation of cycle 1. This evaluation must be completed within 28 days prior to starting therapy, except as indicated.

Table 8.1 Required Data

Parameter	**Pre-study (within 28 days)**	**Prior to Neo-adjuvant therapy**	**Day 1 of cycles 1-4**	**Day 7-10 of cycle 1-4**	**Pre-surgery (2-4 weeks after last protocol chemo)[r]**	**30 days post treatment** (Day 30-33 post tx)	**Within 42 days of last chemo** (Day 30-42 post tx)	**Follow-Up (annually x 3 years)**
Informed consent	X							
Confirmation of BRCA1 and/or BRCA2 mutation	X							
Confirmation of invasive cancer on diagnostic biopsy [a]	X							
Sentinel node biopsy or needle aspiration if clinically suspicious axillary nodes [a]	X							
Mammogram +/or MRI +/or ultrasound	X [b]				X [c]			
Complete medical history	X							
Complete physical exam including tumor measurement	X		X[o]		X [d]			
Weight, vital signs, height [n]	X		X					
Performance status (ECOG)	X							
Adverse Event evaluation			X		X[p]	X[p]	X[p]	
Hematology (CBC, differential, platelets)	X		X[o]	X[e] (Cycle 1 only)				
Serum Glucose	X							
Liver function tests (AST/ALT, Alk Phos, Bili)	X		X[f,o]					
Serum BUN, Cre, K, Mg	X		X[g,o]	X[h]				
MUGA scan or echocardiogram	X							
EKG (12 lead)	X							
Pregnancy test [i]	X							
Excision of tumor or mastectomy and axillary nodes							X [j]	
Research biopsy [k]		X [k]			X[q]			
Research blood sample[m]		X			X			
Bone scan, CT scan, (chest/abd/pelvis) or PET-CT [l]	X							
Survival Assessment (See section 5.14)								X

[a] Pathology report is required for eligibility; 28 day window does not apply. Slides and tumor block of the diagnostic biopsy (and node evaluation if done) can be sent to the coordinating center per section 9.5 at the same time as the specimens from definitive surgery.
[b] **Affected breast imaging that measures the tumor must be done within 28 days prior to study start**; MRI is strongly recommended, although other imaging modalities (mammogram, ultrasound) are permitted. See Section 9.
[c] Affected breast imaging is required prior to breast surgery, between C4D1 and 4 weeks after the last chemotherapy. Mammogram, MRI or ultrasound may be used if being compared to baseline study prior to chemotherapy.
[d] Tumor measurement should be assessed by physical exam prior to surgery, 2-4 weeks after the last protocol chemotherapy.
[e] Participants receiving growth factor support do not need this nadir CBC. A nadir CBC is only required for Cycle 1. This blood draw may be performed at the treating hospital or closer to home.
[f] For participants receiving AC only
[g] For participants receiving Cisplatin only
[h] For participants receiving Cisplatin only; the blood draw may be performed at the treating hospital or closer to home 7-10 days after chemo each cycle. If K or Mg levels are low, supplementation should be administered. If Cre is elevated, this should be managed per institutional guidelines.
[i] Women of childbearing potential only if there is a suspicion of possible pregnancy.
[j] Slides and tumor block of the tumor excision should be sent to BIDMC per section 9.5 within 60 days of surgery.
[k] Optional; prior to beginning study therapy. (See section 5.3 and Appendix E).
[l] Staging Studies are not required for this protocol and are at the discretion of the treating physicians. It is recommended that NCCN guidelines for staging patients receiving pre-operative chemotherapy be followed.
[m] See Section 8.2 and 8.3
[n] Height should only be collected at baseline.
[o] Physical exam and Labs may be performed up to 48 hours prior to the start of a new cycle.
[p] The pre-surgery AE assessment may be performed by surgeons trained on the study or by telephone call. Any AEs documented at pre-surgery should be recorded on the Cycle 4 AE form. The 30 day post AE assessment and any continuing AE follow-up may occur via phone call. See Section 5.13 and Section 10.
[q] This biopsy is required for patients who receive additional therapy prior to definitive surgery. It must be performed prior to the initiation of additional therapy.
[r] For patients with significant residual disease after 4 cycles of assigned protocol chemotherapy who will receive additional treatment. These assessments should be obtained 2-4 weeks after the last protocol assigned chemotherapy and before additional non-protocol assigned chemotherapy is administered.

8.2 Research Specimen Submission

Table 8.2 Required Specimen Submissions

Specimen Type	Time Point				Shipping Condition	Ship to
	Pre-study (Diagnostic biopsy)	Pre-treatment (Optional Research biopsy)	Post protocol treatment	Definitive Surgery		
Representative H&E slide from each block from diagnostic biopsy*	X				Room temperature	[redacted] at BIDMC
Tumor block from diagnostic biopsy*	X				Room temperature	[redacted] at BIDMC
Biopsy core frozen in OCT**		X			Store in -80C freezer; ship on dry ice	DF/HCC Core Blood and Tissue Bank
Biopsy core in RNA Later**		X			Room temperature	DF/HCC Core Blood and Tissue Bank
Biopsy core in formalin		X**	X#		Room temperature	DF/HCC Core Blood and Tissue Bank
Two 10mL lavender top (EDTA) tubes of blood***	X				Use thermos provided in research kit	DF/HCC Core Blood and Tissue Bank
Two 10 mL Streck tubes of blood***		X	X		Room temperature- DO NOT FREEZE/ REFRIGERATE	DF/HCC Core Blood and Tissue Bank
H&E slides from definitive surgery*				X	Room temperature	[redacted] at BIDMC
H&E slides from axillary lymph node dissection*		+/- X		+/- X	Room temperature	[redacted] at BIDMC
Tumor block from definitive surgery*				X	Room temperature	[redacted] at BIDMC
Block of normal breast tissue, skin or uninvolved LN*				X	Room temperature	[redacted] at BIDMC

* For more information see protocol section 9.5.
** For more information see protocol section 5.3
*** For more information see protocol section 8.3
# Only required for patients who received additional chemotherapy before surgery; a biopsy to prove residual cancer is encouraged in this situation. H&E slides will be reviewed by the study team in lieu of definitive surgery slides.

8.3 Research Blood Sample Collection

Blood will be collected, processed, and banked in the DF/HCC Core Blood and Tissue Bank for future research purposes. These specimens will become the property of the DF/HCC. Participants will be informed that their specimens may be used for future research by DF/HCC investigators. Specimens will be identified with a linked sample ID number; all participant identification will be removed.

8.3.1 Lavender Top (EDTA)

Blood will be collected and banked in order to extract germline DNA to be used as normal DNA reference for tumor tissue-based studies. At baseline (or at any point during chemotherapy) collect:

- Two 10 mL lavender top (EDTA Fisher #366643) tubes

These tubes will be supplied in the research biopsy kit that is provided by CORE Prognostex. 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 together to the DF/HCC Core Blood and Tissue Bank. The research tubes should be placed in the thermos provided in the research kit for shipment.

Email the DF/HCC Core Blood and Tissue Bank and DFCI BOC Inform Study Coordinator with the sample information and tracking information the day before shipping specimens.

[redacted]

Specimens should be shipped to DF/HCC Core Blood and Tissue Bank:

Attn: DF/HCC Core Blood and Tissue Bank
Dana-Farber Cancer Institute
[redacted]

Please include a copy of the 12-258 Specimen Requisition (Research Biopsy and Blood) form with your shipment which can be found in Appendix H.

8.3.2 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 and at the end of protocol therapy.

The end of therapy Streck tubes may be collected at the pre-surgery or post treatment biopsy visit but must be collected prior to initiation of 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 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 investigators. Shared specimens will be identified with a sample ID number; all patient identifying material will be removed.

Complete the 12-258 SPECIMEN REQUISITION (Research Biopsy and Blood) form found in Appendix H 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:

Attn: DF/HCC Core Blood and Tissue Bank
Dana Farber Cancer Institute
[redacted]

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.

All research blood samples should arrive during the week by Friday morning. Specimens should be labeled with the assigned sample ID number, date of collection, time point of collection, and protocol number (please send with requisition form found in Appendix H). The coordinating center study coordinator will track the research blood specimens using CaTissue. Upon receipt of an email notification regarding sample shipment from a participating site, the coordinating center study coordinator will log in the sample information into CaTissue. When the sample is received by the staff in the DF/HCC Core Blood and Tissue Bank, the confirmation of receipt and specimen location information will be added to CaTissue. CaTissue will contain a complete record of the research blood samples that are collected as part of this study.

8.4 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.

9. MEASUREMENT OF EFFECT

A baseline and presurgical radiographic study of the breast is required; MRI is recommended. The same radiographic modality should be used consistently. The baseline scan must be obtained within 28 days of beginning therapy. The presurgical scan should occur between C4D1 and up to 4 weeks after the last chemotherapy administration. If the participant clinically progresses, repeat imaging is required. If there is discordance (clinical progression, but radiographic stable disease or response), contact the study chair.

