Title: Phase II Trial of Paclitaxel Combined with Trastuzumab and Pertuzumab as Preoperative Therapy for Inflammatory Breast Cancer

NCT Number: NCT01796197

IRB Approval Date: 04/23/2021

NCI Protocol #: Not Applicable

DF/HCC Protocol #: 12-497

TITLE: Phase II Trial of Paclitaxel Combined with Trastuzumab and Pertuzumab as Preoperative Therapy for Inflammatory Breast Cancer

Coordinating Center: Dana Farber Cancer Institute

***Principal Investigator (PI):** *Filipa Lynce, MD*
Dana-Farber Cancer Institute
450 Brookline Avenue, YC-1255
Boston, MA 02215
Tel: 617-632-3800
Fax: 617-632-1930
Email: Filipa_Lynce@dfci.harvard.edu

Statistician: *Meredith Regan, ScD*
Department of Biostatistics and Computational Biology
Dana-Farber Cancer Institute
450 Brookline Avenue, (CLS 11007)
Boston, MA 02215
Tel: 617-632-2471
Fax: 617-632-2444

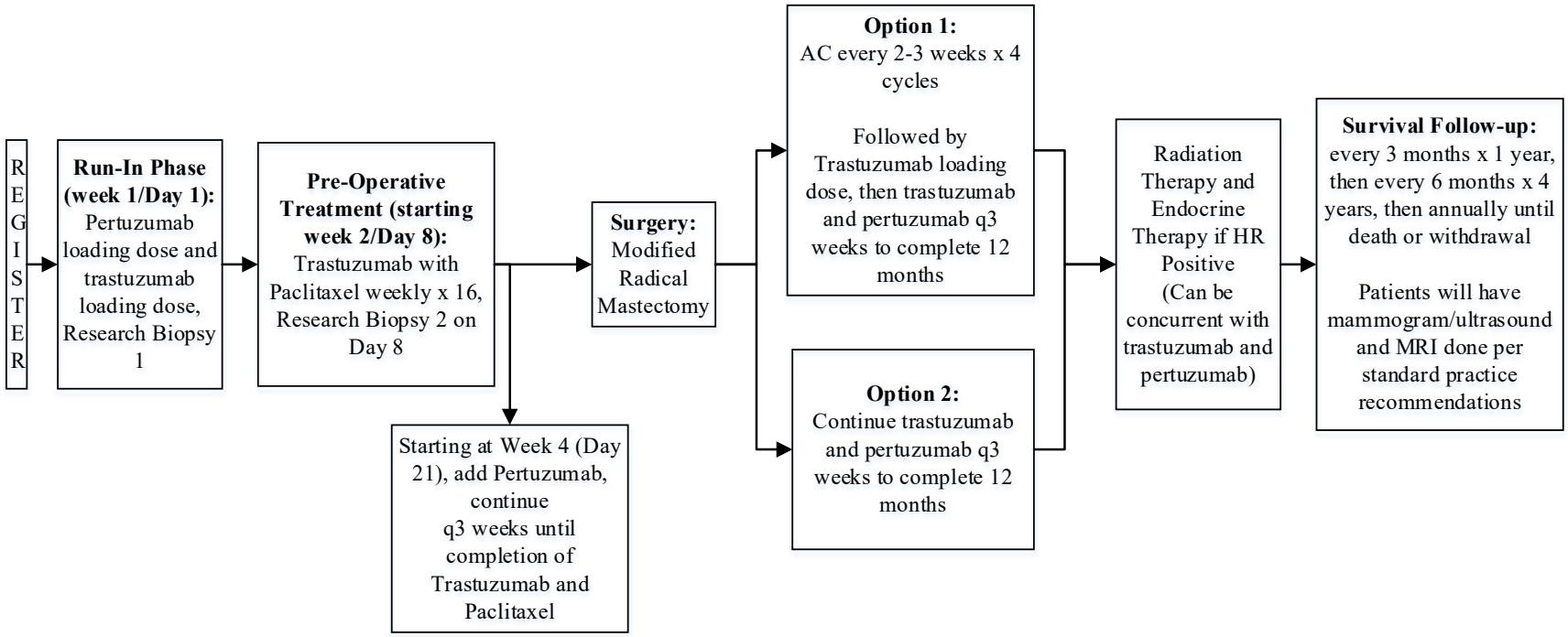
Data Coordinator: *Claire Remolano*
Dana-Farber Cancer Institute
450 Brookline Ave., DA-157
Boston, MA 02215
Tel: 617-582-9380
Fax: 617-632-3550
Email: marie_remolano@dfci.harvard.edu

Agent(s): HERCEPTIN (trastuzumab) [Supplied by Genentech], PERJETA (pertuzumab) [Supplied by Genentech], Paclitaxel [Commercial], Doxorubicin [Commercial], Cyclophosphamide [Commercial]

IND #: 117932
IND Sponsor: Filipa Lynce, MD

Protocol Type / Version # / Version Date: *Amendment 20 / Protocol Version # 20/ Version Date: April 23, 2021*

SCHEMA



AC=Doxorubicin and cyclophosphamide

Non-randomized phase II study, N = 30
Patient Characteristics:

- Newly diagnosed inflammatory breast cancer
- HER2+

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Study Design

Patients with untreated inflammatory breast cancer undergo a research breast biopsy and whole blood sample for correlative studies at baseline. They then receive both trastuzumab and pertuzumab for a loading dose (day 1, week 1). One week later (week 2, day 8), a second research biopsy from breast tissue and whole blood sample for correlative studies is obtained. On week 2, day 8, patients begin weekly paclitaxel for 16 doses. Weekly trastuzumab continues with paclitaxel to complete 16 doses of paclitaxel. Pertuzumab is given every 3 weeks beginning on week 1, day 1 and continues until 16 doses of paclitaxel are completed. Following 16 doses of paclitaxel, trastuzumab and pertuzumab can continue every 3 weeks until surgery. Four to five (5) weeks following the completion of preoperative therapy, patients deemed surgically operable proceed to total mastectomy and axillary lymph node dissection, where residual cancer is obtained for correlative studies.

Patients have two options for post-operative therapy as determined by treating physician. Option 1: Four to five (4-5) weeks following surgery, patients then receive doxorubicin and cyclophosphamide every 14-21 days for 4 cycles. Three to 4 weeks following the completion of doxorubicin and cyclophosphamide, trastuzumab and pertuzumab is administered every 3 weeks to complete 12 months of HER2-directed therapy. Option 2: Trastuzumab and pertuzumab is administered every 3 weeks to complete 12 months of HER2-directed therapy.

Radiation is given to the post-surgical chest wall and regional lymph nodes beginning approximately 3-6 weeks after surgery or following completion of doxorubicin and cyclophosphamide, concurrent with trastuzumab and pertuzumab administration.

Endocrine therapy with either tamoxifen or an aromatase inhibitor is given in standard fashion for hormone receptor positive disease, either beginning with the initiation of radiation therapy, or at the completion of radiation therapy.

Patients with Stage IV disease by virtue of nodal involvement alone will continue on trastuzumab and pertuzumab until disease progression.

Patients whose disease has not achieved the criteria for surgical resection following pre-operative study therapy with 16 doses of paclitaxel combined with pertuzumab and trastuzumab, yet the disease has not progressed, may receive doxorubicin and cyclophosphamide every 14-21 days for 4 cycles.

Patients whose disease is then deemed surgically resectable may proceed to total mastectomy and axillary lymph node dissection 4 to 5 weeks following completion of doxorubicin and cyclophosphamide. They may then continue on study treatment as outlined above (i.e., radiation therapy and continued pertuzumab and trastuzumab).

Patients whose disease is not surgically resectable following doxorubicin and cyclophosphamide every 14-21 days for 4 cycles, may proceed to definitive radiation given to the breast and

regional lymph nodes beginning approximately 4 to 5 weeks after completion of doxorubicin and cyclophosphamide.

If their disease is then deemed surgically resectable, they may proceed to total mastectomy and axillary lymph node dissection 5 – 6 weeks following completion of radiation therapy. They then continue on study treatment as outlined above (i.e., continue on pertuzumab and trastuzumab).

CLARIFICATION NOTE: Patients will only receive doxorubicin and cyclophosphamide and radiation therapy once during their course of treatment; either pre- or post-mastectomy.

1.2 Primary Objectives

- To determine pathologic complete response (pCR) rate after preoperative therapy with combination paclitaxel (T), trastuzumab (H) and pertuzumab (P) in HER2+ inflammatory breast cancer.

- To assess the residual cancer burden (RCB) after preoperative therapy with combination paclitaxel (T), trastuzumab (H) and pertuzumab (P) in HER2+ inflammatory breast cancer.

1.3 Secondary Objectives

- To assess the toxicity of protocol therapy, including clinically-significant CHF.

- To determine the efficacy of therapy defined as disease-free survival (DFS), time to treatment failure (TTF), and overall survival (OS).

- To assess microarray analysis on pre-treatment breast cancer biopsy specimens using PAM50 to correlate the intrinsic subtype with pCR.

- To assess microarray analysis on residual disease within the breast accessed at the time of mastectomy and by using PAM50 correlate the intrinsic subtype of the resistance clone with residual disease.

- To identify early adaptive responses that are associated with resistance to HER2 directed therapies, i.e., trastuzumab and pertuzumab, but analyzing breast tissue specimens obtained 8 days after exposure to only HER2 directed treatment.

- To assess changes in circulating biomarkers, including ctDNA, with pCR.

- To develop patient-derived xenograph (PDX) models of HER2 positive inflammatory breast cancer in order to facilitate extensive research on this disease.

2. BACKGROUND

2.1 Study Disease(s)

2.1.1 Inflammatory Breast Cancer

Inflammatory breast cancer (IBC) accounts for 2-5% of all invasive breast cancer. IBC is classified as a "cliniopathologic" diagnosis, whereby documentation of invasive breast carcinoma is established in the setting of unique clinical characteristics including a rapid onset of breast enlargement, pain, diffuse erythema and edema (peau d'orange) usually occurring within 3-6 months. The breast cancer often presents without a palpable mass, and dermal lymphatic involvement with cancer is demonstrated in approximately 75% of the cases.[1] It is the effect of dermal lymphatic involvement, not infiltration of inflammatory cells that result in the clinical changes observed in IBC. The median age at presentation is less than that seen in non-IBC, and there is a greater incidence of IBC among African American women. [2, 3]

The intrinsic biology of IBC is such that advanced disease is present at the time of diagnosis. Approximately 55-85% of patients present with metastasis to the axillary and / or supraclavicular lymph nodes, and distant metastatic disease is present at diagnosis in approximately 20-40% of women. [4] Even in the absence of metastatic disease at presentation, the likelihood of developing distant metastasis is extremely high, supporting a role for chemotherapy as the mainstay of treatment. Historically, surgery and radiation therapy alone resulted in a 54% relapse rate within 18 months, translating into a median survival of 1.2 years. [5] The addition of chemotherapy to the initial treatment of IBC, i.e. trimodality therapy, improved the median overall survival to 3.8 years, translating into an approximately 50% 5-year overall survival. [1]

The extent of locoregional disease precludes mastectomy as primary treatment for IBC, therefore preoperative chemotherapy has become the standard of care; however the optimal chemotherapy regimen has yet to be determined. Anthracycline-containing regimens employed preoperatively have resulted in 40-45% 5-year overall survival, whereas the addition of taxanes to these regimens improved the pathologic complete response rate and overall survival. [6-8] The current chemotherapy regimens utilized as pre-operative treatment for IBC most commonly include anthracyclines and taxanes, yet the poor overall survival rates still necessitate ongoing investigation into improved pre-operative regimens.

High-throughput molecular technologies have identified five intrinsic molecular subtypes of breast cancer: luminal A, luminal B, HER2 positive, triple negative, and normal. [9] Molecular analysis of IBC demonstrates a tendency for this disease to segregate into the more proliferative intrinsic subtypes, i.e. triple negative (estrogen receptor (ER), progesterone receptor (PR) and HER2 negative) and HER2 positive. [10] Several studies have shown a 40-50% incidence of HER2 positive disease among IBC, which is more than 2-fold greater than the incidence seen in non-IBC. [11, 12] The prevalence of HER2 positive disease among patients with IBC and the recent availability of effective agents targeting the HER2 domain support further investigation into the optimal preoperative regimen incorporating HER2 targeting agents in the treatment of IBC.

2.1.2 HER2 and Breast Cancer

Growth factors and their receptors play critical roles in development, cell growth, differentiation, and apoptosis.[13] Such receptors span the cell membrane, with the extracellular domain binding specific growth factors and the intracellular domain transmitting growth signals. Interaction of the extracellular domain with its cognate ligand often results in intracellular activation of tyrosine kinase activity. Overexpression of human epidermal growth factor receptor 2 (HER2, also known as *erb*B2, neu, and p185HER2) is observed in approximately 25-30% of human breast cancers. [14] HER2 overexpression has been reported to only rarely occur in the absence of gene amplification. [15],[16] High level of HER2 expression has been correlated with poor clinical outcome. [14]

Several lines of evidence support a direct role for HER2 overexpression in the pathogenesis and poor clinical course of human tumors.[17] When the mutated gene is transfected into murine fibroblast (NIH 3T3) cells, it causes transformation, and the resulting cells are tumorigenic in the nude mice. [18],[19] Additionally, transgenic mice that overexpress the rodent homolog of the human HER2 gene develop breast cancer. [20] Finally, specific antibodies to the extracellular domain of HER2 inhibit the experimental growth of tumors that overexpress the gene. [21],[22] These data suggest a direct role for HER2 in both malignant transformation and enhanced tumorigenicity. Therefore a strategy to antagonize the abnormal function of overexpressed HER2 was developed to improve the course of patients with HER2-overexpressing tumors. Monoclonal antibodies directed against the HER2 protein were developed and humanized to minimize the likelihood of immunogenicity. One of these antibodies (trastuzumab) was very effective in inhibiting both in vitro and in vivo proliferation of human breast cancer tumor cells overexpressing the HER2 protein and in mediating antibody-dependent cellular cytotoxicity in the presence of human effector cells.[23]

There is substantial preclinical evidence that inhibition of signal transduction pathways can potentiate the cytotoxic activity of chemotherapeutic drugs. Indeed, trastuzumab has been shown to have synergy, in vitro and in vivo, with several chemotherapeutic drugs including cisplatin, doxorubicin, thiotepa, etoposide, vinorelbine, and taxanes.[24],[25],[26],[27],[28],[29] Given this promising preclinical data, trastuzumab was tested in the clinic both as a single agent and in combination with chemotherapy.

2.2 Trastuzumab Clinical Experience

2.2.1 Trastuzumab Clinical Experience in Metastatic Breast Cancer:

The clinical benefit of trastuzumab in women with metastatic breast cancer has been demonstrated in two pivotal studies. A large Phase II trial (H0649g) assessed the activity of trastuzumab as a single agent in 222 women with HER2 overexpressing metastatic breast cancer with progressive disease after one or more chemotherapy regimens.[30] A blinded, independent response evaluation committee identified 8 complete and 26 partial

responses, for an objective response rate of 15% in the intent-to-treat population (95% confidence interval, 11% to 21%). The median duration of response was 9.1 months, and the median duration of survival was 13 months. The most common adverse events, which occurred in approximately 40% of patients, were mild to moderate infusion-associated fever and/or chills. These symptoms usually occurred only during the first infusion. The most clinically significant event was cardiac dysfunction, which occurred in 4.7% of patients.