9.1 Target Lesions

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).

9.2 Pathologic Complete Response

Pathological Complete Response (pCR): (Miller-Payne method)[1]	Complete disappearance of invasive tumor in the breast at the time of surgery. Pathologic complete response in the lymph nodes is defined as no detectable invasive tumor by H&E.
Pathological Complete Response (pCR): (MD Anderson RCB method)[2]	Complete disappearance of invasive tumor in the breast and axillary nodes at the time of surgery.

All patients with significant residual disease who proceed to alternative chemotherapy will be considered to belong to the poor response group.

9.3 Radiographic Assessment

Each participant will have a pre-therapy baseline radiographic tumor measurement, preferably by MRI, however if logistic, financial, or practical issues preclude MRI use, mammogram or ultrasound may be substituted, as long as the tumor lesion can be adequately measured. 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.

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

9.4 Clinical Assessments

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 “yes”, the study chair should be notified in order to determine whether the participant should come off protocol treatment.

9.5 Pathology Response Central Review and Archival Tissue for Future Correlative Studies

Central review of pathology will be performed by Dr. Stuart Schnitt of DF/HCC. Dr. Schnitt will be blinded to the treatment assignment of each participant.

For each participant, submit the following:

- Clinical material:

  a) A representative H&E slide from each block from the diagnostic breast biopsy (pre-chemotherapy)

  b) All H&E slides from the definitive surgery (post-chemotherapy or mastectomy)

  c) All H&E slides from axillary nodes (if applicable)

  Pathologic complete response will be assessed. For those without a pCR, the semi-quantitative pathologic response scores and grade will be determined using the Miller-Payne criteria and the Residual Cancer Burden measure.

  More slides may be requested from the participating site if needed to complete the pathology response central review.

  The clinical material will be returned to the site after the completion of the pathology central review.

- Tumor Tissue for future research:

  a) One representative tumor block from the diagnostic breast biopsy (pre-chemotherapy)

  b) One representative tumor block from the definitive surgery (post-chemotherapy or mastectomy)

  c) One block of normal breast tissue, skin or uninvolved axillary node

  This tissue will be used by the study pathologist for future correlative studies to identify biomarkers of response to cisplatin and doxorubicin and cyclophosphamide.

*It is recommended that participating sites request all archival tissue specimens, including clinical material and tissue for future research after the definitive surgery clinical case has been signed out by pathology.

**If an institutional policy exists that precludes release of tissue blocks, please send 15 sections (5 micron thickness) on charged slides from: a) the diagnostic tumor biopsy (pre-chemotherapy); b) the post-chemotherapy definitive surgery (excision or mastectomy); and c) normal breast tissue, skin or uninvolved lymph node (i.e. negative for cancer).

Tumor blocks will be kept for future research at DF/HCC; however, if a tumor block is needed for clinical care purposes, the participating site should contact the coordinating center study coordinator to request that the applicable block be returned.

The coordinating center study coordinator will maintain a specimen tracking log of all archival tissue specimens received.

All archival tissue material should be sent to the coordinating center study coordinator, [redacted] at:

Beth Israel Deaconess Medical Center
[redacted]

Please email [redacted] when archival tissue material is being sent. Please include a copy of the 12-258 Specimen Requisition (Diagnostic and Surgery Blocks and Slides) form with your shipment which can be found in Appendix G.

9.6 Biomarkers of Response to Cisplatin and AC chemotherapy

Pretreatment tumor biopsies will be analyzed using genome wide allele-specific copy number analysis to determine number of regions of telomeric allelic imbalance (NtAI) and chromosome 15q26 copy number, and chromosome 8q22 copy number. The first two biomarkers have been found to be associated with cisplatin sensitivity and the 8q22 copy number is associated with doxorubicin resistance. Copy number analysis may be performed by several potential methods. The Affymetrix Oncoscan FFPE Express is a SNP array-based molecular inversion probe assay that gives genome-wide allele-specific copy numbers from DNA extracted from fresh frozen, RNA Later preserved, or FFPE- tumor samples for detection of gene amplifications, losses and NtAI in tumors. The current version is a service test provided by Affymetrix, Inc. and performed on DNA that is sent to them. The tumor sections will be examined to find the most pure areas of the tumor for DNA extraction. DNA is extracted using Argylla DNA/RNA extraction kits and sent to Affymetrix to run the OncoScan assay. Data analysis is performed using established algorithms reported in our prior studies for measuring NtAI and determination of DNA alterations in the tumors. An alternative method that may be more feasible and desirable by the time of study tissue analysis is next generation sequencing from FFPE extracted DNA to determine genome-wide allele-specific copy numbers and derivative measure of NtAI.

Gene expression profiling will be performed to determine intrinsic subtype (basal-like, claudin-low, etc.) and to measure biomarker genes including BLM and FANCI associated with cisplatin sensitivity or LAPTM4B and YWHAZ associated with anthracycline resistance. Gene expression profiling may be performed by several alternative methods including Affymetrix U133plus 2.0 arrays, Nanostring nCounter system, RT-PCR, or RNA Sequencing.

Tumor cells will be microdissected and subjected to exon sequencing of the specific BRCA1 or BRCA2 mutation for each patient in order to determine whether or not there has been loss of the wild type allele in the tumor. Associations between loss of wtBRCA alleles and response to cisplatin or AC chemotherapy will be sought.

Exploratory analysis will be performed to seek new measures of therapy response using the data from DNA copy number and gene expression profiles. In addition, we will plan to perform whole exome and possibly whole genome sequencing of tumors to identify potential single gene modifiers of response to therapy and to measure global point mutation and insertion/deletion mutation burden.

10. ADVERSE EVENT REPORTING REQUIREMENTS

It is of the utmost importance that all staff involved in the study is familiar with the content of this section. The Protocol Chair is responsible for ensuring these guidelines are followed.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 10.1) and the characteristics of an observed AE (Section 10.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

10.1 Adverse Event Lists

10.1.1 Adverse Event (AE) Lists for Study Agents

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

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

10.2 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.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **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.

- **Expectedness** of the AE:

  - **Expected** adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
  - Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

  - **Unexpected** adverse event are those that vary in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in these documents or in the informed consent document as a potential risk.

10.3 Adverse Event Reporting

10.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI, within 24 hours of first awareness of the event.

Nadine Tung, MD
[redacted]

Coordinating Center
[redacted]

10.3.2 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.

10.3.3 For Multi-Center Trials 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.

10.3.4 DF/HCC Adverse Event Reporting Guidelines

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

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

Attribution	DF/HCC Reportable Adverse Events(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/Coordinating Center will submit AE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

10.3.5 Protocol Specific Adverse Event Reporting Exclusions

For this protocol only, the AEs listed below do not require expedited reporting to the Overall PI or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (case report forms).

Grade 4 expected events do not need to be reported by expedited reporting to the IRB, however, the Overall PI should be notified of any Grade 4 events within 24 hours of first awareness of event.

Any events that are unequivocally due to progression of disease should not be reported as an adverse event.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

10.4 Reporting to Hospital Risk Management

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

10.5 Routine Adverse Event Reporting

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) must <u>also</u> be reported in routine study data submissions.** Any adverse events noted during the pre-surgery evaluation should be included on the cycle 4 Adverse Event form.

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification. This AE evaluation can be documented by phone call.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

11. DATA AND SAFETY MONITORING

11.1 Data Reporting

11.1.1 Method

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

11.1.2 Data Submission

The schedule for completion and submission of electronic case report forms to the ODQ is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with ODQ

On Study Form	Within approximately 14 days of registration
Baseline Assessment Form	Within approximately 14 days of registration
Treatment Form	Within approximately 10 days of the last day of the cycle
Adverse Event Report Form	Within approximately 10 days of the last day of the cycle
Response Assessment Form	Within approximately 10 days of the completion of the 4th cycle (or last cycle if subject does not complete 4 cycles of chemotherapy) of preoperative chemotherapy.
Off Treatment/Off Study Form	Within approximately 14 days of completing treatment or being taken off study for any reason

11.2 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.

11.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

Refer to Appendix F, Sections 7.0 and 9.0, of the Data and Safety Monitoring Plan for a detailed description of the study's auditing and monitoring plan.

12. REGULATORY CONSIDERATIONS

12.1 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.

12.2 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.

12.3 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.

12.4 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.

12.5 Records Retention

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

12.6 Multi-center Guidelines

This protocol will adhere to the policies and requirements of the Dana-Farber/Harvard Cancer Center. The specific responsibilities of the DF/HCC Overall Principal Investigator (or Protocol Chair), Coordinating Center, and Participating Institutions are presented in the Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (see Appendix F).

- The DF/HCC Overall Principal Investigator/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 agent(s) directly from the 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.

13. STATISTICAL CONSIDERATIONS

13.1 Analysis of Primary Endpoint

The primary endpoint is pCR [pCR in breast and nodes (i.e. RCB 0) or pCR in breast (i.e. Miller Payne 5) if nodes are not evaluable (i.e. positive nodes were removed surgically before chemo)] assessed by the Miller-Payne method[1] and to determine if the pCR rate after cisplatin chemotherapy is 20% higher than the pCR rate after doxorubicin and cyclophosphamide chemotherapy.