A large, open-label, randomized Phase III study (H0648g) in 469 patients with HER2-positive metastatic breast cancer was conducted to evaluate the efficacy of trastuzumab in combination with chemotherapy as first-line treatment. Patients who were anthracycline-naïve were randomized to receive either anthracycline plus cyclophosphamide (AC) or trastuzumab plus AC. Patients who had received prior anthracyclines in the adjuvant setting were randomized to receive either paclitaxel or trastuzumab plus paclitaxel. Patients randomized to trastuzumab and chemotherapy measurably benefited in comparison to patients treated with chemotherapy alone in terms of time to disease progression, overall response rate, median duration of response, and survival. As determined by an independent Response Evaluation Committee (REC), trastuzumab prolonged median time to disease progression from 4.6 months to 7.4 months ($p<0.001$), improved the overall response rate (complete and partial responses) from 32% to 50% ($p<0.001$), and increased median duration of response from 6.1 to 9.1 months ($p<0.001$). Compared to chemotherapy alone, the addition of trastuzumab significantly lowered the incidence of death at one year from 33% to 22% ($p=0.008$) and increased median overall survival 24% from 20.3 months to 25.1 months ($p=0.046$). The observed survival advantage remained despite crossover of 66% of patients initially randomized to chemotherapy alone who elected to receive trastuzumab upon disease progression.[31] Fever/chills were observed with the initial trastuzumab infusion in approximately 25% of patients. Class III or IV cardiac dysfunction was observed in 16% of the trastuzumab + AC subgroup; increasing age was an associated risk factor for the development of cardiotoxicity in this treatment cohort.

Based on these data, trastuzumab was approved by the U.S. Food and Drug Administration (FDA) for use in HER2-overexpressing metastatic breast cancer in combination with paclitaxel for first-line treatment and as a single agent for patients failing prior chemotherapy for metastatic disease. However, current usage patterns of trastuzumab indicate that the drug is now being used in a broader array of circumstances that in the pivotal clinical trials.

2.2.2 Trastuzumab Clinical Experience in Adjuvant Breast Cancer

Four large, randomized, phase III trials showed significant reduction in risk of disease recurrence with the addition of 1 year of trastuzumab to adjuvant therapy in patients with HER2-positive, early breast cancer. The 3-year planned joint interim analysis of the National Surgical Adjuvant Breast and Bowel Project (NSABP B-31) and the North Central Cancer Treatment Group (NCCTG N-9831) trials demonstrated significant improvements in disease-free survival (DFS) (hazard ratio [HR] 0.48, $p<0.0001$) and

overall survival (OS) (HR 0.67, p=0.015) when 1-year of trastuzumab is added to adjuvant chemotherapy in patients with HER2-positive breast cancer.[32] At the 4-year follow-up, DFS and OS results were consistent.[33] In HERA (trastuzumab adjuvant), single agent trastuzumab after adjuvant chemotherapy demonstrated significant improvements in DFS (HR 0.64, p<0.0001) and OS (HR 0.66, p=0.0115) compared with observation alone at a median follow-up of 23.5 months.[34] At a median follow-up of 48.4 months, a DFS benefit was observed (HR 0.66, p=0.0115) with 1 year of trastuzumab, however, the OS benefit was not statistically significant at 4 years (HR 0.85, p=0.11). At the time of analysis, over 50% of patients in the observation arm had crossed-over to receive trastuzumab.[35] The third protocol-specified analysis of the Breast Cancer International Research Group (BCIRG) 006 study continued to show that the addition of 52 weeks of trastuzumab to docetaxel-based adjuvant regimens significantly improved DFS. At a median follow-up of 65 months, 5-year DFS rates were 84% (HR 0.64, p<0.001) and 81% (HR 0.75, p=0.04) in the doxorubicin-containing trastuzumab and non-anthracycline-containing trastuzumab arms, respectively.[36]

2.3 Pertuzumab Clinical Experience

Pertuzumab, a humanized monoclonal antibody to the HER2 receptor, represents a promising new anti-HER2 agent with a novel mechanism of action targeting inhibition of HER2 dimerization. Nonclinical and clinical data to date indicate that pertuzumab provides a broader HER2 blockade through inhibition of HER2 heterodimerization. Pertuzumab has been shown in preclinical experiments to have superior anti-tumor effects when combined with other anti-HER2 treatments such as trastuzumab than when used as monotherapy.

Trastuzumab and pertuzumab monoclonal antibodies bind to distinct epitopes on the HER2 receptor without competing with each other, resulting in distinctive mechanisms for disrupting HER2 signaling. These mechanisms are complementary and result in augmented therapeutic efficacy when pertuzumab and trastuzumab are given in combination.

Preclinical data indicate at least additive efficacy when the two agents are administered together, resulting in significantly reduced tumor volume compared with either agent alone. Clinically, pertuzumab may have optimal therapeutic effects when given in combination with trastuzumab to patients with HER2-positive cancers, evidenced by data generated in a Phase II study of patients with previously treated HER2-positive MBC.[37] A recently published meta-analysis of pertuzumab phase II trials concluded that pertuzumab has a low cardiac risk and there is no notable increase in cardiac events when pertuzumab is used in combination with other anticancer agents.[38]

2.4 Trastuzumab and Pertuzumab Combination Therapy in Patients with HER2-positive Metastatic Breast Cancer

In the Phase III, pivotal study WO20698/TOC4129g (CLEOPATRA; N=808) in patients with previously-untreated HER2-positive MBC, a statistically significant and clinically meaningful improvement in progression-free survival (PFS) was observed in patients treated with

pertuzumab, trastuzumab and docetaxel (N=406) compared to those receiving placebo, trastuzumab and docetaxel (N=402). PFS was prolonged at the median by 6.1 months and the risk of disease progression or death was reduced by 38% (Hazard ratio [HR] = 0.62; 95% CI = 0.51, 0.75; $p < 0.0001$) with an improvement in median PFS from 12.4 months to 18.5 months.[39] In a Phase II, single-arm study (BO17929; N= 66) in patients with previously-treated HER2-positive MBC, four complete responses and 12 partial responses (24% objective response rate) were observed following combined treatment with pertuzumab and trastuzumab.[37]

2.5 Trastuzumab and Pertuzumab Safety

2.5.1 Trastuzumab Safety

Experience with trastuzumab administration has shown that the drug is relatively safe. The most significant safety signal observed during clinical trials was cardiac dysfunction (principally clinically significant heart failure (CHF), particularly when trastuzumab was given in combination with an anthracycline-containing regimen. Much of the cardiac dysfunction was reversible on discontinuation of trastuzumab.

In addition, during the first infusion with trastuzumab, a symptom complex most commonly consisting of fever and/or chills was observed in approximately 40% of patients. The symptoms were usually mild to moderate in severity and controlled with acetaminophen, diphenhydramine, or meperidine. These symptoms were uncommon with subsequent infusions. However, in the postapproval setting, more severe adverse reactions to trastuzumab have been reported. These have been categorized as hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events. Rarely, these severe reactions culminated in a fatal outcome.

Trastuzumab appears to be relatively nonimmunogenic. Only 1 of 903 patients evaluated developed neutralizing antibodies to trastuzumab. The development of anti-trastuzumab antibodies in this patient was not associated with clinical signs or symptoms.

2.5.2 Pertuzumab Safety

As of 7 November 2011, 1757 patients with cancer have been treated with pertuzumab in all company-sponsored pertuzumab trials, and an additional 114 patients have received pertuzumab in combination studies with trastuzumab emtansine. Overall, data indicate that pertuzumab is well-tolerated as monotherapy and that it can be given in combination with trastuzumab and a range of other therapeutic agents with manageable additional toxicity. No new or unexpected toxicities were encountered other than those that are known for agents that target the HER family of receptors. Serious or severe infusion-associated symptoms have been rarely observed in patients receiving pertuzumab. A low level of cardiac toxicities, predominantly asymptomatic declines in left ventricular ejection fraction (LVEF), has been reported. In the pivotal Phase III trial WO20698/TOC4129g the rates of symptomatic and asymptomatic left ventricular systolic dysfunction (LVSD) were not higher in patients receiving pertuzumab, trastuzumab and docetaxel than in those receiving placebo, trastuzumab and docetaxel.

No fetal studies in humans have been performed but pertuzumab caused oligohydramnios, delayed renal development and embryo-fetal deaths in pregnant cynomolgus monkeys. Moreover, in the post-marketing setting, cases of oligohydramnios, some associated with fatal pulmonary hypoplasia of the fetus, have been reported in pregnant women receiving trastuzumab (for further details, see trastuzumab prescribing information). Therefore, pertuzumab should not be used in pregnant women. Protocols for ongoing pertuzumab studies indicate that highly effective contraceptive measures must be used; continuous pregnancy monitoring must be performed during the trials and for seven months after the last dose of study drug is administered. Because of the long half-life of pertuzumab women should be warned not to become pregnant for at least seven months after completion of treatment.

2.6 Rationale for the Use of Pertuzumab and Trastuzumab in IBC

The goal of neoadjuvant or preoperative systemic therapy for breast cancer is to render inoperable disease operable without compromising overall survival. In addition, the pathologic response, i.e. residual carcinoma in the breast and/or lymph nodes, often correlates with the disease prognosis. [40] Patients with IBC treated with neoadjuvant therapy also have a more favorable outcome when a complete pathologic response (pCR) is achieved. [41] Therefore, pCR is often used as a primary outcome in clinical trials investigating novel neoadjuvant therapy, because it is an acceptable surrogate marker for disease-free survival (DFS) and overall survival (OS).

The majority of studies evaluating the efficacy of trastuzumab added to neoadjuvant chemotherapy include other subtypes of breast cancer in addition to, or excluding IBC. The NOAH (NeOAdjuvant Herceptin) study included 63 patients with HER2 positive IBC and found that the addition of trastuzumab to neoadjuvant chemotherapy resulted in a54.8% pathologic complete response (pCR) rate compared with chemotherapy alone (pCR = 19.3%), which translated into a HR = 0.27 for event-free survival. [42], [43]

Combination pertuzumab, trastuzumab and chemotherapy were investigated neoadjuvantly in the NeoSphere trial. [44] This study also included patients with IBC, though only 29 among 417 patients carried this diagnosis. The NeoSphere trial compared the pCR rate among four groups of patients receiving neoadjuvant treatment: trastuzumab and docetaxel vs. pertuzumab, trastuzumab and docetaxel vs. pertuzumab and trastuzumab vs. pertuzumab and docetaxel. The combination arm including pertuzumab, trastuzumab and docetaxel achieved the highest pCR rate of 39.9%. This favorable response suggests that combination HER2 targeting therapy using pertuzumab and trastuzumab with chemotherapy should be investigated as neoadjuvant treatment for with IBC.

2.7 Correlative Studies Background

2.7.1 Gene Expression Microarrays

The efficacy of trastuzumab and pertuzumab is dependent upon HER2 over-expression,

which clinically can be detected by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) on breast cancer tissue. However, documentation of HER2 positive disease is not always associated with a clinical response to HER2-targeted therapy, either due to the development of resistance pathways or because of imprecision in the determination of true HER2-driven disease. More specific techniques of molecular profiling have been able to determine subtle changes in gene expression which may impact the selection of appropriate patients to receive specific targeted therapy, such as trastuzumab and pertuzumab.

DNA microarray analysis using a 50-gene assay (Prediction Analysis of Microarray or PAM-50) found that IHC or FISH assessment of HER2 status does not provide as complete a classification of the HER2 enriched intrinsic subtype. [45]In fact, a significant proportion of HER2-positive breast cancers determined by clinical methods are not classified within the HER2-enriched intrinsic subtype, and this may have implications overall tumor response to neoadjuvant therapy and on choosing the most efficacious chemotherapy regimen to combine with trastuzumab. [46, 47] To build on these principles, next-generation sequencing technology will be able to explore a larger array of gene expression which may offer more insight into disease response of HER2 positive IBC.

Both de novo and acquired mechanisms of resistance to HER2-directed therapies result in disease recurrence or progression. There are many mechanisms of resistance postulated to exist including the presence of truncated p95HER2 fragments, changes in downstream signaling pathways including loss of PTEN function and mutations in PI3KCA, and the activation of other signaling pathways including IGR1R and HER3 signaling pathways. [48],[49], [50], [51] Investigating the development of these compensatory mechanisms of resistance will allow further exploration into optimizing therapeutic interventions and provide a greater understanding of the utilizing multiple agents simultaneously to target HER2.

The setting of pre-operative therapy provides an optimal opportunity to use expression profiling and examine mechanisms of drug resistance. Initially, tumors have not been exposed to extensive therapies, thus avoiding resultant additional genetic changes. The endpoints are readily measurable, are reached in a short time frame, and are clinically relevant (complete and partial response, measured clinically, radiographically, or pathologically). [52] Sequential analysis of tumor tissue allow for comparison of signal pathway activation and induction of resistance mechanisms. The application of this translational research to IBC is much needed and the information gained will extend to non-inflammatory breast cancer.

2.7.2 Circulating Biomarkers

In order to develop and test novel therapeutic agents for inflammatory breast cancer, better model systems that faithfully recapitulate the human disease at the molecular and cellular level are needed. In the past we have developed new breast cancer cell lines from biopsy specimens, and these cells lines are being used all over the world to study breast cancer biology. To develop even better models of breast cancer that are free from cell culture artifacts, we would now like to develop patient-derived xenograft (PDX) models

by transplanting small pieces of breast cancer tissue orthotopically into the gonadal fat pads of immunodeficient, hormone supplemented mice (e.g., NOD *scid* gamma (NSG) mice).[55-60] This methodology permits development of a renewable resource which retains the morphology and molecular profile of each original patient tumor. These models will therefore be useful tools for studies of breast cancer genetics, cell biology, and experimental therapeutics. Importantly, once tumors develop in the primary hosts, these tumors can be serially transplanted over many generations.

In addition to developing new techniques to analyze tumor tissues, it has become increasingly recognized that mutations that are present in the tumor can be detected in circulating cell-free tumor DNA (ctDNA).[61-63] More than 90% of patients with metastatic breast cancer have detectable ctDNA. Serial assessment of ctDNA via detection of these mutations may be useful for monitoring of patients for disease recurrence or progression.[61-63] The majority of studies published thus far are from the metastatic setting. Few studies have been reported examining the use of ctDNA in patients with early stage breast cancer, and most of those have been conducted in patients with intact disease who are receiving primary systemic therapy. In one study, 29 patients had ctDNA isolated from before and after surgery.[64] Fifteen had PIK3CA mutations identified in pre-surgery ctDNA, and 14 of the 15 had the corresponding mutation in the surgical sample. Ten of the 15 had post-surgical blood samples collected, of which 5 (50%) still had detectable PIK3CA mutations.

3. PARTICIPANT SELECTION

3.1 Inclusion Criteria

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

3.1.1 Participants must have histologically confirmed invasive breast cancer. All histologic subtypes are eligible.

3.1.2 HER2-positive breast cancer, defined by ASCO CAP 2013 guidelines

- IHC 3+ based on circumferential membrane staining that is complete, intense

-AND/OR-

- FISH positive based on one of the following three criteria:
  - Single-probe average HER2 copy number ≥ 6.0 signals/cell **OR**
  - Dual-probe HER2/CEP17 ratio < 2.0 with an average HER2 copy number ≥ 6.0signals/cell; **OR**
  - Dual-probe HER2/CEP17 ratio ≥ 2.0

3.1.3 Patients must have the clinical diagnosis of inflammatory breast cancer as evidenced by the onset of all signs and symptoms noted below within a 6 month time-period:

- Erythema of the breast
- Edema of the skin of the breast
- Enlargement of the breast

3.1.4 Patients must be without visceral or bone involvement with metastatic breast cancer on physical exam or any diagnostic study. Patients with extensive nodal involvement classified as Stage IV disease, are eligible.

3.1.5 Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of trastuzumab and pertuzumab in participants <18 years of age.

3.1.6 ECOG performance status 0 or 1.

3.1.7 The effects of pertuzumab on the developing human fetus are unknown. For this reason and because these agents are known to be teratogenic, 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 and for at least 7 months after the last dose of study treatment. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.8 Willingness to undergo a research biopsy of the effected breast.

- Biopsies may be done with local anesthesia or intravenous conscious sedation, according to standard institutional guidelines.
- If a biopsy requires general anesthesia, then it is not allowed on this protocol.
- Patients who undergo a research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, *are still eligible* and are not required to undergo a repeat biopsy in order to enter the study.
- Some patients may have had a clinically indicated biopsy upon recent disease progression and agreed to submit tissue to their institution's frozen tumor bank. If the tissue was processed as specified in this protocol, no additional pre-treatment biopsy is required as that specimen can be used for the purposes of participation in this clinical trial.