We hypothesize that the incidence of pCR will be 50% among participants randomized to neoadjuvant cisplatin and 30% in the AC treatment arm[62]. Despite the pCR rate of 72% with cisplatin reported by Gronwald et al [54], it is anticipated that the response rate in a multicenter randomized phase 2 study will be lower than that reported initially in a single institution trial. In addition, the effect of introducing high grade ER+ breast cancers in BRCA2 carriers is not certain. Assuming a two-sided alpha of 0.10 and accounting for the interim analysis described below, we would need 76 evaluable participants in each treatment arm to have 80% power to detect the specified difference (50% vs. 30%). We will aim to enroll 85 participants in each treatment arm to account for 10% potential drop out when not evaluable. We will compare the frequency of pCR in the two treatment arms using log-binomial regression to calculate the risk ratio and 95% confidence interval; we will include a term in the model for ER status to evaluate its effect on the outcome. It is anticipated that the duration of the study will be approximately three years.

13.2 Interim Analysis

One interim analysis is planned after 50% (43) of the participants in each arm have completed the study. Results of the interim analysis will be reported to the Dana-Farber/Harvard Cancer Center Data Safety Monitoring Board.

The O'Brien-Fleming group sequential design was used to determine stopping boundaries for a two-sided hypothesis for the interim and final analyses (see table below). The probability of stopping at the first interim analysis under the alternative hypothesis is 0.27. The interim analysis of the primary endpoint will be conducted as described above in 13.1.

	Interim Analysis	Final Analysis
Participants completed	86 (50.6%)	170 (100.0%)
Boundary values (standardized Z)	2.37	1.68
Accumulated alpha	0.018	0.10
Accumulated beta	0.0002	0.2

13.3 Analysis of Secondary Endpoints

13.3.1 pCR and Residual Cancer Burden by MD Anderson RCB method [2]

The RCB method of assessing residual cancer burden, including pCR, requires assessment of both the breast as well as axillary nodes after neoadjuvant chemotherapy. Given the results of the Z0011 (Z-11) study by the American College of Surgeons Oncology Group[69], it was felt that for those participants with one or two sentinel nodes involved with tumor prior to chemotherapy, completion axillary dissection could not be mandated. Therefore this objective was made a secondary rather than a primary objective.

It is anticipated that very few subjects (< 5) in the study will **not** get a completion axillary dissection after chemotherapy if pre-chemotherapy sentinel nodes are involved with cancer since most subjects will not fit the criteria of subjects in the Z-11 study [69] . Therefore, it is believed that we will have adequate power to assess pCR by this method, though less than with the Miller-Payne method that requires only response in the breast.

Therefore a secondary objective is to compare the rates of RCB 0 (i.e.pCR) and RCB1 combined in patients who have received cisplatin and AC neoadjuvant chemotherapy. There is data to support that long term outcomes in patients with RCB1 is equivalent to those with RCB 0.[2]

There is also data that achievement of a near pCR after neoadjuvant therapy (i.e. Miller Payne 4) predicts for good outcomes in women with breast cancer. Therefore, a secondary objective is to assess the rate of Miller Payne 4 and 5 (combined) between those patients who received neoadjuvant cisplatin and those who received neoadjuvant AC[1].

13.3.2 Clinical complete response (CCR)

The CCR to cisplatin and to AC neoadjuvant chemotherapy will be determined in this population. CCR will be determined with 95% confidence interval for both regimens.

13.3.3 Toxicity: frequency and severity of adverse effects

Analyses of safety will be completed for all participants who receive at least 1 dose of study treatment The National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) criteria version 4.03 will be used to classify and grade toxicities observed during and following treatment (up to 30 days after last

chemotherapy). Participants will be tabulated according to the maximum grade for each organ system or preferred term. Safety endpoints will be summarized with descriptive statistics for the participants in the safety analysis dataset.

13.3.4 Biomarkers of response to cisplatin and AC chemotherapy

We will conduct an exploratory analysis to evaluate the utility of NtAI, BLM+FANCI mRNA levels, chr 15q26 copy number, chr 8q22 copy number, and YWHAZ + LAPTM4B mRNA levels to predict response to chemotherapy. The comparison of the results in the two arms will be descriptive and we will not attempt to show marker interaction with a specific drug response or make further claims about the marker performance. Other exploratory analyses based on gene expression profiling, DNA profiling, or sequencing will be for discovery purposes and statistical planning for this part cannot be determined at this time. Other biomarkers of response to cisplatin and/or AC that will be assessed if sufficient tissue exists include: homologous recombination defect assay by Myriad Genetic Laboratories, assessment of BRCA mutation reversion, BRCA1/(avg BLM + FANCI) 3-gene mRNA signature, BLM/ chromosome 15q26 copy number, gene expression profiling to assess breast cancer subtype, lymphocytic infiltration and circulating DNA.

13.3.5 3-year Recurrence-Free Survival

We will compare the frequency of recurrence between the two treatment arms of the INFORM trial (DF-HCC 12-258; TBCRC 031) using log-binomial regression to calculate the risk ratio and 95% confidence interval. We will also compare the frequency of recurrence between patients who achieved pathological complete response and those who did not using log-binomial regression to calculate the risk ratio and 95% confidence interval.

14. PUBLICATION PLAN

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 Protocol Chair will be the final arbiter of the manuscript content.

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Appendix A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status

These scales and criteria are used by doctors and researchers to assess how a subject's disease is progressing, assess how the disease affects the daily living abilities of the subject, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

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

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix B: FORMULA FOR CREATININE CLEARANCE

Creatinine clearance may be calculated by 24 hour urine

Or by the formula below:

Creatinine clearance (CrCl) will be calculated with the Cockroft-Gault equation [47] as follows:

$$\text{CrCl (ml/min)} = \frac{(140\text{-age})(\text{weight (actual) kg})}{[72 \times \text{serum creatinine (mg/dl)}]}$$

If using this formula, use 85% of calculated CrCl value for females.

Appendix C: NCI COMMON TOXICITY CRITERIA FORADVERSE EVENTS (CTCAE) v4.03

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

Appendix D: STUDY SPONSORED BRCA1/2 TESTING

Participants must meet one of the following criteria to be eligible for genetic testing through the study (NCCN guidelines)[70]:

- Breast cancer diagnosed at age ≤ 45 years;
- Diagnosed at age ≤ 50 years with first-, second-, or third-degree blood relative with breast cancer diagnosis ≤ 50 years and/or epithelial ovarian/fallopian peritoneal cancer at any age;
- Diagnosed at age ≤ 60 years with a triple negative breast cancer;
- Two breast primaries (i.e., bilateral disease or two or more clearly separate ipsilateral primary tumors) when first breast cancer diagnosis occurred prior to age 50;
- Personal history of epithelial ovarian/fallopian tube/primary peritoneal Cancer.
- Diagnosed at any age, with ≥ 2 first-, second-, or third-degree relatives with breast and/or epithelial ovarian/fallopian tube/primary peritoneal cancer at any age;
- First- or second-degree relative of an individual known to carry a deleterious BRCA1 or BRCA2 mutation;
- Ashkenazi Jewish descent;
- Male breast cancer; or
- First-, second-, or third-degree male relative with breast cancer.

Note: Participants who pre-qualify with one or more of these criteria will be eligible to screen for BRCA1/BRCA2 mutation by Myriad Genetics through the study. These subjects may be eligible for randomization upon receipt of a confirmed deleterious germline BRCA1/BRCA2 mutation by Myriad Genetics.

BRACAnalysis Test Request Forms will be provided by the Coordinating Center. The genetic test request form should be submitted with BRCA specimen tubes. Genetic counselors or study coordinators should email [redacted] when a BRCA testing specimen is submitted to Myriad to ensure a quick turnaround time.

Appendix E: TISSUE ACQUISITION AND SHIPPING GUIDELINES FOR RESEARCH BIOPSIES

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

1. Ideally two core biopsies will be placed into RNA Later. Both can be placed in the same specimen collection container. These biopsies should be at room temperature when stored and shipped.
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 RNA Later and 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 RNA Later and formalin should be stored at room temperature until shipment. All specimens should be shipped to:

Attn: DF/HCC Core Blood and Tissue Bank
Dana Farber Cancer Institute
[redacted]

*Please email to the following:
DF/HCC Core Blood and Tissue Bank [redacted] and DFCI BOC Inform Study Coordinator [redacted] with the sample information and tracking information the day before shipping specimens.

Appendix F: DF/HCC MULTI-CENTER DATA AND SAFETY MONITORING PLAN

DFCI IRB Protocol #: 12-258

Table of Contents

1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for a DF/HCC Multi-Center research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center (DF/HCC) Multi-center protocol will comply with Federal regulations; and Health Insurance Portability and Accountability Act (HIPAA) requirements in accordance with the CTEP Multi-center Guidelines.