3.1.9 Prior Therapy for the treatment of breast cancer is not allowed.

3.1.10 Participants must have normal organ and marrow function as defined below:

- Leukocytes > 3,000/mcL
- Absolute neutrophil count > 1,500/mcL

- Platelets > 100,000/mcL
- total bilirubin within normal institutional limits
- AST (SGOT)/ALT (SGPT) < 2.5 X institutional upper limit of normal
- Adequate renal function, as indicated by creatinine ≤1.5× upper limit of normal (institutional ULN).

3.1.11 Cardiac ejection fraction, as assessed by either MUGA scan or echocardiogram, greater than or equal to 50%, within 28 days prior to registration.

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

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 Participants may not be receiving any other investigational or commercial agents or therapies other than those described in this protocol to treat their malignancy.

3.2.2 Participants with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.3 Symptomatic intrinsic lung disease or extensive tumor involvement of the lungs, resulting in dyspnea at rest.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to paclitaxel, trastuzumab, pertuzumab. Patients with minor (grade 1 or 2) allergic reactions to these agents, controlled with standard supportive measures, are eligible.

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

3.2.6 Pregnant women are excluded from this study because paclitaxel, trastuzumab, and pertuzumab have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued if the mother is treated on study. These potential risks may also apply to other agents used in this study.

3.2.7 Individuals with a history of a different malignancy are ineligible except for the following circumstances. 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. 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.

3.2.8 HIV-positive individuals on combination antiretroviral therapy are eligible so long as they meet all other criteria. HIV-positive individuals who are not on combination antiretroviral therapy are not eligible because these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.9 Patients with metastatic disease involving viscera or bones are ineligible. Patients with extensive nodal involvement alone classified as Stage IV disease, are eligible.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

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

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

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

Following registration, participants may begin protocol therapy. 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 must be canceled. Registration cancellations must be made in OnCore as soon as possible.

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

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the DFCI Coordinating Center by the

Project Managers. Following registration, participants should begin protocol therapy within 5 business 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 must be canceled. The Project Managers should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the research nurse or data manager and faxed (617-632-5152) or e-mailed (ctopm@dfci.harvard.edu) to the Project Manager:

- Signed participant consent form
- HIPAA Authorization form (if separate from the informed consent document)
- Eligibility Checklist
- Clinic visit note documenting consent process, history, and physical exam documenting criteria for diagnosis of inflammatory breast cancer
- Copy of required laboratory test including: Hematology (CBC with differential), Serum Chemistries (Sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total protein, albumin, total bilirubin, SGOT (AST), SGPT (ALT), and Alkaline Phosphatase, pregnancy test (for women of child-bearing potential only), PR/INR/PTT.
- Breast MRI and Mammogram or Breast Ultrasound
- PET/CT, CT of Chest/Abdomen/Pelvis
- MUGA or Echocardiogram
- EKG
- Pathology report and documentation of HER2+ status

To complete the registration process, the Project Manager will follow DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol. The project manager will fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site.

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

5. TREATMENT PLAN

5.1 Treatment Regimen

During the pre-operative treatment phase, trastuzumab will be administered weekly, pertuzumab will be administered every 3 weeks, and paclitaxel will be administered weekly. Every 3 weeks with 21 consecutive days will defined as the pre-operative treatment cycle. Pertuzumab and trastuzumab will begin on Cycle 1 Day 1 (also known as Week 1). Beginning on Cycle 1 Day 8

(Week 2), paclitaxel will be given weekly for a total of 16 weekly doses. Pre-operative treatment will conclude with the initiation of surgery.. Treatment will be administered on an outpatient basis.

Following the pre-operative treatment phase, patients will move on to surgery. After surgery, there are two options for further treatment. In option 1, doxorubicin and cyclophosphamide will be given every two or three weeks with every two weeks (14 consecutive days) or three weeks (21 consecutive days) defined as this part of the post-operative treatment cycle. Doxorubicin and cyclophosphamide will conclude after 4 cycles, at which time trastuzumab and pertuzumab will be administered every three weeks (21 consecutive days) to complete 12 months of treatment with trastuzumab and pertuzumab (12 months = total of 18 doses of pertuzumab). Treatment will be administered on an outpatient basis.

In option 2 of post-operative treatment, trastuzumab and pertuzumab will be administered every three weeks (21 consecutive days) to complete 12 months of treatment (12 months = total of 18 doses of pertuzumab). Treatment will be administered on an outpatient basis.

Minor schedule changes due to observed holidays, inclement weather, etc. are permitted. Patients may interrupt therapy for protocol-directed reasons (i.e. toxicity) or for personal preferences (holidays, vacations, etc). Treatment should resume according to protocol guidelines and the Study Flowchart found in Section 10.

Refer to Section 5.3 for detailed administration instructions. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Pre-Operative Treatment Phase					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Trastuzumab	No premedication is necessary.	2 mg/kg (4 mg/kg loading dose)	IV	Weekly	21 days (3 weeks)
Pertuzumab	No premedication is necessary.	420 mg (840 mg loading dose)	IV	Every 3 weeks	
Paclitaxel	Dexamethasone 10 mg po or IV; diphenhydramine 12.5-50 mg po or IV; famotidine 20 mg IV; all agents administered per institutional guidelines	80 mg/m^2	IV	Begin on week 2, Weekly x 16	

Post-Operative Treatment Phase (Option 1)					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Doxorubicin	No premedication is necessary.	60 mg/m^2	IV push	Every 2-3 weeks x 4	14-21 days (2-3 weeks)
Cyclophosphamide	No premedication is necessary.	600 mg/m^2	IV	Every 2-3 weeks x 4	
Trastuzumab	No premedication is necessary.	6 mg/kg (8 mg/kg loading dose)	IV	Every 3 weeks to complete 12 months total	21 days (3 weeks)
Pertuzumab	No premedication is necessary.	420 mg (840 mg loading dose)	IV	Every 3 weeks to complete 12 months total	

Post-Operative Treatment Phase (Option 2)					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Trastuzumab	No premedication is necessary.	6 mg/kg (no loading dose required; 8mg/kg dose if reloading)	IV	Every 3 weeks to complete 12 months total	21 days (3 weeks)
Pertuzumab	No premedication is necessary.	420 mg (no loading dose required; 840mg dose if re-loading)	IV	Every 3 weeks to complete 12 months total	

5.2 Pre-Treatment Criteria

5.2.1 Run-In Phase, Cycle 1 Day 1 (Week 1)

During the run-in phase, prior to administration with pertuzumab and trastuzumab on Cycle 1 Day 1 (Week 1), the following parameters must be met:

- ANC ≥1,000/µl
- Platelet count ≥100,000/µl
- Bilirubin ≤ 1.5 x institutional ULN
- ALT and AST < 2.5 X institutional ULN
- Serum β-HCG Negative (in female patients unless S/P hysterectomy or menopausal or no menses for 24 consecutive months).
- LVEF ≥ 50%.

5.2.2 Preoperative Treatment Phase, Week 2, Day 8 and then weekly x 16 doses

During the pre-operative treatment phase starting on Cycle 1 Day 8 (Week 2), and then weekly for a total of 16 additional weeks, the following parameters must be met prior to administration with paclitaxel:

- ANC ≥1,000/µl
- Platelet count ≥100,000/µl
- Bilirubin ≤ 1.5 x institutional ULN
- ALT and AST < 2.5 X institutional ULN

NOTE: pertuzumab and trastuzumab do not require specific laboratory criteria for administration.

5.2.3 Post-operative, Day 1 of Every Doxorubicin/Cyclophosphamide Cycle

During the post-operative treatment phase with doxorubicin and cyclophosphamide, the following parameters must be met on day 1 of every cycle:

- ANC ≥1,000/µl
- Platelet count ≥100,000/µl
- Bilirubin ≤ 1.5 x institutional ULN
- ALT and AST < 2.5 X institutional ULN
- LVEF ≥ 50% - only assessed prior to cycle 1.

5.2.4 Subsequent Cycles of Post-operative Trastuzumab/Pertuzumab

The following parameters apply to both option 1 and option 2 post-operative treatments.
- No laboratory criteria apply.
- See Appendix A for LVEF criteria

5.3 Treatment Administration

5.3.1 Trastuzumab During Pre-Operative Phase

Trastuzumab is administered on Cycle 1 Day 1 (Week 1) at a loading dose of 4 mg/kg IV per institutional guidelines. Following the initial infusion, subsequent trastuzumab infusions will be administered at 2 mg/kg IV per institutional guidelines weekly (+/- 2 days) during the 16 doses of paclitaxel until the patient's surgery. Post-infusion observation times will be per institutional guidelines. On days when pertuzumab or paclitaxel are also administered, trastuzumab will be administered first or per institutional guidelines.

Following the completion of trastuzumab and paclitaxel 16 concurrent doses, additional trastuzumab may be administered at a dose of 6 mg/kg IV every 3 weeks (+/- 1 week)

along with pertuzumab until surgery based upon physician discretion. Three weekly doses of trastuzumab must be completed prior to starting the every 3 week dose of trastuzumab and pertuzumab.

Dosing should be made based on institutional guidelines. Trastuzumab reactions will be managed per institutional guidelines. Additional management guidelines can be found in Section 6.1.

5.3.2 Pertuzumab During Pre-Operative Phase

Pertuzumab is administered on Cycle 1 Day 1 (Week 1) at a loading dose of 840 mg IV Infusion time and post-infusion observation period are per institutional guidelines. Subsequent doses will be administered at 420 mg IV every 3 weeks (+/- 2 days) with an observation period per institutional guidelines until surgery. If the first dose of pertuzumab was well tolerated, post-infusion observation times may be discontinued as per institutional guidelines. On days when trastuzumab or paclitaxel are also administered, pertuzumab will be administered following the completion of trastuzumab through a separate administration set. Pertuzumab will be administered prior to paclitaxel administration or per institutional guidelines.

Dosing should be made based on institutional guidelines. Pertuzumab reactions will be managed per institutional guidelines. Additional management guidelines can be found in Section 6.1.

5.3.3 Paclitaxel During the Pre-Operative Phase

Beginning on Cycle 1 Day 8 (Week 2), paclitaxel is administered at a dose of 80 mg/m2 IV weekly (+/- 2 days) per institutional guidelines for a total of 16 doses. Missed doses should be made up to equal 16 doses. Paclitaxel is administered following the completion of trastuzumab and pertuzumab administrations or per institutional guidelines. Premedications should be administered per institutional guidelines prior to paclitaxel and may include:

- dexamethasone 10 mg po or IV
  - If treatment is tolerated through completion of the first 2 cycles, Dexamethasone may be decreased or discontinued.
- diphenhydramine 12.5-50 mg PO or IV
- famotidine 20 mg IV

If the patient does not experience an allergic reaction, the premedication regimen may be altered at the discretion of the treating physician. Anaphylaxis precautions should be observed during paclitaxel infusion. Paclitaxel reactions will be managed per institutional guidelines. Additional management guidelines can be found in Sections 6.2 and 6.3.

Dosing should be made based on institutional guidelines.

If discontinuation of paclitaxel is required or requested, patients should be assessed for eligibility for mastectomy. If they are deemed surgical candidates, then they should proceed to mastectomy and continue on treatment as per protocol.

5.3.4 Surgery

Prior to surgery, patients should be assessed for clinical response to preoperative treatment by physical examination and imaging of the breasts (MRI, mammogram, and ultrasound if indicated). Primary breast surgery should be performed within 4 - 5 weeks after the last dose of paclitaxel combined with trastuzumab. Surgery must be performed at one of the participating institutions. Patients should undergo surgery with a total mastectomy; removal of the breast and level 1 and 2 axillary lymph node dissection. Pathological specimens will be analyzed for tumor extent and grade, ER and PR status, HER2 expression, and other markers of tumor biology. Given the high risk of local regional disease recurrence with IBC, reconstruction surgery is to be delayed for at least 6 months following the completion of radiation therapy.

5.3.5 Post-operative Option #1: Doxorubicin and Cyclophosphamide followed by Trastuzumab and Pertuzumab

Starting within 4-5 weeks following surgery, doxorubicin and cyclophosphamide is administered every two or three weeks for a total of 4 doses. Doxorubicin is administered at a dose of 60 mg/m^2 IV over approximately 3-5 minutes (or per institutional guidelines) every 14-21 days (+/- 2 days) for 4 cycles. Doxorubicin is administered prior to cyclophosphamide. Cyclophosphamide is administered at a dose of 600 mg/m2 IV infusion over approximately 30 minutes (or per institutional guidelines) every 14-21 days (+/- 2 days) x 4 cycles. Cyclophosphamide is administered after doxorubicin.

Dosing for doxorubicin and cyclophosphamide should be per institutional guidelines. Patients should not receive doxorubicin/cyclophosphamide treatment any sooner than 14 days from administration of Neulasta (if applicable). Patients who need to discontinue doxorubicin or cyclophosphamide because of toxicity, can start radiation with trastuzumab and pertuzumab as outlined in the protocol. Reactions should be managed per institutional guidelines. Additional management guidelines can be found in Section 6.3.

Beginning 3-4 weeks following the completion of doxorubicin and cyclophosphamide administration (post-operative Option #1), trastuzumab and pertuzumab maintenance therapy will be administered every 3 weeks to complete 12 months of HER2-directed therapy (i.e., patients will typically complete around 8 months of HER2-directed therapy during the post-operative phase since they will have completed around 4 months in the

pre-operative phase; 12 months = 18 doses of pertuzumab). Trastuzumab will be administered prior to pertuzumab or as per institutional guidelines.

Trastuzumab is administered at a loading dose of 8 mg/kg IV per institutional guidelines for the first infusion. Subsequent trastuzumab infusions are given at a dose of 6 mg/kg IV every 3 weeks (+/- 1 week) per institutional guidelines. Post-infusion observation times for trastuzumab will be per institutional guidelines. Pertuzumab is administered at a loading dose of 840 mg IV with infusion timing and post-infusion observation period per institutional guidelines. Subsequent pertuzumab infusions may be given at 420 mg IV per institutional guidelines every 3 weeks (+/- 1 week). If the first dose of pertuzumab was well tolerated, post-infusion observation times may be discontinued as per institutional guidelines.

Reactions to maintenance therapy will be managed per institutional guidelines. Additional management guidelines can be found in Section 6.1.

Cardiac evaluation by MUGA or echocardiogram is to be completed within 1 week prior to initiating trastuzumab and pertuzumab maintenance therapy and again every 12 weeks (+/- 1 week) until maintenance therapy is completed.

5.3.6 Post-operative Option #2: Trastuzumab and Pertuzumab

Starting within 4-5 weeks following surgery, trastuzumab and pertuzumab maintenance therapy will be administered every 3 weeks (+/- 1 week) to complete 12 months of HER2-directed therapy (i.e., patients will typically complete around 8 months of HER2-directed therapy during the post-operative phase since they will have completed around 4 months in the pre-operative phase; 12 months = 18 doses of pertuzumab).

There are no loading doses required for trastuzumab or pertuzumab when begun as the first treatment after surgery, however, patients can be re-loaded with pertuzumab and trastuzumab at physician discretion or per institutional guidelines. Trastuzumab is administered at a dose of 6 mg/kg IV every 3 weeks (+/- 1 week) with observation periods per institutional guidelines. Pertuzumab is administered at a dose of 420 mg IV every 3 weeks (+/- 1 week) with an observation period per institutional guidelines. If the first dose of pertuzumab was well tolerated, post-infusion observation times may be discontinued as per institutional guidelines.

If re-loading, trastuzumab is administered at a loading dose of 8 mg/kg IV per institutional guidelines for the first infusion. Subsequent trastuzumab infusions are given at a dose of 6 mg/kg IV every 3 weeks (+/- 1 week) per institutional guidelines. Post-infusion observation times for trastuzumab will be per institutional guidelines. Pertuzumab is administered at a loading dose of 840 mg IV with infusion timing and post-infusion observation period per institutional guidelines. Subsequent pertuzumab infusions may be given at 420 mg IV per institutional guidelines every 3 weeks (+/- 1 week).