1.2 Multi-Center Data and Safety Monitoring Plan Components

The Multi-Center Data and Safety Monitoring Plan includes the following components:

DF/HCC Multi-center Protocol: One or more outside institutions collaborating with Dana-Farber/Harvard Cancer Center on a research protocol where DF/HCC is the Lead Institution. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center sites (DFCI, MGH, BIDMC, CHB, BWH) will be the Lead Institution and will be 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 (CTEP, FDA, OBA etc.). The Lead Institution is the home of the Overall PI.

DF/HCC Contract Principal Investigator: Investigator located at the Lead Institution who will be charged with the responsibility of the administration of the DF/HCC Project. This most often will be the Protocol Chair, but occasionally this may be the overall grant or contract holder, as applicable.

Protocol Chair: The Protocol Chair is the Principal Investigator for the DF/HCC protocol submitted as the Lead Institution. The Protocol Chair may also be referred to as the Overall Principal Investigator or DF/HCC Sponsor. For applicable protocols, the Protocol Chair will be the single liaison with any regulatory agencies (i.e. CTEP Protocol and Information Office (PIO), FDA, OBA etc.).

Participating Institution: A Participating Institution is an institution that desires to collaborate with DF/HCC and commits to accruing participants to a DF/HCC protocol. The Participating Institution acknowledges the Protocol Chair as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-center Protocol. The Coordinating Center will provide the administrative support to the Protocol Chair in order that he/she may fulfill the responsibilities outlined in the DSMP and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In addition to the Lead Institution, the Quality Assurance Office for Clinical Trials (ODQ) provides support services to assist the Protocol Chair.

Clinical Trials Office: The clinical trials offices of the DF/HCC consortium members support investigators and their study teams with the coordination, submission and ongoing conduct of research protocols involving human subjects. Specifically, these offices support four core service areas including; pre-review of PI initiated protocols; assistance in the preparation and management of Investigational New Drug (IND) applications and subsequent required reporting to the FDA; regulatory consultation and guidance in the interpretation of local, federal, and ICHguidelines and policies; and the orientation and ongoing training support of clinical research personnel.

DF/HCC Quality Assurance Office for Clinical Trials: The DF/HCC ODQ is a unit that has been developed to computerize, manage, and QC & QA data and DF/HCC trials. The DF/HCC ODQ is located administratively in the office of the Senior Vice President for Clinical Research, at Dana-Farber Cancer Institute. The ODQ uses DF/HCC computerized institutional databases for participant registrations and for the management of trial data as well as a set of quality assurance programs designed to audit DF/HCC trials.

2.0 GENERAL ROLES AND RESPONSIBILITIES

In accordance with the CTEP Multi-center Guidelines, the Protocol Chair, Coordinating Center (Lead Institution or designee), and the Participating Institutions will all agree to the general responsibilities as follows (specific procedures for these general responsibilities are detailed in the DSMP):

2.1 Protocol Chair (DF/HCC Principal Investigator)

The Protocol Chair, Dr. Nadine Tung, will accept responsibility for all aspects of the Multi-Center Data and Safety Monitoring Plan 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 inclusion 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 subjects.
- For international trials, assure that the protocol is provided to Participating Institutions in the primary language spoken at the site.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.
- Ensure all DFCI IRB and DF/HCC requirements are met.
- Review data and maintain timely submission of data for study analysis.

2.2 Coordinating Center (Lead Institution)

The Coordinating Center is the DF/HCC Lead Institution's study team or designee (i.e Medical Monitor, Clinical Research Organization). The DF/HCC Lead Institution Dana-Farber Cancer Institute will ensure that all Participating Institutions within the Multi-Center Protocol demonstrate their intent and capability of complying with Federal Regulations and HIPAA requirements. To assist the Protocol Chair in meeting his/her responsibilities as required by the DSMP, the DF/HCC Lead Institution's study team or designee will assume the following general responsibilities:

- Assist in protocol review.
- Maintain copies of FWA and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Collect data on protocol specific CRFs.
- Prepare all submitted data for review by the Protocol Chair.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to Protocol Chair for timely review.
- Distribute Serious Adverse Event safety reports (both IND Safety reports and protocol specific SAEs).
- Monitor at Participating Institutions either by on-site inspection of selected participant records and/or with source documents and research records submitted to the Lead Institution.

In addition to the Lead Institution, the DF/HCC Quality Assurance Office for Clinical Trials provides the following support services to assist the Protocol Chair:

- Develop protocol specific case report forms (eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide Central Participant Registration.
- Verify that eligibility has been confirmed by the investigator and that appropriate consent has been obtained.
- Provide auditing services (funding and ODQ approval required).

2.3 Participating Institution

Each Participating Institution will provide to the Coordinating Center a list of the key personnel assigned to the role for oversight of data management at their site. All sites must have office space, office equipment, and internet access that meet HIPAA standards.
The general responsibilities for each Participating Institution are as follows:

- Commit to accrual to the Lead Institution's (DF/HCC) protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder.
- Update Coordinating Center with research staff changes on a timely basis.

- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- Submit Serious Adverse Event reports to local IRB and directly to the Coordinating Center.
- Submit deviations and violations to local IRB and the Coordinating Center.

3.0 PROTOCOL DEVELOPMENT

3.1 Activation of a Protocol

The Protocol Chair is responsible for the coordination, development, and approval of the protocol as well as its subsequent amendments, and reporting SAEs, violations and deviations per DFCI IRB guidelines.

To meet these requirements, the Protocol Chair will be responsible for the following minimum standards:

- Inclusion of the DF/HCC Multi-Center Data and Safety Monitoring Plan in the protocol as an appendix.
- Identify, qualify and initiate Participating Institutions and obtain accrual commitments.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the Protocol Chair.
- Ensure that there is only one version of the Protocol and that all Participating Institutions use the correct version.
- Oversee the development of data collection forms (case report forms) that are of common format for use at all the Participating Institutions.

3.2 Coordinating Center Support Function

The DF/HCC Lead Institution's study staff or designee will provide administrative and clerical support to the Protocol Chair for the development and distribution of the protocol.

The tasks to be performed by the DF/HCC Lead Institution's study staff or designee include:

- Maintain Regulatory documents for all Participating Institutions.
- Review of the protocol and consent to check for logistics, spelling, and consistency. Provide the Protocol Chair a list of queries related to any inconsistencies.
- Provide necessary administrative sections, including paragraphs related to registration logistics, data management schedules, and multi-center guidelines.
- Maintenance of contact list of all Participating Institutions in the DF/HCC Multi-center Protocol and the distribution of updates to the sites as needed.
- Derivation of the study calendar, if applicable.
- Assistance in preparation and maintenance of case report forms.

- Conduct regular communications with all Participating Institutions (conference call, emails, etc)
- Maintain documentation of all communications.

4.0 PROTOCOL MANAGEMENT

The Coordinating Center is responsible for assuring that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP). Additionally, the Coordinating Center must maintain copies of all IRB approvals, for each Participating Institution.

4.1 Protocol Distribution

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

4.2 Protocol Revisions and Closures

The Participating Institutions will receive phone, fax, mail or e-mail notification of protocol revisions from the Lead Institution or designee. 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 Lead Institution or designee. Non-life-threatening protocol revisions should be IRB approved and implemented within 90 days from receipt of the notification.

Revisions for life-threatening Causes: Participating Institutions will receive telephone notification from the Lead Institution or designee concerning protocol revisions required to protect lives with follow-up by fax, mail or e-mail. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval

Protocol Closures and Temporary Holds: Participating Institutions will receive fax, e-mail, or phone notification of protocol closures and temporary holds from the Lead Institution or designee. Closures and holds will be effective immediately. In addition, the Lead Institution or designee will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

4.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent from participating institutions. As best a possible, the template should be followed with the specifications outlined in the DF/HCC guidance document on Model Consent Language.

Participating sites are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Lead Site for their revision prior to submission to the participating site's 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. **It is DF/HCC policy that only attending physicians can obtain informed consent and re-consent to drug and/or device trials.**

4.4 IRB Documentation

The following must be on file with the DF/HCC Lead Institution or designee and must be submitted and approved by the DFCI IRB prior to participant registration:

- Approval Letter of the institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution's IRB
- IRB approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the DF/HCC Lead Institution their IRB approval for Amendments to a protocol.

4.5 IRB Re-Approval

Annual IRB re-approval from the Participating Institution is required in order to continue research and register participants onto a protocol. There is no grace period for continuing approvals.

Protocol registrations will not be completed if a re-approval letter is not received by the DF/HCC Lead Institution from the Participating Institutions on or before the anniversary of the previous approval date.

4.6 Participant Confidentiality and Authorization Statement

The HIPPA of 1996 contains, as one of its six major components, the requirement to create privacy standards for health care information that is used or disclosed in the course of treatment, payment or health care operations. The original Privacy Rule, as it has come to be known, was published in December 2000. The Final Rule was published on August 14, 2002, which modified the privacy rule in significant ways vis-à-vis research.

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

The DF/HCC Multi-Center Protocol 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 National Cancer Institute 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.

4.7 Participant Registration and Randomization

Refer to section 4 of the protocol for information regarding participant registration and randomization.

4.8 DF/HCC Multi-center Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for ODQ CRF/eCRF completion and written on all data and ODQ correspondence for the participant.

4.9 DF/HCC Multi-center Protocol Registration Policy

4.9.1 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 a faxed or e-mailed copy of the participant's Registration Confirmation memo from the DF/HCC ODQ. Therapy must be initiated per protocol guidelines. The Protocol Chair and DFCI IRB must be notified of any exceptions to this policy**.**

4.9.2 Eligibility Exceptions: The DF/HCC ODQ will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. In addition, the Cancer Therapy Evaluation Program (CTEP) specifically prohibits registration of a participant on any NCI Sponsored protocol that does not fully and completely meet all eligibility requirements. The DF/HCC ODQ requires each institution to fully comply with this requirement.

4.9.3 Verification of Registration, Dose Levels, and Arm Designation: A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one working day of the registration.

Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

4.9.4 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 Lead Institution or designee must have the participant's full name & social security number "blacked out" and the assigned DF/HCC ODQ case number and protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification.

4.10 Schedule of Data Submission

The DF/HCC ODQ develops a set of either paper or electronic case report forms, (CRF/eCRFs) for use with the DF/HCC Multi-Center Protocol. ODQ provides a web based training for eCRF users. These forms are designed to collect data for each study.
Note: It is necessary to send only ONE copy of all paper Case Report Forms, if applicable.

4.10.1 Eligibility Checklist

Purpose - Outlines protocol-specific eligibility criteria and includes the following:

Participant Demographics (address, zip code, sex, race, ethnicity, initials, date of birth)
1) Parameters for eligibility
2) Parameters for exclusion
3) Parameters for stratifications

If a time frame is not specified in the protocol, tests must be completed as follows:
- Lab tests required for eligibility must be completed within 28 days prior to study enrollment by the ODQ.
- Non-lab tests required for eligibility must be performed within 30 days prior to study entry. Example: radiological scans

4.10.2 On-study Form(s)

Purpose - documents the following items:
- Demographic data
- Prior therapy
- Past medical and surgical history
- Description of participant's physical status at protocol registration
- Disease site specific data

4.10.3 Baseline Assessment Form(s)

Purpose – Documents objective and subjective disease status as defined by the protocol**.** Records all pertinent radiographic and laboratory measurements of disease utilized in determining response evaluations.

4.10.4 Treatment Form(s)

Purpose - Records the following information related to the time the participant receives protocol treatment:

- Participant, Protocol information
- Protocol treatment and supportive therapy per treatment cycle
- Protocol specific laboratory values per treatment cycle
- All medications other than protocol chemotherapy agents used to treat concomitant diagnoses, if applicable

4.10.5 Adverse Event Report Form(s)

Purpose – Documents adverse events that occur while the participant is receiving treatment and for up to 30 days after the last dose of treatment. All adverse events are to be graded by number using the toxicity grading scale required by the protocol. This form is not for IRB submission, but for recording the AE in the research database.

4.10.6 Off Treatment and Off Study Form(s)

Purpose - The Off Treatment and Off Study Forms are submitted when the participant is removed from the study or has completed all protocol treatment. Note: If the participant dies while on protocol**,** the Off Study Form is the last form submitted.

4.11 Data Form Review

When data forms arrive at the DF/HCC ODQ, they are reviewed for:

Completeness:
Is all the information provided as required per protocol?

Protocol Treatment Compliance:
Are the body surface area (BSA) and drug dosage calculations correct? The dose must be within 10% of the calculated protocol dose.

Adverse Events (Toxicities):
Did the participant experience adverse events (toxicities or side effects) associated with the treatment? Was the treatment delayed due to the adverse event? What was the most severe degree of toxicity experienced by the participant?

Notations concerning adverse events will address relationship to protocol treatment for each adverse event grade. All adverse events encountered during the study will be evaluated according to the NCI Common Toxicity Criteria assigned to the protocol and all adverse events must be noted on the participant's Adverse Event (Toxicity) Forms.

Response:
Did the participant achieve a response? What level of response did they achieve? On what date did the participant achieve the response and how was the response determined?

Response criteria are defined in the protocol. A tumor assessment must be performed prior to the start of treatment and while the participant is on treatment as specified by the protocol.

Objective responses must have documentation such as physical measurements, x-rays, scans, or laboratory tests.

A subjective response is one that is perceived by the participant, such as reduction in pain, or improved appetite.

4.12 Missing and Deficient Memorandum

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

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written query from the DF/HCC ODQ Data Analyst. Responses to the query should be completed and returned within 14 days. Responses may be returned on the written query or on an amended case report form. In both instances 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 DF/HCC ODQ noting the missing forms. These reports are compiled by the DF/HCC ODQ and distributed a minimum of three times a year.

5.0 REQUISITIONING STUDY DRUG

All study drugs are commercially available. Participating Institutions are responsible for checking with the local Director of Pharmacy to ensure that the agent is in stock.

6.0 SAFETY ASSESSMENTS AND TOXICITY MONITORING

All participants receiving investigational agents 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 to the investigator by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol and recorded prior to each course of therapy. Life-threatening toxicities should be reported immediately to the Protocol Chair and Institutional Review Board (IRB).

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

6.1 Serious Adverse Events

A serious adverse event (SAE) is any adverse drug experience at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions in a participant who has never had seizure activity in the past that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

6.2 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Serious Adverse Events (SAEs) will be followed as is delineated in the protocol in section 10**.**

The Lead Institution will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating all SAEs to all sites conducting the trial.

Participating Institutions must report the AEs to the Protocol Chair and the Coordinating Center following the DFCI IRB SAE Reporting Requirements.

6.3 Guidelines for Processing IND Safety Reports

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent. The Protocol Chair will review all IND Safety Reports and is ultimately responsible for forwarding the IND Safety Reports to the Participating Institutions. The Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

7.0 PROTOCOL VIOLATIONS AND DEVIATIONS

Neither the FDA nor the ICH guidelines define the terms "protocol violation" or "protocol deviation." All DF/HCC Protocol Chairs must adhere to those policies set by the DFCI IRB, the definitions for protocol violation and deviation as described by the DFCI IRB will be applied for reporting purposes for all Institutions Participating in the DF/HCC Multi-center Protocol.

7.1 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 subject 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.

7.2 Reporting Procedures

The Protocol Chair: 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 Protocol Chair 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 DFCI IRB. The Participating institution must submit the deviation request to the Protocol Chair or designee, who will submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation should be submitted to the Participating Institution's own IRB, per its institutional policy.
A copy of the Participating Institution's IRB report and determination will be forwarded to the DF/HCC Lead Institution or designee by mail, facsimile, or via e-mail within 10 business days after the original submission.

All protocol violations must be sent to the DF/HCC Lead Institution Protocol Chair or designee in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the DF/HCC Lead Institution or designee will submit the report to the Protocol Chair 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.

8.0 MONITORING: QUALITY CONTROL

1) 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.

8.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit participant 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-going monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and subject safety. Additional 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, a plan will be formulated to provide regular and ongoing communication to Participating Institutions about study related information which will include participation in regular coordinating center initiated teleconferences. Teleconferences will occur approximately once a month beginning as soon as external sites are activated and will continue regularly until the completion of accrual. Upon completion of accrual, teleconferences will occur quarterly thereafter until study completion. Additional communication may be distributed via “Newsletter” or email as deemed appropriate by the protocol chair.

Monitoring will occur before the clinical phase of the protocol begins and will continue during protocol performance through study completion. Each participating site will have one on-site monitoring visit after at least 3 participants have been enrolled at the site. Virtual monitoring should occur approximately every 6 months and may occur more frequently if there are significant findings or discrepancies. On-site and virtual monitoring will be performed by the Coordinating Center’s Clinical Research Specialist (CRS). The data will be reviewed for completeness, quality, and adherence to the protocol requirements. Sites will be asked to provide source documentation via fax, email, or mail as specified by the Clinical Research Specialist for all virtual monitoring visits. A virtual site initiation visit (SIV) will be conducted with each participating site prior to study activation. Participating sites many not begin enrolling until the SIV has occurred.

All data submitted to the DF/HCC ODQ will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Coordinating Center or designee and if applicable ODQ Data Analysts assigned to the Protocol will perform the ongoing protocol data compliance monitoring with the support of the Participating Institution’s Coordinators, the Principal Investigators, and the Protocol Chair.

9.0 Evaluation of Participating Institution Performance

9.1.1 Eligibility Checklist:

Eligibility criteria are checked on a protocol-specific eligibility checklist and faxed to the DF/HCC ODQ prior to registration on protocol. The checklist and informed consent document are reviewed by a DF/HCC ODQ Protocol Registrar before the participant can be registered on a protocol. The DF/HCC ODQ cannot make exceptions to the eligibility requirements.