Reactions to maintenance therapy will be managed per institutional guidelines. Additional management guidelines can be found in Section 6.1.

Cardiac evaluation by MUGA or echocardiogram is to be completed prior to initiating trastuzumab and pertuzumab maintenance therapy; to avoid repeating, pre-surgical cardiac evaluation may fulfill this requirement. Cardiac evaluation will continue to be completed every 12 weeks (+/- 1 week) from the start of maintenance therapy until maintenance therapy is completed.

5.3.7 Radiation Therapy

Radiation therapy should be initiated within 3-6 weeks following the last dose of doxorubicin and cyclophosphamide (Post-operative Option #1) or within 3-6 weeks following surgery (Post-Operative Option #2). Radiation therapy may start and continue during maintenance therapy with trastuzumab and pertuzumab.

Radiation should be delivered to the chest wall and regional lymph nodes. Treatment will be administered per institutional guidelines for the treatment of inflammatory breast cancer. Radiation can be administered at a local facility.

5.3.8 Endocrine Therapy

Appropriate postoperative adjuvant endocrine therapy should be administered in patients with ER and/or PR positive disease. Either tamoxifen or an aromatase inhibitor should be administered per institutional standards as determined by the patient's treating physician. Endocrine therapy should be initiated 3-4 weeks following the completion of doxorubicin and cyclophosphamide (Post-operative Option #1), or concurrent with maintenance therapy of trastuzumab and pertuzumab. Endocrine therapy may begin concurrent with or after completion of radiation therapy per physician preference.

5.3.9 Additional Therapy for Stage IV Disease

Patients with Stage IV disease by virtue of nodal involvement alone will continue trastuzumab with pertuzumab every 3 weeks (+/- 1 week) until disease progression (beginning maintenance therapy per post-operative options noted in Sections 5.3.5 and 5.3.6).

5.4 General Concomitant Medication and Supportive Care Guidelines

Filgrastim or Peg- filgrastim:

- May be administered during the treatment with doxorubicin / cyclophosphamide per institutional guidelines and at the treating physician's discretion.
- Not to be administered with paclitaxel.

Anti-emetics:

- May be administered per physician discretion.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until the conclusion or post-operative therapy or until one of the following criteria applies:

- Disease progression as evidenced by clinical examination and confirmed with imaging with MRI of the breast.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s), including grade 4 congestive heart failure (CHF).
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements.
- Participant decides to withdraw from the protocol.
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

A ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the ODQ website or obtained from the DFCI Coordinator Center Project Managers.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Filipa Lynce, MD at 617-632-3800, or filipa_lynce@dfci.harvard.edu.

5.6 Duration of Follow Up

Following the completion of the study treatment or other items noted above in Section 5.5., participants will be followed every 3 months (+/- 1 month) for 1 year, then every 6 months (+/- 1 month) for 4 years, and then annually (+/- 1 month) after removal from protocol therapy or until death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

Patients who are no longer being followed at the treating institution will be contacted either directly or their current treating physician will be contacted every 6 months for 4 years, and then annually to determine disease status until death.

5.7 Criteria for Taking a Participant Off Study

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

- 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).

A ODQ Treatment Ended/Off Study Form will be filled out when a participant comes off study. This form can be found on the ODQ website or obtained from the DFCI Coordinator Center Project Managers.

5.8 Study Discontinuation

The Overall Principal Investigator has the right to terminate this study at any time. Reasons for terminating the study may include the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to subjects.
- Subject enrollment is unsatisfactory
- Data recording are inaccurate or incomplete
- Study protocol not followed

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s) and paragraphs. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website 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.).

It is optimal to administer all study drugs as outlined, however each drug may be held individually as per outlined in the protocol. For example, pertuzumab and trastuzumab may continue as scheduled if paclitaxel is held for toxicity. Patients who discontinue chemotherapy due to toxicity should not systematically be withdrawn from all study treatments.

6.1 Trastuzumab and Pertuzumab Dosage Modification

Pertuzumab or trastuzumab dose modifications are not permitted. Pertuzumab and trastuzumab doses may be delayed due to toxicities. If pertuzumab or trastuzumab are held for more than two cycles or need to be permanently discontinued, the patient will be withdrawn from all study treatment and treated at the discretion of the investigator as clinically indicated. The patient will continue to be followed post-treatment as described in Section 5.6.

6.1.1 Criteria for Continuation and Discontinuation of HER2 Targeted Study Medication based on LVEF Assessments

If the criteria to hold pertuzumab treatment are met (based on Appendix A) repeat LVEF assessment within approximately 3 weeks. If after a repeat assessment, the LVEF has not improved, or has declined further, discontinuation of pertuzumab and trastuzumab should be strongly considered, unless the benefits for the individual patient are deemed to outweigh the risks. For delayed or missed doses, if the time between 2 sequential infusions is less than 6 weeks, the 420 mg IV dose of pertuzumab should be administered. Do not wait until the next planned dose. If the time between 2 sequential infusions is 6 weeks or more, the initial dose of 840 mg pertuzumab should be re-administered as a ~60 minute IV infusion followed every 3 weeks thereafter by a dose of 420 mg IV administered over ~30-60 minutes. Pertuzumab should be withheld or discontinued if trastuzumab treatment is withheld or discontinued due to cardiac toxicity. Most patients who developed heart failure in the adjuvant trastuzumab breast cancer trials improved with standard medical treatment. This included ACE-inhibitors or angiotensin receptor blockers, beta-blockers and diuretics when needed.

6.2 Dose Modifications and Delays for Paclitaxel:

The principle is to attempt to administer full doses of paclitaxel therapy on schedule. Paclitaxel may be modified or held as indicated below. Missed paclitaxel doses should be made up at the end of scheduled pre-operative therapy, prior to surgery to achieve 16 total doses. In the case of discontinuation of paclitaxel, patients should be assessed for eligibility for mastectomy, and if they are deemed surgical candidates, then they should proceed to mastectomy and continue on treatment as per protocol.

All dose modifications for paclitaxel are based on the dose level changes outlined below in Table 1. No more than 1 dose-reduction of paclitaxel chemotherapy should be made for toxicity. If more than 1 dose-reduction is required, paclitaxel should be discontinued. If Paclitaxel is held for > 3 weeks for toxicity, paclitaxel should be discontinued.

TABLE 1: Dose Levels for Paclitaxel

	Dose level 0	Dose Level -1	Dose Level -2

	Starting dose		
Paclitaxel (mg/m2)	80	64	Discontinue

The sections below outline modifications for paclitaxel-related hypersensitivity reactions, neurosensory toxicity, and musculoskeletal pain. Instructions for management of all other toxicities related to paclitaxel are listed in Section 6.3, Table 5. If a Grade 3 or 4 non-hematologic toxicity not otherwise listed below is experienced, paclitaxel may be reduced from 80 mg/m^2 to 64mg/m^2. If further dose reduction is needed, the paclitaxel should be discontinued as indicated above in Table 1.

6.2.1 Hypersensitivity Reactions

If paclitaxel-related hypersensitivity reaction occurs despite pre-medication, treatment as medically indicated will be instituted. For hypersensitivity reaction less than Grade 3, continuation of paclitaxel is at the Investigator's discretion. If Grade 4 hypersensitivity is experienced, paclitaxel must be permanently discontinued.

6.2.2 Neurosensory Toxicity

TABLE 2: Dose Modifications for Paclitaxel-Related Neurosensory Toxicity

Paresthesias/Dysesthesias	**1 – 7 Days Duration**	**Persistent for > 7 Days *or* Caused the Next Cycle to be delayed**
Grade 1 Paresthesias/dysesthesias that do not interfere with function	Maintain paclitaxel dose	Maintain paclitaxel dose
Grade 2 Paresthesias/dysesthesias interfering with function, but not activities of daily living	Maintain paclitaxel dose [a]	Decrease paclitaxel one dose level [b]
Grade 3 Paresthesias/dysesthesias with pain or with function impairment interfering with activities of daily living [C]	**First episode:** Decrease paclitaxel one dose level[a] **Second episode:** Discontinue paclitaxel	Discontinue paclitaxel

[a] Must be resolved to ≤ Grade 1 on Day 1 of the next cycle.
[b] Hold paclitaxel for *persistent* Grade 2 neurotoxicity. When ≤ grade 1, resume treatment with dose modification for paclitaxel. If grade 2 toxicity persists after 3 weeks of delay, discontinue paclitaxel.
[c] For persistent paresthesias/ dysesthesias that are disabling or life-threatening, paclitaxel should be discontinued.

6.2.3 Musculoskeletal Pain

TABLE 3: Dose Modifications for Paclitaxel Musculoskeletal Pain *Not Controlled by Analgesics*[a]

Musculoskeletal Pain	**1 – 7 Days Duration**	**Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed**
Grade 1	Maintain paclitaxel dose	Maintain paclitaxel dose
Grade 2	Maintain paclitaxel dose	Decrease paclitaxel one dose level[b]
Grade 3	**First episode:** Decrease paclitaxel one dose level **Second episode:** Discontinue paclitaxel	**First episode:** Decrease paclitaxel one dose level[b] OR Discontinue paclitaxel **Second episode**: Discontinue paclitaxel

[a] Use of narcotics and NSAIDs is encouraged to maintain dose of paclitaxel if possible.
[b] Hold paclitaxel for persistent Grade 2 or 3 musculoskeletal pain (trastuzumab and pertuzumab should be continued while paclitaxel is held.) When ≤ grade 1, resume treatment with dose modification for paclitaxel. If Grade 2 or Grade 3 toxicity persists after 3 weeks of delay, discontinue paclitaxel.

6.3 Dose Modifications and Delays for Paclitaxel and doxorubicin/cyclophosphamide (AC)

No more than 1 dose-reduction of doxorubicin/cyclophosphamide chemotherapy (AC) should be made for toxicity. If more than 1 dose-reduction would be required, AC should be discontinued, and the patient may proceed with trastuzumab and pertuzumab and radiation. If AC is held for > 3 weeks for toxicity, stop AC and proceed to trastuzumab and pertuzumab and radiation. Patients who need to discontinue AC because of toxicity, can proceed to trastuzumab and pertuzumab and radiation.

TABLE 4: Dose Levels for doxorubicin/cyclophosphamide (AC)

	Dose level 0 Starting dose	**Dose Level -1**	**Dose Level -2**
doxorubicin (mg/m^2)	60	45	discontinue
Cyclophosphamide (mg/m^2)	600	450	discontinue

Table 5 below outlines modifications for various AC-related toxicities. If a Grade 3 or 4 non-hematologic toxicity not otherwise listed below is experienced, doxorubicin may be reduced

from 60 mg/m^2 to 45 mg/m^2, and cyclophosphamide may be reduced from 600 mg/m^2 to 450 mg/m^2. If further dose reduction is needed, the paclitaxel should be discontinued as indicated above in Table 4.

TABLE 5: Dose Modifications and Delays for Paclitaxel and doxorubicin/cyclophosphamide (AC)

NCI CTCAE v 4.0 [Category] Grade	**Modifications for AEs that occur during a cycle but resolve prior to the next treatment cycle[a]**	**Modifications for AEs that require a delay in administration of the treatment cycle (i.e., they occur on Day 1)[b]**
HEMATOLOGICAL:		
Neutrophils count decreased		
Grades 2, 3, 4	Maintain dose	*Hold until ≥ 1000/mm^3 for paclitaxel and for AC.* *If recovery takes:* • 1 wk: maintain dose • 2-3 wks: ↓ one dose level
Platelet count decreased		
Grades 2, 3 Grade 4	Maintain dose ↓ one dose level	*Hold until ≥ 100,000/mm3.* *If recovery takes:* • 1 wk: maintain dose • 2-3 wks: ↓ one dose level ↓ one dose level
BLOOD AND LYMPHATIC SYSTEM DISORDERS:		
Febrile neutropenia		
Grade 3, 4	↓ one dose level or discontinue.	
GASTROINTESTINAL DISORDERS *(if related to chemotherapy)*:		
Diarrhea		
Grade 2 Grade 3 Grade 4	Maintain dose ↓ one dose level discontinue	↓ one dose level ↓ one dose level discontinue
Mucositis oral (stomatitis)		
Grade 2 Grade 3 Grade 4	Maintain dose ↓ one dose level discontinue	↓ one dose level ↓ one dose level discontinue
Vomiting *(despite antiemetics)*		
Grade 2 Grades 3, 4	↓ one dose level (optional) ↓ one dose level or	↓ one dose level discontinue

	discontinue	
HEPATIC FUNCTION:		
Bilirubin or AST or ALP increased		
Grade 2 Grade 3 Grade 4	↓ one dose level ↓ one dose level Discontinue	*Hold until bilirubin returns to the baseline grade, and AST and alkaline phosphatase have returned to ≤ grade 1.* Then ↓ one dose level ↓ one dose level Discontinue
Grade 3 Grade 4	↓ one dose level ↓ one dose level or discontinue	↓ one dose level Discontinue

- Dose modifications must be based on AEs that occur during the cycle (column 2) and AEs present on the scheduled cycle Day 1 (column 3).
- Dose modifications must be based on the AE requiring the greatest modification.

[a] Resolved means that all requiring dose modification are ≤ grade 1 (except ANC/AGC [which must be ≥ 1000/mm3 and bilirubin which must be ≤ the baseline grade]) on each week of paclitaxel or (except ANC/AGC [which must be ≥ 1000/mm3] and bilirubin [which must be ≤ the baseline grade]) on Day 1 of the next scheduled cycle of AC (ie, treatment can be given without delay).
[b] Hold and check weekly. With exception of ANC/AGC and bilirubin, resume treatment when toxicity is ≤ grade 1. If toxicity has not resolved after 3 weeks of delay, discontinue paclitaxel or doxorubicin/cyclophosphamide.
[c] Determination of "clinically significant" AEs is at the discretion of the investigator.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. 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. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

7.1.1 Adverse Events List(s) for Trastuzumab and Pertuzumab

a. **Infusion-Associated Symptoms**

Like other monoclonal antibodies, pertuzumab has been associated with infusion associated reaction (such as chills, diarrhea, fatigue, headache, nausea, and pyrexia), and with hypersensitivity reactions. Close observation of the patient during and for ~60 minutes after the first infusion of pertuzumab or trastuzumab, and during and for ~30 minutes following the first 30 minute infusion of pertuzumab or trastuzumab. If these infusions are tolerated well, post-infusion observation may be avoided following subsequent infusions as per institutional guidelines. If a significant IAR occurs, the infusion should be slowed down or interrupted and appropriate medical therapies should be administered. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms. Permanent discontinuation should be considered in patients with severe infusion reactions. This clinical assessment should be based on the severity of the preceding reaction and response to administered treatment for the adverse reaction.

b. **Serious Infusion-Associated Events**

Serious adverse reactions to trastuzumab infusion including dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation and respiratory distress have been reported infrequently. In rare cases (4 per 10,000), these events were associated with a clinical course culminating in a fatal outcome. Serious reactions have been treated with supportive therapy such as oxygen, beta-agonists, corticosteroids and withdrawal of trastuzumab as indicated.

c. Hematologic Toxicity and Neutropenic Infections

In the clinical trials, an increased incidence of anemia was observed in patients receiving trastuzumab plus chemotherapy compared with patients receiving chemotherapy alone. The majority of these anemia events were mild or moderate in intensity and reversible; none resulted in discontinuation of trastuzumab therapy.

In the clinical trials, the per-patient incidences of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving trastuzumab in combination with myelosuppressive chemotherapy as compared to those who received chemotherapy alone. In the post marketing setting, deaths due to sepsis in patients with severe neutropenia have been reported in patients receiving trastuzumab and myelosuppressive chemotherapy, although in controlled clinical trials (pre- and post-marketing), the incidence of septic deaths was not significantly increased. The pathophysiologic basis for exacerbation of of neutropenia has not been determined; the effect of trastuzumab on the pharmacokinetics of chemotherapeutic agents has not been fully evaluated.