9.2.2 Accrual of Eligible Participants:

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.A total of 170 subjects will be enrolled among all participating sites during a three year period. Participating Institutions are expected to enroll 3 patients annually.

10.0 AUDITING: QUALITY ASSURANCE

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

10.1 DF/HCC Sponsored Trials

On-site audits will be conducted by ODQ at participating sites as needed (as determined by the Protocol chair). An audit may be triggered if deficiencies are noted related to consent practices, eligibility, missing, incomplete, or questionable data submission, or any other issue the protocol chair deems appropriate for audit. If a participating site is selected for audit, approximately 3-4 subjects would be audited at the site over a 2 day period. If violations which impact subject safety or the integrity of the study are found, more subject records may be audited.

10.2 Participating Institution

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

10.3 Coordinating Center (Lead Institution or designee)

The Protocol Chair will review all DF/HCC Multi-Center Protocol Final Audit reports and corrective action plans if applicable. The Lead Institution or designee 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 Protocol Chair to implement recommendations or require further follow-up. For unacceptable audits, the Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

10.4 Sub-Standard Performance

The Protocol Chair, DFCI IRB and the NCI for CTEP trials, is charged with considering the totality of an institution's performance in considering institutional participation in the DF/HCC Multi-Center Protocol.

10.4.1 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 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 Protocol Chair for revocation of participation.

Appendix G: 12-258 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: Beth Israel Deaconess Medical Center, Attn: [redacted]

Specimen Information

Participant Initials (FML): _____ DFCI Participant Study ID Number: _____ Date specimen(s) shipped: _____

Site of Lesion: ☐ Right breast ☐ Left breast Pathology reports included (Mark all that apply): ☐ Pre-chemo ☐ Post-chemo

Specimen Type *(indicate inclusion in shipment by checking box)*	**Pathology Number(s) or Serial Coding**	**Quantity submitted**	**Date specimen obtained**
☐ Representative H&E slide from each block from Diagnostic biopsy			
☐ Block / ☐ Unstained slides from Diagnostic biopsy			
☐ All H&E slides from Definitive surgery (post chemotherapy)			
☐ All H&E slides from Axillary Lymph Nodes			
☐ Block / ☐ Unstained slides from Definitive surgery (post chemotherapy)			
☐ Block / ☐ Unstained slides from Normal breast tissue, skin or uninvolved lymph nodes			
☐ Other, specify:			

Responsible contact: _____

Email: _____

Phone number: _____

Site: _____

Mailing address (to return slides): _____

_____

_____

_____

Appendix H: 12-258 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: Attn: DF/HCC Core Blood and Tissue Bank , Dana Farber Cancer Institute, S[redacted]

Specimen Information

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

Date specimen(s) shipped: _____

Time Point: ☐ Pre-treatment ☐ Post-treatment

Specimen Type *(indicate inclusion in shipment by checking box)*	**Quantity submitted**	**Date specimen obtained**	**Time specimen obtained** *(24 hr clock)*
☐ Biopsy core(s) frozen in OCT			
☐ Biopsy core(s) in RNA Later			
☐ Biopsy core(s) in formalin			
☐ Blood in purple top (EDTA) tube			
☐ Blood in Streck tubes			
☐ Other, specify:			

Responsible Contact: _____

Email: _____

Phone number: _____

Site: _____

Appendix I: INFORM/TBCRC031 Questionnaire for Annual Follow-Up after Breast Surgery
This questionnaire should be utilized by the study team if contacting a participant, their designee, or local provider directly for follow up information.

For the first annual follow up, please complete Questions 1-11. For the second and third annual follow up, only Questions 6-11 need to be completed.

Patient Name: _____

Date of Birth: _____

Date Questionnaire Completed: _____

1) Please provide all the ways in which we may contact you:
   - Phone #s: _____
     home: _____
     cell: _____
   - Email: _____

2) What are the names and contact information of any members of your health care team who have treated you for your breast cancer or followed you since completing surgery on the INFORM trial:

   - Surgeon: _____
     name: _____
     phone #/email: _____

   - Radiation Therapist: _____
     name: _____
     phone #/email: _____

   - Medical Oncologist: _____
     name: _____
     phone/email: _____

   - Primary Care physician: _____
     name: _____
     phone/email: _____

3) Did you have any additional breast surgeries after your first breast surgery to remove your breast cancer on the INFORM trial (Yes/No)? _____
   - If yes, what kind (unilateral mastectomy, bilateral mastectomy, conserving surgery, other)? _____
     i. If unilateral or conserving surgery, which breast? _____
     ii. Date of surgery: ____/_____/____

4) Did you receive chemotherapy after the breast surgery (Yes/No)? _____
   - If yes, which chemotherapy agent(s)? _____
   - If you were not assigned to Cisplatin as part of the INFORM trial, did you receive platinum chemotherapy at any time to treat the breast cancer (Yes/No)? _____
     i. If yes: was platinum given right after your breast surgery or to treat recurrent breast cancer? _____

5) Did you receive any other systemic therapy after surgery (including, but not limited to, immune therapy and PARP inhibitors) (Yes/No)? _____
   - If yes, which agents? _____

6) Did you receive radiation as part of your care (Yes/No)? _____
   - If yes, what was the start date of the radiation? ____/_____/____

7) Have you had your ovaries and fallopian tubes removed (Yes/No)? _____
   - If yes, what was the date of the procedure? ____/_____/____

8) Have you had your breast cancer return (recurrence) anywhere in the body (please complete all applicable from the list below) (Yes/No)? _____
   - Breast (Yes/No) _____
     i. If yes, what date? ____/_____/____
     ii. If yes, was it the same breast as the initial cancer? _____
   - Regional lymph nodes (Yes/No) _____
     i. If yes, what date? ____/_____/____
   - Skin (Yes/No) _____
     i. If yes, what date? ____/_____/____
   - Distant (Yes/No) _____
     i. If yes, what date? ____/_____/____
     ii. If yes, what location(s)? _____

9) Have any of your treatments including a PARP inhibitor (e.g. Olaparib, Talazoparib, Nirapirib)?
   - If yes, what was the name of the PARP inhibitor? _____
   - If yes, what date(s)? ____/_____/____
   - If yes, what was your response (e.g. shrinkage vs stable disease vs. immediate progression)? _____
   - If yes, what was the duration of your treatment on the PARP inhibitor? _____

10) Have you developed any new cancers since completing surgery on the INFORM trial? _____

If contacting designee or local provider:

11) Is the patient to whom we are referring currently living? _____
   - If no, when did she pass away (date of death)? ____/_____/____
   - If no, was the cause of death breast cancer? _____

Potential Participant Information Sheet

What is the INFORM trial?
It is a study evaluating whether a chemotherapy drug, cisplatin is better than currently used standard chemotherapy drugs (Cyclophosphamide/Doxorubicin) for treating patients with newly diagnosed breast cancer due to an inherited abnormality (mutation) in one of the breast cancer genes, BRCA1 or BRCA2.

How do I know if I have breast cancer due to BRCA1 or BRCA2?
Genetic testing is performed on a blood sample after genetic counseling; results usually return in about 10 days. Breast cancer patients who fit one of the criteria below are appropriate candidates for genetic testing. The cost of the test would be covered by insurance or the INFORM study.

What is involved in being in the INFORM trial?
If you are found to have a BRCA1 or BRCA2 abnormality (mutation), you are potentially eligible to be in the study if your cancer is the kind that would otherwise be treated with chemotherapy. If you choose to be in the study, you would receive chemotherapy before your breast cancer is removed surgically. As part of the study you would either receive conventional chemotherapy, or cisplatin, the study drug.

Why is this study being done?
There are some data to show that cisplatin is an effective drug for treating breast cancers in women with BRCA1 and BRCA2 mutations, possibly more effective than currently used chemotherapy. However, this has not been proved in a randomized clinical trial like the INFORM study. Therefore, you would not receive cisplatin to treat your breast cancer outside of a study.

Thank you for considering this study. **Undergoing genetic testing does not obligate you to participate in the study** but does allow us to determine if you are eligible. If you would consider participating in the study, please ask your doctor for a consult with a genetic counselor, possibly even today, so you can hear more about genetic testing. **If you feel that you would prefer to have your tumor removed before any systemic treatment, then this study is not for you. You may still choose to have genetic testing, but there is no rush to do so. It is important that the genetic test be performed before your tumor is removed,** because in the study, chemotherapy would be given before your breast surgery.

For more information about this study, please contact:
Dr Nadine Tung .....[redacted]
Dr Judy Garber.....[redacted]
<Add Site Specific Contact Information Here>

Patients *With* Breast Cancer: Criteria for genetic testing

If you fit one of the following criteria, genetic testing for BRCA1 and BRCA2 is appropriate for you:

- You are age 45 or younger
- You are a man
- You are Ashkenazi Jewish (ancestors from Europe)
- You are age 50 or younger and also have a relative with breast cancer who was diagnosed at age 50 or younger
- You are age 60 or younger and have the type of breast cancer known as triple negative breast cancer (the tumor does not express the estrogen receptor, the progesterone receptor or HER2)
- You have bilateral breast cancer (cancer in both breasts) and are younger than age 50.
- This is your second breast cancer, and the first was diagnosed when you were younger than age 50 (If you received chemotherapy in the past you are ineligible for this study)
- You or a relative have had ovarian cancer. (If you received chemotherapy in the past you are ineligible for this study)
- You have 2 blood relatives (on the same side of the family) who have also had breast cancer
- A blood relative is known to have a BRCA1 or BRCA2 mutation
- One of your male relatives was diagnosed with breast cancer

Provider Information Sheet

INFORM: BRCA1/2 trial is a randomized neoadjuvant trial for women with newly diagnosed breast cancer and BRCA1/2 mutations comparing 4 cycles of Cisplatin to standard "AC" (Adriamycin/Cytoxan) followed by surgery (lumpectomy or mastectomy). There are data suggesting that cisplatin is an extremely effective drug for BRCA+ breast cancers.

Eligibility

- Newly diagnosed breast cancer > 1.5 cm, triple negative or high grade ER+ (Grade 2 or 3 or recurrence score > 31).
- BRCA1 or BRCA2 germline mutation.
- No prior chemotherapy

Genetic Testing Availability

All patients who meet criteria listed below are eligible for genetic testing. The cost of testing will be covered by insurance or the trial: expedited results within 10 days. Patients who would consider the trial should be referred for genetic counseling and testing expeditiously. Please contact <Insert Site Information here > to facilitate an appointment. .

Genetic Testing Criteria (For breast cancer patients)

- Has a blood relative with a BRCA1 or BRCA2 mutation
- Age 45 or younger
- Ashkenazi Jewish (ancestors from Europe)
- Age 50 or younger and has a blood relative with breast cancer who was diagnosed at age 50 or younger
- Age 60 or younger and has triple negative breast cancer (i.e., ER-, PR-, HER2-)
- Bilateral breast cancer (cancer in both breasts) and is younger than age 50
- Had a blood relative with male breast cancer
- Had a blood relative with ovarian cancer
- Has 2 blood relatives (on the same side of the family) who have also had breast cancer
- This is a second breast cancer, the first diagnosed before age 50 (however, prior chemotherapy renders a patient ineligible)
- Also had prior ovarian cancer (however, prior chemotherapy renders a patient ineligible)
- Is a male

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**12-258**
Protocol Name:	**Randomized Phase II trial of Neoadjuvant Cisplatin vs. Doxorubicin/Cyclophosphamide ("AC") in Women with Newly Diagnosed Breast Cancer and Germline BRCA Mutations**
DFCI Site PI:	Judy Garber, MD
DFCI Research RN:	[redacted]

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

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	The main goal of this study is to determine if the pathologic complete response (pCR) rate to neoadjuvant cisplatin is at least 20% greater than the pCR to doxorubicin/cyclophosphamide (AC) in women w/ newly diagnosed breast CA & a germline BRCA mutation. ***Study Design***: Randomized to either cisplatin or AC in a ratio of 1:1 - Sections 1.1 & 5; ***Study Rationale*** – Section 2.6; *A cycle is either 2 weeks or 3 weeks depending on regimen* – Section 5.2
Dose Calc.	• Doxorubicin, Cyclophosphamide and Cisplatin doses are calculated in mg/m^2 – Section 5.4 • Doses should be based on actual body weight. The participant should be weighed each cycle – Section 5.6
Study Drug Administration	*Study Drug Administration* is outlined in Sections 5 and 7 **Cisplatin** • Administered IV every 3 weeks (+/-3 days) for 4 cycles – Sections 5.6.2 • May be administered per institutional guidelines – Sections 5.6,2 • Use of filgrastim or pegfilgrastim is at the treating physician's discretion – Sections 5.6.2 • See Section 5.6.2 for guidance on magnesium (pre & post infusion) & potassium supplementation (post infusion) • Participants should receive prophylactic anti-nausea medication –see Section 5.6.2 for guidelines/regimens • Please remind participants that they will need follow-up labs @ Day 8 (protocol specifies 7-10 days after chemotherapy) – Table 8.1. The Research RN will also follow up with the participant. **Doxorubicin and Cyclophosphamide ("AC")** • Administered IV every 2 or 3 weeks (+/-3 days) for 4 cycles – Sections 5.6 • ER-negative must be treated every 2 weeks; ER-positive can be treated every 2 or 3 weeks – Section 5.6.1 • May be administered per institutional guidelines – Section 5.6.1 • Filgrastim or pegfilgrastin is **mandatory** for the **2 week** schedule & **optional** for the **3 week** schedule – see Section 5.6.1 for guidance. • Antiemetics may be given per institutional guidelines – Section 5.6.1
Dose Mods & Toxicity	*Dose Modifications for toxicity* are outlined in Sections 5.5 and 6 • This protocol uses CTCAE criteria, Version 4.0.3 – Section 6.2 • ***Requirement for Day 1 of any cycle for cisplatin or AC – See section 5.5*** • Maximum time permitted for delay of chemotherapy is up to 3 weeks – Section 5.5 • See Section 6.2.2 for heme and non-heme toxicity management • No more than 2 dose reductions are allowed – Section 6.2; Re-escalation of dose is not allowed – Section 6.2
Con Meds	*Concomitant therapies/meds* are outlined in Section 5.6 and 5.1.1 • Participants should receive optimal supportive care throughout the study • See Section 6.1.1 for possible cisplatin drug interactions
Required Data	*Study Assessments* are outlined in Section 8 • See Table 8.1 for the study calendar
Chart Tips	• Please be sure to DOCUMENT infusion **actual** UP/DOWN times in medical record • Please be sure to also DOCUMENT any additional vital signs and routes of administration

Dear Patient,

We are writing to you because you have previously been seen in [INSERT CLINIC NAME HERE] due to a personal or family history of cancer and have been found to have a BRCA1 or BRCA2 mutation.

As you are likely aware, the medical management recommendations for individuals with a hereditary predisposition to develop cancer are constantly evolving as we learn new information about cancer risks in families, as new technology is developed, and as new genetic testing becomes available. We strongly encourage all of our patients to follow up with our genetics team on a regular basis to ensure we keep you up to date regarding the latest information about screening and prevention. If you have not been seen in our program for greater than one year (and you aren't otherwise followed regularly by your oncologist or other cancer specialist), we encourage you to take the time to make a follow up visit with our program.

At [INSERT CLINIC NAME HERE] we are continually striving to provide our patients with the latest and most advanced information on the treatment of hereditary cancers that may be relevant to you, your family or a community of BRCA carriers with whom you have connected.

[INSERT THIS SECTION FOR DF/HCC AND DF/PCC SITES; DELETE THIS SECTION FOR EXTERNAL SITES]Towards this end, we want to let you know about an important study for BRCA carriers newly diagnosed with breast cancer, the **INFORM BRCA1/2 trial**. This study investigates whether a particular chemotherapy agent, cisplatin is superior to conventional chemotherapy for BRCA carriers with breast cancer. This study will accrue patients at many centers across the country, and is open at several hospitals in Boston including [INSERT HOSPITAL NAME HERE]. Please feel free to share the information about this study with your family members or other individuals you know who are at increased risk for cancer due to an inherited BRCA mutation. Included is a brochure about this trial and contact information for any potentially eligible and interested patients.

[INSERT THIS SECTION FOR EXTERNAL SITES; DELETE THIS SECTION FOR DF/HCC AND DF/PCC SITES] Towards this end, we want to let you know about an important study for BRCA carriers newly diagnosed with breast cancer, the **INFORM BRCA1/2 trial**. This study investigates whether a particular chemotherapy agent, cisplatin is superior to conventional chemotherapy for BRCA carriers with breast cancer. This study will accrue patients at many centers across the country including [INSERT HOSPITAL NAME HERE]. Please feel free to share the information about this study with your family members or other individuals you know who are at increased risk for cancer due to an inherited BRCA mutation.

There are also many other cancer trials for BRCA carriers available at [INSERT HOSPITAL NAME HERE]. For questions regarding these studies, please contact [INSERT CONTACT INFORMATION HERE] (research nurse) at [INSERT PHONE # HERE}, or me, [INSERT DOCTOR NAME HERE] at [INSERT PHONE # HERE].

If you have any questions about the information provided with this letter, or if you would like to discuss making a follow up appointment with our program, please call us directly at [INSERT PHONE # HERE].

Sincerely,

[INSERT PHYSICIAN NAME HERE]

[INSERT TITLE HERE]

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 Advance Cancer Research

Providing Tissue Samples as Part of a Clinical Trial

Research Advocacy Network

Advancing Patient-Focused Research

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 want to ask my doctor about providing tissue samples for research?