Secondary acute leukemia or myelodysplastic syndrome has been reported in 4 of approximately 1200 patients who participated in trastuzumab clinical trials. Patients treated with chemotherapeutic agents are known to be at increased risk for secondary leukemia. The observed incidence of leukemia among trastuzumab-treated patients appears to be consistent with the expected incidence of leukemia among patients treated

with chemotherapy for metastatic breast cancer (7). Therefore, the contribution of trastuzumab to the etiology of acute leukemia or myelodysplastic syndrome in these cases is unclear.

d. Management of Hematologic Toxicities

Care should be taken to carefully monitor the patient's hematologic status throughout the course of the trial. Use of hematopoietic growth factors to ameliorate hematologic toxicity is at the discretion of the physician investigator and should be in accordance with the American Society of Clinical Oncologists (ASCO) guidelines.

Please refer to the trastuzumab and pertuzumab Investigator Brochure for a detailed description of the safety profile of trastuzumab.

e. Risk of Respiratory Events

A low rate of respiratory events that are compatible with an IAR or hypersensitivity reaction/anaphylaxis has been reported. Although pertuzumab targets the HER2 receptor it inhibits heterodimerization with other members of the HER family (eg, EGFR [HER1]). Accordingly, it may cause toxicities associated with the use of EGFR inhibitors,such as ILD. The few reports of ILD occurring in pertuzumab-treated patients received so far also had evidence of alternative causes, eg, concomitant medication, preceding/concurrent neutropenia with potential infection or relevant medical history.

f. Risk of EGFR-Related Toxicities

Although pertuzumab targets the HER2 receptor, it inhibits heterodimerization with other members of the HER family (eg, EGFR [HER1]). Accordingly, it may cause toxicities associated with the use of EGFR inhibitors such as diarrhea, rash and other dermatologic toxicities (eg, dry skin, pruritus, nail disorders, mucositis).

g. Diarrhea

In the 7-week IV and 26-week toxicity studies in cynomolgus monkeys, there was a treatment-related increase in the incidence of diarrhea. Diarrhea has been observed in approximately 60% of patients (treatment-related diarrhea in 50% of patients) being treated with pertuzumab in phase II single-agent studies, and up to approximately 70% of patients in combination therapy studies. Diarrhea was CTC Grade 1 or 2 in the majority of cases. To prevent dehydration, early treatment of diarrhea with anti-diarrheal medication should be considered and patients treated with fluids and electrolyte replacement, as clinically indicated.

h. Rash

Rash has also been observed with EGFR inhibitors, mostly of mild to moderate intensity. Rash has been observed in approximately 17% of patients receiving pertuzumab in Phase

II single-agent studies and up to 73% of patients in combination studies. The rash was generally of CTC Grade 1 or 2 in severity. Treatment recommendations for EGFR associated rash include topical or oral antibiotics, topical pimecrolimus, topical or (for severe reactions) systemic steroids. These agents may be used in patients experiencing pertuzumab-related rash, as clinically indicated, although they have not been studied in this context.

i. Risk of Neutropenia

Neutropenic events are virtually absent with chemotherapy-free Pertuzumab regimens and with single-agent pertuzumab. In the pivotal study WO20698/TOC4129g incidence of neutropenic events was increased in patients receiving pertuzumab, trastuzumab and docetaxel, compared to patients in the placebo-controlled arm. This was largely driven by an increase in Grades 3 and 4 febrile neutropenia. No febrile neutropenia events occurred after docetaxel discontinuation. Pertuzumab, at a dose of 420 mg, was well tolerated in combination with docetaxel up to 75 mg/m2 in the Phase Ib study, BO17021. However, pertuzumab in combination with 100 mg/m2 docetaxel was not well tolerated. Dose-limiting toxicity was observed, including febrile neutropenia. However, the registered dose of docetaxel in combination with trastuzumab ranges from 65 to 100 mg/m2, and there is evidence that outcomes might be improved when higher doses of docetaxel are given. Given that the DLTs in Study BO17021 were not life threatening and that exposure to docetaxel shows interpatient variability, patients receiving pertuzumab in combination with docetaxel in ongoing studies are treated initially with 75 mg/m2 docetaxel, and then dose escalation of docetaxel to 100 mg/m2 is recommended (as described in the protocol dose escalation rules), provided that the patient does not experience significant toxicities at the starting dose. This strategy is intended to ensure optimum individual exposure for patients receiving docetaxel in combination with pertuzumab. The tolerability of the combination therapy at the higher (100 mg/m2) dose of docetaxel is encouraging in those patients who tolerate the 75 mg/m2 starting dose well. Patients receiving pertuzumab in combination with docetaxel or other cytotoxic agents should undergo careful hematological monitoring for neutropenia during treatment, and should be treated promptly with antibiotics and other supportive measures as clinically indicated.

j. Risk of Left Ventricular Dysfunction

Decreases in LVEF have been reported with drugs that block HER2 activity. Trastuzumab and pertuzumab both target HER2, thus there is a risk of cardiac dysfunction with these agents. In the CLEOPATRA pivotal trial WO20698/TOC4129g, pertuzumab in combination with trastuzumab and docetaxel was not associated with increases in the incidence of symptomatic LVSD or decreases in LVEF compared with placebo in combination with trastuzumab and docetaxel. Pertuzumab combined with trastuzumab and chemotherapy also did not result in any significantly greater incidence of symptomatic LVSD or decreases in LVEF than trastuzumab and chemotherapy in patients with EBC (Study WO20697). However, in the pivotal MBC trial (Study WO20698/TOC4129g) a greater proportion of patients who developed symptomatic

LVSD had received prior anthracyclines and/or radiotherapy compared to the proportion of patients receiving prior anthracyclines and/or radiotherapy in the overall pertuzumab-treated population. Therefore patients who have received prior anthracyclines or prior radiotherapy to the chest area may be at higher risk of decreased LVEF.

Pertuzumab has not been studied in patients with: a pretreatment LVEF value of ≤ 50%; a prior history of CHF; decreases in LVEF to <50% during prior trastuzumab adjuvant therapy; conditions that could impair left ventricular function such as uncontrolled hypertension, recent myocardial infarction, serious cardiac arrhythmia requiring treatment or a cumulative prior anthracycline exposure to > 360mg/m2 of doxorubicin or its equivalent.

Cardiac exclusion criteria are specified in this protocol and should be closely followed.

k. Management of Cardiac Safety

All patients must have a baseline evaluation of cardiac function including a measurement of LVEF by either MUGA or ECHO prior to entry into the study. Only patients with an LVEF ≥ 50% should be entered into this study.

All patients should have cardiac monitoring at regular intervals (e.g., every three months) during treatment with pertuzumab and trastuzumab. ECHO or MUGA scans should be scheduled at the same radiology facility where the patient's baseline ECHO or MUGA was conducted. LVEF measurements should be required at protocol-specified time-points and after a patient has any of the following: discontinuation of protocol therapy, congestive heart failure (CHF), breast cancer recurrence, or a second primary cancer. When a cardiac event occurs, the Cardiac Report Form must be submitted within 14 days of learning of the event.

During the course of trastuzumab and pertuzumab therapy, patients should be monitored for signs and symptoms of CHF (i.e., dyspnea, tachycardia, new unexplained cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and rapid unexplained weight gain). The confirmation of the CHF diagnosis should include the same method used to measure LVEF at baseline (either ECHO or MUGA).

Pertuzumab and trastuzumab should be discontinued in any patient who develops clinical signs and symptoms suggesting CHF. CHF should be treated and monitored according to standard medical practice.

At present, there are inadequate data available to assess the prognostic significance of asymptomatic drops in LVEF.

7.1.2 Adverse Events List(s) for Paclitaxel

Myelosuppression, abnormalities on liver function tests (elevated SGOT, SGPT,

bilirubin, alkaline phosphatase), nausea, vomiting, diarrhea, mucositis, peripheral neuropathy, transient asymptomatic bradycardia, and much less frequently, arrhythmias, hypotension, hypersensitivity/anaphylactic reactions (dyspnea, tachycardia, rash, urticaria, hypotension, or hypertension), myalgias, arthralgias, and alopecia.

7.1.3 Adverse Events List(s) for Doxorubicin

a. Hematologic:

Leukopenia (dose-limiting), also thrombocytopenia, and anemia. Nadir at 10-14 days, recovery in 21 days.

b. Dermatologic:

Reversible, usually complete alopecia; increased sensitivity to sunlight; hyperpigmentation of skin and nail beds.

c. Gastrointestinal:

Nausea and vomiting, sometimes severe; anorexia; diarrhea; mucositis (stomatitis and esophagitis)

d. Cardiovascular:

Arrhythmias, EKG changes, rarely sudden death. Cardiomyopathy may occur and is related to the total cumulative lifetime dose. The risk of cardiomyopathy increases with total doses >450 mg/m^2.

7.1.4 Adverse Events List(s) for Cyclophosphamide

a. Hematologic:

Leukopenia, with nadirs about 8-14 days after administration and recovery in 18-25 days, and thrombocytopenia

b. Dermatologic:

Temporary alopecia

c. Gastrointestinal:

Nausea and vomiting

d. Genitourinary:

Hemorrhagic cystitis (onset of cystitis may be delayed for a period ranging from 24 hours

to several weeks). Subject should be well hydrated before, during, and after treatment.

e. Other:

Amenorrhea, which may be long-term; possible irreversible sterility in both sexes; teratogenic effects; in rare cases, anaphylaxis; may cause secondary neoplasms.

7.2 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.

- **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.
  - For the purpose of this study, an adverse event is considered unexpected when it varies 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 the informed consent document as a potential risk.

- Serious Adverse Events: A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:
  - Results in death
  - Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.

- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- Meets the reporting criteria defined in Section 7.3.1, Table 6.

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

7.3 Expedited Adverse Event Reporting

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

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

7.3.1 DF/HCC Expedited Reporting Guidelines

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

Other investigative sites will report SAEs to their respective IRB according to the local

IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to the Overall PI and Project Managers within the timeframes detailed in the table below.

Filipa Lynce, MD
Phone: 617-632-3800
Fax: 617-632-1930
Email: filipa_lynce@dfci.harvard.edu and ctopm@dfci.harvard.edu

TABLE 6: DF/HCC Reportable AEs

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

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

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Reporting to Genentech Drug Safety

7.5.1 AEs of Special Interest (AESIs)

The Trastuzumab and Pertuzumab Events of Special Interest are:

- Left Ventricular Ejection fraction decrease (<50%)
- LV dysfunction
- Heart Failure
- Embryo-fetal Toxicity or Birth Defects

7.5.2 AESI and SAE Reporting to Genentech

Investigators must report all SAEs and AESIs to Genentech Drug Safety within the timelines described below. A completed Medwatch 3500A form should be faxed immediately upon completion with the cover sheet in Appendix B to Genentech Drug Safety at:

(650) 225-4682
OR
(650) 225-5288

Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available. SAE reports that are related to trastuzumab and pertuzumab and AEs of Special Interest (regardless of causality) will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date. Serious AE reports that are unrelated to the trastuzumab and pertuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.

Additional Reporting Requirements to Genentech include the following:

Any reports of pregnancy following the start of administration with the trastuzumab and pertuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.

For questions related to safety reporting, please contact Genentech Drug Safety:

Tel: (888) 835-2555

Fax: (650) 225-4682 OR (650) 225-5288

7.5.3 Non-serious AE Reporting to Genentech

Listings of non-serious AEs from the study will be forwarded to Genentech on a quarterly basis by the DFCI Coordinating Center.

7.6 Expedited Reporting to Hospital Risk Management

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

7.7 Monitoring of Adverse Events and Period of Observation

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.

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.

7.8 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, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

8.1 Pertuzumab

8.1.1 <u>Description, Form, and Preparation</u>

Pertuzumab drug product is provided as a single use formulation containing 30 mg/mL pertuzumab in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20. Each 20 mL vial contains 420 mg of Pertuzumab (14.0 mL/vial).

Prepare the solution for infusion, using aseptic technique, as follows:

- Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.
- Withdraw the appropriate volume of pertuzumab liquid concentrate from the vial(s).
- Dilute into the 250 mL 0.9% sodium chloride PVC or non-PVC polyolefin infusion bags. Do not withdraw saline out of the infusion bag.
- Mix diluted solution by gentle inversion. Do not shake.
- Administer immediately once prepared.
- **Dextrose (5%) solution should not be used to dilute pertuzumab.**

Refer to the Investigator's Brochure for detailed pertuzumab information and FDA approved package for more information.

8.1.2 Storage and Stability

Upon receipt, pertuzumab vials are to be refrigerated at 2°C–8°C (36°F–46°F) until use. Pertuzumab vials should not be used beyond the expiration date provided by the manufacturer. Because the formulation does not contain a preservative, the vial seal may only be punctured once. Any remaining solution should be discarded. Vial contents should be protected from light, and should not be frozen or shaken. The solution of pertuzumab for infusion, diluted in PVC or non-PVC polyolefin bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2°C–8°C for up to 24 hours prior to use. Diluted pertuzumab has been shown to be stable for up to 24 hours (up to 30°C). However, since diluted pertuzumab contains no preservative, the diluted solution should be stored refrigerated (2°C–8°C).

8.1.3 Compatibility

No incompatibilities between pertuzumab and polyvinylchloride, polyethylene or non-PVC polyolefin bags have been observed. Dextrose (5%) in water (D5W) solution should not be used to dilute pertuzumab since it was chemically and physically unstable in such solutions (dilute formulations of pertuzumab liquid formulations in D5W IV bags did not maintain stable pH after storage at room temperature (27-33°C) for 24 hours followed by 24 hours at refrigerator temperature [2°C -8°C]). Pertuzumab should not be mixed or diluted with other drugs.

8.1.4 Handling

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

8.1.5 Availability

Pertuzumab is an investigational agent and will be supplied free-of-charge from Genentech.

8.1.6 Administration

Pertuzumab will be administered in an outpatient setting. The initial dose of pertuzumab is 840 mg administered with infusion timing and post-infusion observation period per institutional guidelines. Subsequent infusions will be given every 3 weeks thereafter by a dose of 420 mg administered with infusion timing and post-infusion observation period per institutional guidelines. If the previous loading dose or previous doses were well tolerated, the post-infusion observation time may be discontinued as per institutional guidelines. **DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.**

If pertuzumab is being administered concomitantly with chemotherapy, monoclonal

antibody administration should precede chemotherapy administration. Patients should be observed for fever and chills or other infusion-associated symptoms. Refer to Sections 5.3.2, 5.3.5, and 5.3.6 for more details.

8.1.7 Ordering

The investigator or those named as sub-investigators agree to supply study drug only to those subjects who are enrolled in the study. The investigator or designee will keep a current and accurate inventory of all clinical drug supplies provided by Genentech. The study site will maintain a dispensing log and compliance with the treatment regimen will be calculated at the site from this information at each visit. Each participating center will order its own inventory of drug. Drug will be shipped directly to that participating site by Genentech.

8.1.8 Drug Supplies and Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form.

8.1.9 Destruction and Return

At the end of the study, unused supplies of pertuzumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 Trastuzumab

8.2.1 Description, Form, and Preparation

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each vial of trastuzumab contains either 440 mg or 150mg of trastuzumab (may vary based upon availability).

Use appropriate aseptic technique. Each 440 mg vial of trastuzumab should be reconstituted with 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied, to yield a multidose solution containing 21 mg/mL trastuzumab. Immediately upon reconstitution with BWFI, the vial of trastuzumab must be labeled in the area marked "Do not use after" with the future date that is 28 days from the date of reconstitution.

If the patient has known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with sterile water for injection. Trastuzumab that has been reconstituted with SWFI must be used immediately and any unused portion discarded. Use of other reconstitution diluents should be avoided.

Each 150 mg vial is reconstituted with 7.4 ml of Sterile Water for Injection (SWFI). The reconstituted vial yields a solution containing 21mg/ml trastuzumab.