- What is the purpose of the research and what do you hope to learn?
- Am I likely to benefit from providing tissue samples?
- 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? Will they be taken at different times? If yes, how many and how often?
- What happens if I provide one tissue sample and then decide I do not want to provide any more? Can I leave the study at any time?
- Do I have to make a special trip to the hospital or clinic to provide the tissue sample or will it be done as part of my regularly scheduled appointments?
- Will these samples be used only in this study or stored for any other purpose in the future?
- How will my privacy (and my family's privacy) be protected?
- Will I be able to find out the results of the tests conducted on my tissue samples?
- Will my insurance cover the costs or is this included in the study?

The answers to these questions depend on the specifics of a trial and your situation and are best addressed by your healthcare team.

TIP SHEET

How You Can Help Advance Cancer Research

Providing Tissue Samples as Part of a Clinical Trial

Research Advocacy Network

This "tip sheet" is intended to provide answers and insight for using the brochure and maximizing the utility of the publication. It should be used by the healthcare provider and not distributed to patients.

TIP SHEET

Overall Tips

- As you know from your experience with patients, a cancer diagnosis is often a traumatic experience. The lack of highly effective and well-tolerated treatments is a major reason that all of us fear cancer. In order to design improved treatments, researchers must be able to study the cancer cells from actual patients to see what problems they show and how they respond to treatment. Patients who participate in a clinical trial that includes tissue samples may help to improve cancer treatments in the future that could benefit others – perhaps even their friends and family.
- Some patients will want to participate in a clinical trial with tissue samples; others will not. The choice to participate is an individual one and is likely influenced by the type of cancer a patient has and the invasiveness of the tissue sampling procedures. Some patients may be willing to provide an initial tissue sample that is used for diagnosis but may not want to provide a subsequent sample that would be used for research.
- If we accept that participation in a clinical trial that includes tissue samples is important in advancing cancer research, the next question becomes how to address this participation with your patients. When speaking with patients, many researchers report that it is best to motivate participation in a clinical trial, before addressing the need for tissue samples. Once patients become interested in the trial, they may perceive the samples as a necessary logistical component instead of as a barrier.

Answers to Questions in the Brochure

- **What is the purpose of the research and what do you hope to learn?**
  The purpose of the research study can be found in the Primary Objective or Study Objective section of the protocol.
- **Am I likely to benefit from providing tissue samples?**
  In general, patients will not necessarily benefit directly from providing tissue samples. However, in rare cases, the tissue sample may provide information relevant to the patient's disease or treatment that can then be shared with the physician. The protocol may specify the process by which the patient's physician can receive this information; if it does not, you may want to ask the principal investigator or the organization sponsoring the trial. However, it is important that patients understand that participation in the study and provision of tissue samples is unlikely to benefit them directly. Information derived from the tissue samples could lead to treatments that would benefit the patient's family and friends, as well as future generations. It is also possible that the information may benefit the patient in the future if they have a recurrence.
- **From which part of my body will the tissue sample(s) be taken?**
  This will depend on the type of cancer, the amount of tissue needed, current levels of technology and other factors. The protocol will describe the part of the body from which tissue is to be taken, the procedures to take the sample, and the risks involved.
- **How will the tissue sampling procedure be performed?**
  This will also depend on many factors, as listed in the preceding answer. The website by Dr. Ed Uthman listed in the sources section at the end of this tip sheet provides a patient-friendly overview of different types of tissue sampling procedures. However, note that these procedures are listed under the heading Biopsies, which technically refers only to samples obtained for diagnostic purposes. The procedures, however, are the same. The websites are listed on a separate sheet at the end of this document that can be copied and given to patients.
- **Will the tissue sampling procedure hurt?**
  The amount of discomfort the patient experiences will depend on the type of procedure performed, as well as patient-specific factors such as tolerance level for pain or discomfort. The patient should ask you, as a part of the healthcare team, for more information about the planned sampling procedure. Also see question above.
- **Is the tissue sampling procedure risky to me?**
  In general, tissue sampling procedures are not unduly risky to patients. However, the patient should be encouraged to discuss potential risks with the doctor. Some of the websites listed at the end of this tip sheet list risks and may be helpful for patients.

  - Information for patients about clinical trials can be found on the National Cancer Institute and United States Food and Drug Administration websites listed at the end of this document.
  - Information about providing tissue samples can be found on the Research Advocacy Network website listed at the end of this document.
- A patient may have already provided a tissue sample as part of his or her diagnostic procedures and thus may only be looking at one additional sample.
- Use of the term biopsy may make some patients uncomfortable, as it is psychologically associated with "looking for cancer". Therefore, it may be better to use the phrase tissue sample. A biopsy is defined as the removal of tissue or cells from the body for examination under a microscope, typically to check for signs of cancer. Tissue samples obtained for research may be used to examine the action of a potential therapeutic agent on the cells.
- The person who discusses clinical trial participation and tissue samples with patients should be thoroughly familiar with the study protocol. This tip sheet offers general suggestions, but the specific features of each trial will differ.
- At the end of the brochure, there is a space for the name and contact information of the person from whom patients can receive further information. Please add this information to each brochure.

- **How soon will I be able to engage in my normal activities after the tissue sampling procedure?**
  This will depend on the type of tissue sample provided. In some cases, the tissue sampling procedure would not be expected to affect the patient's activities at all, whereas in other cases, the patients may need to remain in the hospital for several days after the procedure and slowly resume normal activities over the course of a week (e.g., open lung tissue sampling procedure).

- **How many tissue samples are you requesting of me? Will they be taken at different times? If yes, how many and how often?**
  This should be specified in the study protocol. Often, protocols will have a section entitled Schedule of Assessments that will list the scheduled time for each tissue sampling procedure.

- **What if I provide one tissue sample then decide I do not want to provide any more?**
  Patients can always decide to stop participating in the study and can refuse to provide tissue samples at any time.

- **Do I have to make a special trip to the hospital or clinic to provide the tissue sample or will it be done as part of my regularly scheduled appointments?**
  This will depend on the protocol, as well as the type of procedure. Often, protocols will have a section entitled Schedule of Assessments that will list the scheduled time for each tissue sampling procedure. It can then be determined whether these could correspond with regularly-scheduled appointments.

- **Can my tissue samples be used for more than one study?**
  Under some conditions, patients may be able to request that additional tissue be taken for use in a different study. However, you may want to ask whether they have a specific study in mind. If not, you may want to put them into contact with a tissue bank that will be able to use additional tissue.

- **Will I be able to find out the results of the tests conducted on my tissue samples?**
  In general, the results of the research tests conducted on tissue samples are provided as averages for the entire group under study and not for individual patients. Additionally, the meaning of each patient's results (e.g., high or low levels of a certain protein in their cancer cells) will probably not be known for some time, often years. Studies take time to conduct and the cancer community must be shown to have confidence in the results before any information can be used to make individual patient treatment decisions or prognoses. Healthcare providers should be prepared to tell the patient, based on the protocol, if and when any results will be made available to the patients or their physicians. This information may also be listed in the consent form.

- **Will my insurance cover the costs or is this included in the study?**
  If the tissue sample is for research purposes only, the procedure will be covered by the study and the cost should be spelled out under the "Cost Section" in the Informed Consent Document. In order to adequately address cost and payment issues, the study coordinator or nurse should be thoroughly familiar with the study protocol.

Sources of Information For Patients About Tissue Sampling and Clinical Trials

Tissue Sampling Procedures

Note that the many of the procedures listed on these sites can be the same for tissue that is sampled for diagnostic and research purposes.

Mayo Clinic. Biopsy procedures used to detect cancer. Available at: http://www.mayoclinic.com/health/biopsy/CA00083. Accessed August 14, 2008.

Uthman EO. The biopsy report: a patient's guide. Types of biopsies. Available at: http://www.cancerguide.org/pathology.html. Accessed August 14, 2008.

Clinical Trials

Coalition of Cancer Cooperative Groups. Available at: http://www.cancertrialshelp.org/. Accessed August 15, 2008.

United States Food and Drug Administration. Clinical trials. Available at: http://www.fda.gov/oashi/clinicaltrials/default.htm. Accessed August 15, 2008.

National Cancer Institute. What is a clinical trial? Available at: http://www.cancer.gov/clinicaltrials/learning/what-is-a-clinical-trial. Accessed August 15, 2008.

Providing Tissue for Research

National Cancer Institute. Providing tissue for research. What you need to know. Available at: http://www.cancer.gov/clinicaltrials/resources/providingtissue. Accessed September 2, 2008.

Research Advocacy Network. Why is it important for me to consider donating my tissue for research? Available at: http://www.researchadvocacy.org/publications/posters.php. Accessed August 14, 2008.

Research Advocacy Network. The importance of tissue samples in research. Available at: http://www.researchadvocacy.org/publications/posters.php. Accessed August 14, 2008.

Research Advocacy Network. Understanding pathology and tissue research. Available at: http://www.researchadvocacy.org/publications/posters.php. Accessed August 14, 2008.

Research Advocacy Network

Advancing Patient-Focused Research

6505 W. Park Blvd., Suite 305, PMB 220 • Plano, TX 75093 • Phone & Fax: 877.276.2187

www.researchadvocacy.org