Determine the dose of trastuzumab needed, based on a loading dose of 8 mg trastuzumab/kg body weight for q3wk dosing schedules or 4 mg trastuzumab/kg body weight for weekly dosing schedules; or a maintenance dose of 6 mg/kg trastuzumab/kg body weight for q3w dosing schedules or 2 mg trastuzumab/kg body weight for weekly dosing schedules. Calculate the correct dose using 21 mg/mL trastuzumab solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

No incompatibilities between trastuzumab and polyvinylchloride or polyethylene bags have been observed.

Trastuzumab should not be mixed or diluted with other drugs. Trastuzumab infusions should not be administered or mixed with Dextrose solutions.

8.2.2 Storage and Stability

Vials of trastuzumab are stable at 2°C–8°C (36°F–46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of 440 mg trastuzumab reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2°C–8°C (36°F–46°F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. A vial of 150 mg trastuzumab once reconstituted with SWFI should be used immediately, as it contains no preservative and is intended for single-dose only. If not used immediately, store the reconstituted Herceptin solution for up to 24 hours at 2°C–8°C; discard any unused Herceptin after 24 hours.

DO NOT FREEZE TRASTUZUMAB THAT HAS BEEN RECONSTITUTED.

The solution of trastuzumab for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% sodium chloride for injection, USP, may be stored at 2°C–8°C (36°F–46°F) for up to 24 hours prior to use. Diluted trastuzumab has been shown to be stable for up to 24 hours at room temperature 15°C–25°C; however, since diluted trastuzumab contains no effective preservative the reconstituted and diluted solution should be stored refrigerated (2°C–8°C).

8.2.3 Compatibility

No incompatibilities between trastuzumab and polyvinylchloride, polyolefin or polypropylene bags have been observed. Dextrose 5% solution should not be used since it causes aggregation of the protein. Trastuzumab should not be mixed or diluted with other drugs.

8.2.4 Handling

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

8.2.5 Availability

Trastuzumab is an investigational agent and will be supplied free-of-charge from Genentech.

8.2.6 Administration

Trastuzumab will be administered in an outpatient setting. The recommended initial loading dose for weekly administration is 4 mg/kg trastuzumab administered per institutional guidelines. The recommended maintenance weekly trastuzumab dose is 2 mg/kg and should be administered per institutional guidelines. DO NOT ADMINISTER AS AN IV PUSH OR BOLUS (see ADMINISTRATION).

The recommended initial loading dose for every 3 week administration is 8 mg/kg trastuzumab administered per institutional guidelines. The recommended every 3 week maintenance trastuzumab dose is 6 mg/kg q3wk and should be administered per institutional guidelines. Trastuzumab may be administered in an outpatient setting. DO NOT ADMINISTER AS AN IV PUSH OR BOLUS (see ADMINISTRATION).

Post-infusion observation periods may be done as per institutional guidelines. If the previous loading dose or previous doses were well tolerated, the post-infusion observation time may be discontinued as per institutional guidelines.

If trastuzumab is being administered concomitantly with chemotherapy, monoclonal antibody administration should precede chemotherapy administration. Patients should be observed for fever and chills or other infusion-associated symptoms. Refer to Sections 5.3.1, 5.3.5, and 5.3.6 for more details.

8.2.7 Ordering

The investigator or those named as sub-investigators agree to supply study drug only to those subjects who are enrolled in the study. The investigator or designee will keep a current and accurate inventory of all clinical drug supplies provided by Genentech. The study site will maintain a dispensing log and compliance with the treatment regimen will be calculated at the site from this information at each visit. Each participating center will

order its own inventory of drug. Drug will be shipped directly to that participating site by Genentech.

8.2.8 Drug Supplies and Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form.

8.2.9 Destruction and Return

At the end of the study, unused supplies of pertuzumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.3 Paclitaxel

8.3.1 Description, Form, and Preparation

Paclitaxel is commercially available in 30 mg/5ml, 100 mg/16.7 ml, and 300 mg/50 ml multidose vials containing a clear colorless to slightly yellow viscous solution. Each ml of sterile nonpyrogenic solution contains 6 mg of paclitaxel, 527 mg of purified Cremophor EL (polyoxyethylated castor oil), and 49.7% (v/v) dehydrated alcohol, USP. Please refer to the FDA-approved package insert for complete product information.

Paclitaxel must be diluted before administration with 0.9% sodium chloride for injection, USP; 5% dextrose for injection, USP for a final concentration of 0.3 to 1.2 mg/ml.

Paclitaxel should be prepared and stored in glass, polypropylene, or polyolefin containers because of the leaching of DEHP [di(2ethylhexyl)phthalate] plasticizer from polyvinyl chloride (PVC) containers. Non-PVC-containing tubing and connectors should be used, such as the IV administration sets (polyethylene or polyolefin) used to infuse parenteral nitroglycerin. In-line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 micron (e.g. IVEX-2) into the IV fluid pathway distal to the infusion pump. The Chemo Dispensing Pin device and similar devices with spikes should not be used with vials of paclitaxel since they can cause the stopper to collapse, resulting in loss of sterile integrity of the paclitaxel solution.

8.3.2 Storage and Stability

Intact vials should be stored between 20°- 25°C (68°-77°F) in the original package to protect from light, and remain stable until the expiration date on the label. Neither freezing nor refrigeration adversely affects stability. Upon refrigeration components in the paclitaxel vial may precipitate, but will redissolve upon reaching room temperature with little or no agitation. There is no impact on product quality under these circumstances. If the solution remains cloudy or if an insoluble precipitate is noted, the vial should be discarded. Solutions for infusion prepared as recommended are stable at

ambient temperature (approximately 25°C) and lighting conditions for up to 27 hours.

8.3.3 Compatibility

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

8.3.4 Handling

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

8.3.5 Availability

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

8.3.6 Administration

Paclitaxel will be administered as an IV infusion with the use of an in-line 0.22-micron filter. Refer to Section 5.3.3 for more details including the premedication regimen.

8.3.7 Ordering

Paclitaxel is a commercially available agent and therefore ordering should be performed as standard policy in the investigational site.

8.3.8 Accountability

Paclitaxel is a commercially available agent and therefore accountability should be performed as standard policy in the investigational site.

8.3.9 Destruction and Return

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.

8.4 Doxorubicin

8.4.1 Description, Form, and Preparation

Commercially available as lyophilized powder for reconstitution in 10-, 20-, 50-, 100-and 150-mg vials. Also available as solution (2 mg/ml) in 10-, 20-, 50- and 200-mg vials for

injection. Please refer to FDA-approved package insert for complete product information.

Reconstitute the vials with 5, 10, 25, 50, or 75 ml, respectively, of sodium chloride for injection, USP.

8.4.2 Storage and Stability

Intact vials of doxorubicin 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.

8.4.3 Handling

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

8.4.4 Availability

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

8.4.5 Administration

Administer doxorubicin intravenously, either peripherally as a bolus injection or through a central venous line. Avoid extravasation, since severe local tissue necrosis may result. Refer to Section 5.3.5 for more details.

8.4.6 Ordering

Doxorubicin is a commercially available agent and therefore ordering should be performed as standard policy in the investigational site.

8.4.7 Accountability

Doxorubicin is a commercially available agent and therefore accountability should be performed as standard policy in the investigational site.

8.4.8 Destruction and Return

Doxorubicin is a commercially available agent and therefore drug destruction should be performed as standard policy in the investigational site.

8.5 Cyclophosphamide

8.5.1 Description, Form, and Preparation

Commercially available as powder for injection in 100-mg, 200-mg, 500-mg, 1-g, and 2-g vials. Please refer to the FDA-approved package insert for complete product information.

Reconstitute 100-mg, 200-mg, 500-mg, 1-g, and 2-g vials with 5,10, 25, 50, or 100 ml of sterile water for injection respectively, for a final concentration of 20 mg/ml. Vigorous shaking an/or gentle warming may be necessary. Bacteriostatic water (paraben-preserved only) may also be used for reconstitution. 0.9% sodium chloride or 5% dextrose may also be used for reconstitution.

8.5.2 Storage and Stability

Store intact vials of powder at room temperature (15° to 30°C). Reconstitute lyophilized cyclophosphamide is chemically and physically stable for 24 hours at room temperature or for 6 days in the refrigerator (2° to 8°C). It does not contain any antimicrobial preservative, and care must therefore be taken to ensure the sterility of prepared solution.

8.5.3 Handling

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

8.5.4 Availability

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

8.5.5 Administration

Cyclophosphamide is administered via intravenous injection per institutional guidelines. Refer to Section 5.3.5 for more details.

8.5.6 Ordering

Cyclophosphamide is a commercially available agent and therefore ordering should be performed as standard policy in the investigational site.

8.5.7 Accountability

Cyclophosphamide is a commercially available agent and therefore accountability should be performed as standard policy in the investigational site.

8.5.8 Destruction and Return

Cyclophosphamide is a commercially available agent and therefore drug destruction should be performed as standard policy in the investigational site.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biospecimen Collection

Biopsies will be performed prior to treatment and after 1 week on treatment. If there is residual disease on gross exam, an additional sample of tissue will be obtained at the time of breast surgery. Collection guidelines are described in Section 9.4.
Blood collection will also be done to look for potential interference with drug metabolites. Blood and biopsy sample acquisition, processing, and shipping details are outlined in section 9.3.

Samples will be stored at the DF/HCC Core Blood and Tissue Bank until requested for further processing. RNA and DNA will be made from the core biopsy samples. Other genomic analysis may also be performed as part of a hypothesis generating practice.

Tumor tissue may be stored at -80°C until exhaustion. There are multiple checks on specimen/data quality at various steps in the procedure.

9.2 Tissue Studies

9.2.1 Gene Expression Microarrays

Frozen invasive breast adenocarcinoma is evaluated by frozen section to determine tumor percentage and stroma/fat and necrosis percentages. An adjacent section of the tumor is homogenized and RNA is purified. Tumor RNA and universal reference RNA are separately converted to cDNA and then to cRNA in which the tumor and reference are labeled with two different fluorescent dyes. These two RNA populations are incubated together and compete for binding to an array surface covered by single stranded DNA 60-mers which represent multiple copies of nearly every human gene. Analysis by the Agilent scanner produces data which indicates relative expression levels for all of the genes compared to the universal reference in known locations on the array. A Single Sample Predictor (SSP) algorithm is applied to classify the tumor into one of the 5 major intrinsic subtypes of breast cancer. [54]

This analysis will take place in collaboration with Nanostring Technologies, whereby de-identified tissue samples will be shared and analyzed.

9.2.2 Cyclic Immunofluorescence (cyCIF)

FFPE tissue samples collected from participants will be sectioned and assessed by IHC and/or IF assays for various markers, as deemed appropriate and informative.

This analysis will take place in collaboration with Dr. Peter Sorger at Harvard Medical School, whereby de-identified tissue samples will be shared and analyzed.

9.2.3 Whole Exome, Whole Genome Sequencing, DNA Methylation

All DNA sequencing will be performed at the Broad Institute; this may include SNP-arrays for germline genomic analyses, as well as exome and/or whole genome sequencing. Specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed. Potential assays that may be performed on frozen or FFPE samples include:

- Whole Genome sequencing depth of ≥ 40X coverage, or Whole Exome sequencing depth of ≥ 500X coverage.
- AFFY SNP 6.0, or Illumina SNP arrays for genotypes and DNA copy number changes may be performed.
- DNA methylation: Illumina Infinium 450K methylation arrays.

9.3 Circulating Biomarkers

We plan to collect samples for analysis of circulating biomarkers, including ctDNA, on this clinical protocol and indicated in Section 2.7.2. Blood and biopsy sample acquisition, processing, and shipping details are outlined in Section 9.3. We will evaluate associations between change in ctDNA during therapy and residual disease at the time of definitive surgery. In addition we will be able to explore genotypic and phenotypic changes that occur during therapy, which may yield insights into mechanisms underlying resistance to therapy.

Samples will be stored at the DF/HCC Core Blood and Tissue Bank until requested for processing, at DF/HCC or the Broad Institute. Samples transferred to the Broad will be de-identified specimens.

9.4 Sample Acquisition

TABLE 7. Specimen Collection

	Baseline (at screening or on Week 1 (Day 1) prior to tx start)	Pre-Operative Therapy Week 2 (Day 8 prior to tx)	After paclitaxel Pre-operatively	Surgery	Post mastectomy	Ship to
Lavender Top Blood Sample	x					DFCI Lab
Streck Tubes	x	x	x		x	DFCI Lab

Breast Tissue	x	x		X[a]		DFCI Lab and Michigan Lab

[a] If adequate residual disease present; per pathology discretion

9.4.1 Blood Samples

a. Lavender Top Blood Sample

One 10mL lavender top (EDTA Fisher #366643) tube of whole blood will be collected at baseline or on Week 1, Day 1 prior to treatment start. This specimen 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.

Following blood collection, label the specimens with the assigned DFCI participant study ID, date of collection, time point of collection, and protocol number. Include a copy of the 12-497 DFCI Specimen Requisition Form (Appendix D) with your shipment.

All blood samples should be shipped ambient overnight Monday through Thursday at ambient temperature by either FedEx or UPS to:

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

If blood specimens must be collected on Friday, the specimens should be stored over the weekend and shipped on Monday. When storing tubes over a weekend, lavender top tubes should be frozen at -80ºC until shipment.

Email the DFCI study coordinator, Grace Winship, and Project Managers with the sample information and tracking information the day before shipping specimens: gwinship@partners.org and ctopm@dfci.harvard.edu.

b. Streck Tubes

Circulating biomarkers will be collected via two 10 mL Streck tubes each at the following time points:

- Week 1 (Day 1): Before the start of trastuzumab and pertuzumab
- Week 2 (Day 8): Before the start of paclitaxel, trastuzumab and pertuzumab
- After completing paclitaxel preoperatively
- At the first visit post-mastectomy

Streck tubes are to be kept at room temperature, and centrifuged within 2 hours of collection if possible.

- Place Streck Cell-Free Blood Collection Tubes in centrifuge with swinging-out rotor and appropriate buckets.
- Centrifuge blood samples for 10 min at 1900 x g [3000 rpm] at room temperature.
- Carefully collect plasma supernatant without disturbing the buffy coat layer. About 4–5ml of plasma can be obtained from one 10 ml primary blood tube.
- Aspirated plasma is transferred into multiple labeled 1.5 or 2mL RNase/DNase free tubes for use with the Beckman Coulter Microfuge 22R, or transfer aspirated plasma into fresh 15 ml centrifuge tubes with conical bottom for use with the Sorvall RC5C plus (*Check RCF factor of 15mL tubes).
- Centrifuge plasma samples for 10 min at 16,000 x g [in fixed-angle rotor] at room temperature setting. This will remove additional cellular nucleic acids attached to cell debris. Carefully remove plasma supernatant without disturbing any possible debris at the bottom of each tube. There will end up being residual volume of plasma that is not used.
- Store plasma and buffy coat at -80°C freezer in RNase/DNase free tubes in 1-5mL aliquots until shipment.
- Label the specimens with the assigned DFCI participant study ID, date of collection, time point of collection, and protocol number.

Samples will be stored in the Core Blood and Tissue bank until requested for processing.

9.4.2 Biopsy Collection

Core needle tumor biopsies (14-gauge) will be acquired with 5-8 cores each at the following timepoints:

- Week 1 (Day 1): Before the start of trastuzumab and pertuzumab
- Week 2 (Day 8): Before the start of paclitaxel, trastuzumab and pertuzumab
- If adequate residual tumor is present (per pathology discretion) in the mastectomy specimen, samples of it will also be obtained.

a. Processing biopsy samples for DFCI:

Following biopsy collection, use at minimum 4 core samples for research at DFCI (one core will be used for research at Michigan). The tissue will be immediately snap-frozen in dry ice and stored at -80°C with the suggested supplies and procedure listed below. The tumor section size should be about 20 mg. Only residual tumor measuring $\geq$ 1 cm found within the mastectomy specimen will be snap frozen in dry ice and stored at -80°C.

- **Supplies:**
    - Tissue-Tek® Cryomold,

        - Standard size 25x20x5mm (#4557), Fisher Scientific Cat# NC9511236
        -OR-
        - Intermediate size 15x15x5mm (#4566), Fisher Scientific Cat# NC9542860
    - Tissue-Tek® O.C.T. Compound, 4oz (#4583): Fisher Scientific Cat# NC9638938
    - Small specimen bag: Fisher Cat# 01-002-37 (or equivalent)
    - Forceps: Fisher Cat# NC9832137 (or equivalent)
    - Dry Ice and Cooler
    - Cryoware Pen: Fisher Cat# 13-382-88

- **Procedure:**
    - Prepare cryomolds ahead of time: Squeeze small amount of OCT into cyromolds* – enough for there to be a thin layer covering the bottom let settle on a flat surface for a minute or two. It is *crucial* for future sectioning that these core biopsies are placed on a very flat OCT surface, so the best way to make sure these mold are flat is the *freeze the bottom layer on a -80 freezer shelf.*
        - *If time permits, the cryomolds can be placed onto the dry-ice once a thin layer of OCT has been put it but before the tissue is put in. Once this is frozen or begins to freeze, the tissue can be placed on top of the now frozen OCT and then covered with more liquid OCT and then placed back onto the dry ice to freeze completely.
    - Bring all supplies needed to the biopsy as the tissue needs to be frozen immediately.
    - As soon as the specimen is ready, use disposable forceps to gently place only 1 core into each cryomold. Gently pick up the tissue with the forceps from one end of the core biopsy, being careful not to crush the tissue and immediately lay out the fresh biopsy tissue onto the center of the mold. Be sure to lay the tissue as flat to the mold, and as straight as possible. Keep molds on dry ice at all times.
    - You should ideally collect between 3 and 5 separate cores.
    - Once the specimen is in the cryomold, cover with more OCT making sure the tissue is entirely submerged.
    - Immediately place cryomold with OCT and tissue onto dry-ice making sure the cryomold is level and will not tip over.
    - The OCT will freeze into a solid white block within 5-10 minutes
    - Once the blocks have completely frozen they can be put into a specimen bag and sealed. More than one block can be put into a bag.
    - The bag should be labeled with:
        - Patient name
        - DFCI Study #: 12-497
        - DFCI MRN #
        - Date of biopsy
        - Time Point (Biopsy #1, Biopsy #2, or Surgery)

- Number of blocks in the bag
- Samples should be kept on dry ice at all times until they can be placed in a -80ºC freezer. Samples should remain frozen at -80ºC until shipment.

All biopsy samples should be shipped frozen overnight Monday through Thursday at frozen temperature by either FedEx or UPS to:

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

If biopsy specimens must be collected on Friday, the specimens should be stored over the weekend and shipped on Monday. When storing tubes over a weekend, biopsy tubes should be frozen at -80ºC until shipment.

Email the DFCI study coordinator, Grace Winship, and Project Managers with the sample information and tracking information the day before shipping specimens: gwinship@partners.org and ctopm@dfci.harvard.edu.

b. Processing biopsy samples for Michigan:

From the biopsy samples obtained above, at most one core from each time point will be used for Michigan samples (i.e., Week 1 (Day 1), Week 2 (Day 8), and residual tumor). Place the pieces separately into 2 ml cryogenic vials, then add 90% FBS and 10% DMSO to the vials so the will be slowly frozen. Place the vials on dry ice.

Following processing, label the specimens with the assigned DFCI participant study ID, date of collection, time point of collection, and protocol number.

Samples will be shipped frozen to Dr. Merajver's laboratory overnight as soon as possible post-biopsy. Include a copy of the 12-497 Michigan Specimen Requisition Form (Appendix E) with your shipment.

Dr ZhiFen Wu
c/o Merajver Lab
7121 CC
1500 East Medical Center Drive
Ann Arbor, MI 48109

9.5 Future Use of Biospecimens and Data

All biospecimens and data will be banked for future research and may be made available to other researchers for proposed research projects. These projects will be approved by the PI, Breast Users Committee, and/or the IRB, as appropriate.

9.6 Consent to Sharing and Future Use

In November 2019, the protocol and consent form were updated with language about correlative plans due to new regulations pertaining to the use of biospecimens and data in collaboration with external entities. At the time of this amendment, all samples have been collected and no participants remain on active treatment. In total, 23 participants took part on this study; currently 2 participants have passed away and 1 participant is lost to follow-up. Therefore, notification of deceased subjects is not feasible and locating the lost participant likely puts them at a larger risk than in sharing their de-identified samples for analysis. As such, the study team would like to move forward with utilizing these participant's samples as described in Section 9.0 given the lack of feasibility in notification of this new information.

The study team will practice due diligence in locating and notifying the remaining 20 subjects by letter. Should the patient actively decline this information, samples and data will not be shared. If due diligence is exercised and the patient cannot be reached, the study team would like permission to share these de-identified samples and data, given that it puts the patient at no more than minimal additional risk.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 28 days prior to registration except pregnancy test (within 14 days prior to registration). Baseline scans must be done ≤4 weeks (28 days) prior to registration. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

During pre-operative therapy, patients will be seen by exam at Week 1, Week 2, and each time point they receive Pertuzumab (i.e., week 4, week 7, week 10, week 13, week 16, and then every three weeks during pertuzumab/trastuzumab therapy prior to surgery). The pre-surgical evaluation is to be done at week 18. or at the conclusion of pertuzumab/trastuzumab, as this therapy may be given after week 16 per investigator discretion.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered according to the schedule below, unless otherwise noted.

TABLE 8: Study Calendar

	Screening	Preoperative Therapy		Pre-Surgical Evaluation	Post-Operative Therapy Option 1 (AC)	Maintenance Therapy Options 1 and 2 (H/P)	Follow-up [g] (+/- 1 month)
Tests and Procedures	**≤28 Days Prior to Registration**	**Wk 1 (Day 1)**	**Wk 2 to Wk 16**	**Wk 18 (+/- 1 wk)**			
Complete medical history	x						
Complete physical exam, including tumor assessment	x	x	Wk 2, Wk 4, then Every 3 wks (+/- 2 days)	x	Every 2 to 3 wks (+/- 2 days)	Every 9 wks (+/- 1 wk) and at last treatment	
Weight, vital signs, height, and ECOG PS	x	x		x			
Toxicity evaluation	x	x		x			
EKG (12 lead)	x			x			
Clinical breast imaging [a]	x			x			x[h]
PET/CT, CT of Chest/Abdomen/Pelvis	x			x[i]			
MUGA or Echocardiogram [b]	x			x		Before H/P start, Every 12 wks (+/- 1 wk)[f]	
Serum pregnancy test[c]	x [e]						
Hematology (CBC with differential)	x		Weekly (+/- 2 days)	x	Every 2 to 3 wks (+/- 2 days)	Every 9 wks (+/- 1 wk)	
Serum chemistry [d]	x			x			
PT/INR/PTT	x						
Survival status							x
Research Specimens (Blood, Biopsy, or Tissue (See Section 9.3)	x	x	x	x	x	x	

AC= doxorubicin/cyclophosphamide H/P= Trastuzumab and Pertuzumab

[a] Breast imaging includes a bilateral MRI, Mammogram, and ultrasound if indicated.

[b] Cardiac evaluations should consistently use one modality across all study evaluations.

[c] Women of childbearing potential.

[d] Sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total protein, albumin, total bilirubin, SGOT(AST), SGPT(ALT), alkaline phosphatase.

[e] Serum pregnancy testing at screening is required within 14 days prior to registration.
[f] If Option 2, pre-surgical MUGA/ECHO can be used; does not require repeating prior to H/P start. Further MUGA/ECHOs every 12 weeks from Day 1 of H/P maintenance therapy (Section 5.3.6).
[g] See section 5.6.
[h] Breast imaging per standard practice recommendations.
[i] PET/CT and/or CT required for Stage 4 disease; otherwise optional

11. MEASUREMENT OF EFFECT

Inflammatory breast disease is considered non-measurable. The major effect to be measured in this study is the tumor/pathological response and response on exam to study drugs.

11.1 Antitumor Effect

A baseline and presurgical imaging study of the breast is required; mammogram, ultrasound (if indicated), and bilateral MRI. The baseline imaging must be obtained within 28 days prior to registration. The pre-surgical imaging should occur at week 18 +/- 1 week during pre-operative treatment. Patients will also be examined at baseline within 28 days prior to registration, every 3 weeks during pre-operative treatment with Trastuzumab, Pertuzumab, and Paclitaxel, and again at week 18 (+/- 1 week). Photographs of the affected breast may be obtained.

If the patient demonstrates clinical progression at any time, repeat imaging may be necessary. If there is discordance (clinical progression, but radiographic stable disease or response), the study PI should be contacted to solve the discordance.

Tumor response will be evaluated based upon resolution of edema, erythema and any density palpable within the effected breast.

11.1.1 Evaluable for objective response definition.

All participants who initiate the preoperative treatment phase will be considered as evaluable for the two preoperative treatment endpoints.

11.1.2 Disease Parameters

Non-measurable disease. Inflammatory breast disease is considered non-measurable.

Non-target lesions. All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring > 10 mm to < 15 mm in short axis, as well as truly non-measurable lesions, which include inflammatory breast disease.

11.1.3 Response Criteria

Pathologic Complete Response pCR

Complete pathologic disease response (pCR) is defined as absence of invasive carcinoma within the breast and axillary lymph nodes following preoperative THP therapy. Participants whose disease is not surgically resectable following preoperative treatment are considered as not having pCR.

Residual cancer burden (RCB)

RCB after preoperative therapy will be determined, as defined by Symmans et al.[36]

Clinical Progressive Disease

Subjects, who in the opinion of the treating physician investigator have had a substantial decline in their performance status and have clinical evidence of progressive disease may be classified as having progressive disease.

11.1.4 Evaluable for Toxicity

All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

11.1.5 Disease-Free Survival(DFS), Time to Treatment Failure (TTF), and Overall Survival (OS)

- Disease-free survival (DFS) will be defined among participants who undergo surgery, as the duration of time from surgery until ipsilateral local-regional, contralateral or distant invasive recurrence or death from any cause; in the absence of an event, DFS will be censored at the date last know alive and free from recurrence.
- Time to treatment failure (TTF) will be defined among all participants, as the duration of time from treatment initiation to a DFS event or progressive disease during preoperative therapy or treatment disease that is not surgically resectable; in the absence of an event, TTF will be censored at the date last know alive and free from recurrence or progression.
- Overall survival (OS) will be defined among all participants, as the duration of time from treatment initiation to death from any cause, or is censored at date last known alive. Post-surgery OS will be defined among the participants who undergo surgery, as the duration of time from treatment initiation to death from any cause, or is censored at date last known alive.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

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

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

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

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

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

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

12.4 Collaborative Research and Future Use of Data and Samples

Tissue, blood, and other materials derived from these will be collected in this study to analyze genes, DNA, RNA, proteins and cells for the study's correlative endpoints and potential future research, utilizing new types of biomarker testing as it becomes available.

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entitites, with whom we collaborate. When samples or data are sent to collaborators

and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, birthday, or MRNs.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one of more publicly-accessible scientific databases, such as the National Institutes of Health's Database for Genotypes and Phenotypes (dbGaP). The results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The primary objective of this phase II trial is to determine pathologic complete response (pCR) rate after preoperative therapy with combination paclitaxel (T), trastuzumab (H) and pertuzumab (P) in HER2+ inflammatory breast cancer, as well as to assess the residual cancer burden (RCB) after preoperative therapy. Participants are enrolled using a single-arm, two-stage Simon minimax design.

Following surgery, participants will be treated with 4 cycles of adjuvant AC chemotherapy, as per physician preference, and HP for 12 a total of 12 months; radiation therapy and endocrine therapy are given according to standard of care. Participants will be followed post-therapy for recurrence and survival (see Section 10). Efficacy of protocol therapy, defined as disease-free survival (DFS), time to treatment failure (TTF), and overall survival (OS), will be assessed.

For correlative studies, research biopsies and whole blood will be obtained prior to start of preoperative therapy, and prior to day 8 of preoperative therapy. A sample of residual disease within the breast at time of surgery will be obtained.

13.2 Design and Sample Size

The study was planned using Simon minimax two-stage design. Pathologic complete response (pCR) is the primary endpoint. We propose that if the proportion of participants experiencing pCR is ≤ 0.15 then the preoperative THP regimen is considered minimally effective, versus an alternative hypothesis that the TPH regimen is worthy of further study if proportion pCR ≥ 0.40. After testing the regimen on 16 participants in the first stage, the study will be terminated if ≤ 2 have pCR, and will proceed if ≥ 3 of 16 have pCR. If the study goes on to the second stage, the regimen is rejected as not if ≤7 of 27 participants in total have pCR. If the preoperative THP regimen is actually not effective, there is an α=0.039 probability of concluding that it is (target α=0.05); if the THP regimen is actually effective, there is an 0.099 probability of concluding that it is not (target β=0.10). Up to 30 participants will be enrolled, allowing up to 3 participants not evaluable for the pCR endpoint (see below).

13.3 Analysis of Primary Endpoints

Primary endpoint definitions

- Pathologic complete response (pCR) is defined as absence of invasive carcinoma within the breast and axillary lymph nodes following preoperative THP treatment. Participants whose disease is not surgically resectable following preoperative THP treatment are considered as not having pCR.
- Residual Cancer Burden following preoperative treatment is defined according to Symmans et al[53].
- All participants who initiate the preoperative treatment phase will be considered as evaluable for the two preoperative treatment endpoints.

Statistical analysis

- The number and proportion of participants achieving pCR following preoperative THP treatment will be summarized with 90% confidence interval (CI) that accounts for the two-stage design.
- The number and proportion of participants achieving RCB-0, I, II, III will be summarized with 90% exact binomial CI.

13.4 Analysis of Secondary Endpoints, Reporting and Exclusions

13.4.1 Evaluation of Toxicity

The frequencies of adverse events (AE) while on study treatment will be summarized and tabulated according to treatment phase (preoperative vs. post-surgical), and at any time. Enrolled patients who received at least one dose of study treatment will be included in the analyses.

13.4.2 Efficacy of Protocol Therapy

- Disease-free survival (DFS) will be defined among participants who undergo surgery, as the duration of time from surgery until ipsilateral local-regional, contralateral or

distant invasive recurrence or death from any cause; in the absence of an event, DFS will be censored at the date last know alive and free from recurrence.

- Time to treatment failure (TTF) will be defined among all participants, as the duration of time from treatment initiation to a DFS event or progressive disease during preoperative therapy or treatment disease that is not surgically resectable; in the absence of an event, TTF will be censored at the date last know alive and free from recurrence or progression.
- Overall survival (OS) will be defined among all participants, as the duration of time from treatment initiation to death from any cause, or is censored at date last known alive. Post-surgery OS will be defined among the participants who undergo surgery, as the duration of time from treatment initiation to death from any cause, or is censored at date last known alive.

Time-to-event distributions will be summarized using the method of Kaplan-Meier and two-sided 90% CI for the medians will be summarized. Exploratory analyses will summarize DFS and post-surgery OS distributions according to pCR and of RCB, and estimates hazards ratios with 90% CI using Cox proportional hazards models.

13.4.3 Correlative Studies

- PAM50 analysis will be performed on the pre-treatment biopsy specimen. Participants' pCR will be tabulated according to the intrinsic subtype with 90% CI, and the association of intrinsic subtype (HER2-enriched vs. other) with pCR will be assessed using Fisher's exact test.
- Gene expression profiling will be assessed on pre-treatment and day-8 biopsies to identify early adaptive responses that are associated with resistance to HER2 directed therapies. Differential expression analysis, using standard procedures in R/Bioconductor package limma, will seek expression at either time point, or changes in expression between time points, consistently associated with pCR.
- Residual disease within the breast at time of mastectomy will be assessed by PAM50 and microarray analysis. Changes in gene expression, including those genes used for definition of intrinsic subtype, will be analyzed to investigate the intrinsic subtype of the resistance clone with residual disease.
- Samples will be collected for analysis of circulating biomarkers, including ctDNA. Associations between change in ctDNA during therapy and residual disease at the time of definitive surgery will be evaluated.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three

(3) years after the end of the study.

REFERENCES

1. Kim T, Lau J, Erban J. Lack of uniform diagnostic criteria for inflammatory breast cancer limits interpretation of treatment outcomes: a systematic review. Clin Breast Cancer 2006;7(5): 386-95.
2. Woodward WA, Cristofanilli M. Inflammatory breast cancer. Semin Radiat Oncol 2009;19(4): 256-65.
3. Anderson WF, Chu KC, Chang S. Inflammatory breast carcinoma and noninflammatory locally advanced breast carcinoma: distinct clinicopathologic entities? J Clin Oncol 2003;21(12): 2254-9.
4. Walshe JM, Swain SM. Clinical aspects of inflammatory breast cancer. Breast Dis 2005;22: 35-44.
5. Zucali R, Uslenghi C, Kenda R, Bonadonna G. Natural history and survival of inoperable breast cancer treated with radiotherapy and radiotherapy followed by radical mastectomy. Cancer 1976;37(3): 1422-31.
6. Ueno NT, Buzdar AU, Singletary SE, et al. Combined-modality treatment of inflammatory breast carcinoma: twenty years of experience at M. D. Anderson Cancer Center. Cancer Chemother Pharmacol 1997;40(4): 321-9.
7. Baldini E, Gardin G, Evagelista G, Prochilo T, Collecchi P, Lionetto R. Long-term results of combined-modality therapy for inflammatory breast carcinoma. Clin Breast Cancer 2004;5(5): 358-63.
8. Cristofanilli M, Gonzalez-Angulo AM, Buzdar AU, Kau SW, Frye DK, Hortobagyi GN. Paclitaxel improves the prognosis in estrogen receptor negative inflammatory breast cancer: the M. D. Anderson Cancer Center experience. Clin Breast Cancer 2004;4(6): 415-9.
9. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406(6797): 747-52.
10. Van Laere SJ, Van den Eynden GG, Van der Auwera I, et al. Identification of cell-of-origin breast tumor subtypes in inflammatory breast cancer by gene expression profiling. Breast Cancer Res Treat 2006;95(3): 243-55.
11. Sawaki M, Ito Y, Akiyama F, et al. High prevalence of HER-2/neu and p53 overexpression in inflammatory breast cancer. Breast Cancer 2006;13(2): 172-8.
12. Turpin E, Bieche I, Bertheau P, et al. Increased incidence of ERBB2 overexpression and TP53 mutation in inflammatory breast cancer. Oncogene 2002;21(49): 7593-7.
13. Cross M, Dexter TM. Growth factors in development, transformation, and tumorigenesis. Cell 1991;64(2): 271-80.
14. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235(4785): 177-82.
15. Kallioniemi OP, Kallioniemi A, Kurisu W, et al. ERBB2 amplification in breast cancer analyzed by fluorescence in situ hybridization. Proc Natl Acad Sci U S A 1992;89(12): 5321-5.
16. Pauletti G, Godolphin W, Press MF, Slamon DJ. Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. Oncogene 1996;13(1): 63-72.
17. Hynes NE. Amplification and overexpression of the erbB-2 gene in human tumors: its involvement in tumor development, significance as a prognostic factor, and potential as a target for cancer therapy. Semin Cancer Biol 1993;4(1): 19-26.
18. Di Fiore PP, Pierce JH, Kraus MH, Segatto O, King CR, Aaronson SA. erbB-2 is a potent oncogene when overexpressed in NIH/3T3 cells. Science 1987;237(4811): 178-82.
19. Hudziak RM, Schlessinger J, Ullrich A. Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells. Proc Natl Acad Sci U S A 1987;84(20): 7159-63.

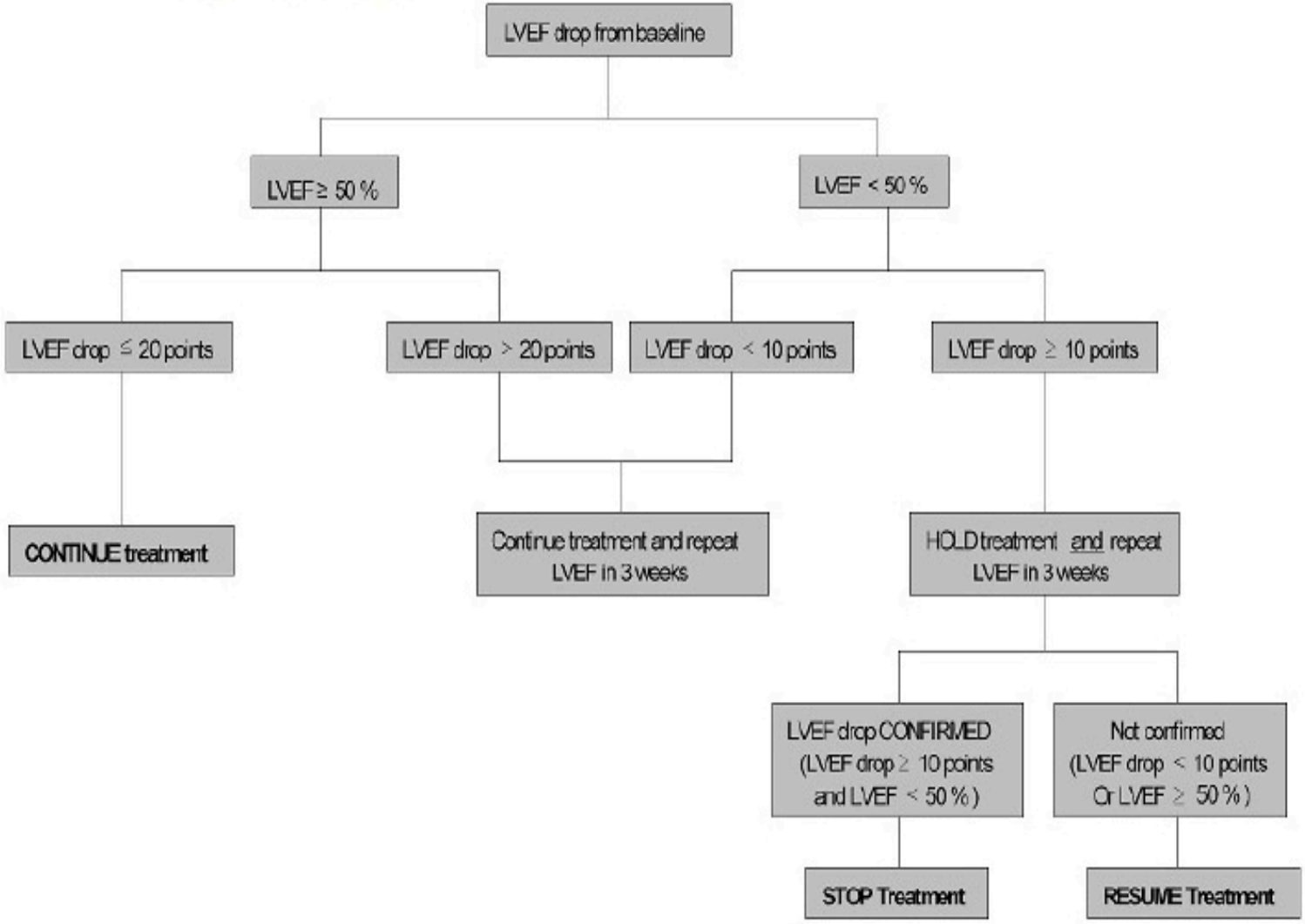
20. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc Natl Acad Sci U S A 1992;89(22): 10578-82.
21. Drebin JA, Link VC, Stern DF, Weinberg RA, Greene MI. Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. Cell 1985;41(3): 697-706.
22. Fendly BM, Winget M, Hudziak RM, Lipari MT, Napier MA, Ullrich A. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. Cancer Res 1990;50(5): 1550-8.
23. Jurianz K, Maslak S, Garcia-Schuler H, Fishelson Z, Kirschfink M. Neutralization of complement regulatory proteins augments lysis of breast carcinoma cells targeted with rhumAb anti-HER2. Immunopharmacology 1999;42(1-3): 209-18.
24. Pegram MD, Lopez A, Konecny G, Slamon DJ. Trastuzumab and chemotherapeutics: drug interactions and synergies. Semin Oncol 2000;27(6 Suppl 11): 21-5; discussion 92-100.
25. Pietras RJ, Fendly BM, Chazin VR, Pegram MD, Howell SB, Slamon DJ. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. Oncogene 1994;9(7): 1829-38.
26. Arteaga CL, Winnier AR, Poirier MC, et al. p185c-erbB-2 signal enhances cisplatin-induced cytotoxicity in human breast carcinoma cells: association between an oncogenic receptor tyrosine kinase and drug-induced DNA repair. Cancer Res 1994;54(14): 3758-65.
27. Hancock MC, Langton BC, Chan T, et al. A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. Cancer Res 1991;51(17): 4575-80.
28. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer Res 1998;58(13): 2825-31.
29. Pegram MD, Finn RS, Arzoo K, Beryt M, Pietras RJ, Slamon DJ. The effect of HER-2/neu overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Oncogene 1997;15(5): 537-47.
30. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999;17(9): 2639-48.
31. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344(11): 783-92.
32. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005;353(16): 1673-84.
33. Perez EA, Romond EH, Suman VJ, et al. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 2011;29(25): 3366-73.
34. Smith I, Procter M, Gelber RD, et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. Lancet 2007;369(9555): 29-36.
35. Gianni L, Dafni U, Gelber RD, et al. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. Lancet Oncol 2011;12(3): 236-44.
36. Slamon D, Eiermann W, Robert N, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011;365(14): 1273-83.
37. Baselga J, Gelmon KA, Verma S, et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. J Clin Oncol 2010;28(7): 1138-44.

38. Lenihan D, Suter T, Brammer M, Neate C, Ross G, Baselga J. Pooled analysis of cardiac safety in patients with cancer treated with pertuzumab. Ann Oncol 2012;23(3): 791-800.
39. Baselga J, Cortes J, Kim SB, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012;366(2): 109-19.
40. Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. J Natl Cancer Inst Monogr 2001(30): 96-102.
41. Hennessy BT, Gonzalez-Angulo AM, Hortobagyi GN, et al. Disease-free and overall survival after pathologic complete disease remission of cytologically proven inflammatory breast carcinoma axillary lymph node metastases after primary systemic chemotherapy. Cancer 2006;106(5): 1000-6.
42. Baselga J, Semiglazov V, Manikahs A, et al. Efficacy of neoadjuvant trastuzumab in patients with inflammatory breast cancer: data from the NOAH (NeOAdjuvant Herceptin) Phase III trial. ECCO 14- the European Cancer Conference. Barcelona, Spain, 2007:193.
43. Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 2010;375(9712): 377-84.
44. Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13(1): 25-32.
45. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27(8): 1160-7.
46. Cheang MC, Prat A, Fan C, Perou CM. S5-2: PAM50 HER2-Enriched subtype enriches for tumor response to neoadjuvant anthracyclines/taxane and trastuzumab/taxance containing regimens in HER2-positive breast cancer. Cancer Res 2011;71(24 Suppl): S5-2, 110s. .
47. de Ronde JJ, Hannemann J, Halfwerk H, et al. Concordance of clinical and molecular breast cancer subtyping in the context of preoperative chemotherapy response. Breast Cancer Res Treat 2010;119(1): 119-26.
48. Sperinde J, Jin X, Banerjee J, et al. Quantitation of p95HER2 in paraffin sections by using a p95-specific antibody and correlation with outcome in a cohort of trastuzumab-treated breast cancer patients. Clin Cancer Res 2010;16(16): 4226-35.
49. Razis E, Bobos M, Kotoula V, et al. Evaluation of the association of PIK3CA mutations and PTEN loss with efficacy of trastuzumab therapy in metastatic breast cancer. Breast Cancer Res Treat 2011;128(2): 447-56.
50. Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J Natl Cancer Inst 2001;93(24): 1852-7.
51. Sergina NV, Rausch M, Wang D, et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. Nature 2007;445(7126): 437-41.
52. Burstein HJ. Preoperative therapy as a model for translational research in breast cancer. Cancer Invest 2008;26(3): 217-21.
53. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25(28): 4414-22.
54. Hu Z, Fan C, Oh DS, et al. The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics 2006;7: 96.
55. Landis MD, Lehmann BD, Pietenpol JA, Chang JC. Patient-derived breast tumor xenografts facilitating personalized cancer therapy. Breast Cancer Res 2013;15:201.
56. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res 2013;73:5315-9.

57. DeRose YS, Wang G, Lin YC, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med 2011;17:1514-20.
58. Marangoni E, Vincent-Salomon A, Auger N, et al. A new model of patient tumor-derived breast cancer xenografts for preclinical assays. Clin Cancer Res 2007;13:3989-98.
59. Tentler JJ, Tan AC, Weekes CD, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol 2012;9:338-50.
60. Zhang X, Claerhout S, Prat A, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res 2013;73:4885-97.
61. Forshew T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med 2012;4:136ra68.
62. Murtaza M, Dawson SJ, Tsui DW, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 2013;497:108-12.
63. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199-209.
64. Beaver JA, Jelovac D, Balukrishna S, et al. Detection of cancer DNA in plasma of patients with early-stage breast cancer. Clin Cancer Res 2014;20:2643-50

APPENDIX A. LVEF ASSESSMENTS ALGORITHM

Algorithm for Continuation and Discontinuation of Pertuzumab and Trastuzumab Based on LVEF Assessments.



APPENDIX B. GENENTECH SAFETY REPORTING FAX COVER SHEET

Genentech
A Member of the Roche Group

SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

AE / SAE FAX No: (650) 225-4682

Alternate Fax No: (650) 225-5288

Genentech Study Number	
Principal Investigator	
Site Name	
Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

APPENDIX C. DF/HCC MULTI-CENTER DATA AND SAFETY MONITORING PLAN

DFCI IRB Protocol #: 12-497

APPENDIX C

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

TABLE OF CONTENTS

1. INTRODUCTION

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

1.1 Purpose

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

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

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

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Boston Children's Hospital (BCH), Brigham and Women's Hospital (BWH)) 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, Food and Drug Administration (FDA), Office of Biotechnology Activities (OBA) etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

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

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

Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research Organization (CRO), etc) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as

specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines). In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol. Should the DF/HCC Sponsor decide to use a CRO, the CRO will be deemed the Coordinating Center.

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

2. GENERAL ROLES AND RESPONSIBILITIES

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

2.1 DF/HCC Sponsor

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

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

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

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

2.3 Participating Institution

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

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

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.

- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

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

3.1 Protocol Distribution

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

3.2 Protocol Revisions and Closures

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

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

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

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

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC

Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

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

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

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

3.5 IRB Re-Approval

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

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

3.6 Participant Confidentiality and Authorization Statement

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

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an authorization statement. This authorization statement may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB, will provide a consent template, with information regarding authorization for the disclosure of protected health information.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be

collected. DF/HCC has chosen to use authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

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

3.7 DF/HCC Multi-Center Protocol Registration Policy

Please refer to Section 4.4 for participant registration information.

3.7.1 Participant Registration and Randomization

Please refer to Section 4.4 for participant registration information. Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

3.7.2 Initiation of Therapy

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

3.7.3 Eligibility Exceptions

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

3.8 DF/HCC Protocol Case Number

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

3.8.1 Protocol Deviations, Exceptions and Violations

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

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe departures from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.8.2 Definitions

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

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

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

3.8.3 Reporting Procedures

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

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

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

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines. DF/HCC will forward all violation reports to CTEP via an internal DF/HCC process, as applicable.

3.9 Safety Assessments and Toxicity Monitoring

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

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

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

3.9.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol Section 7.

Participating Institutions must report the SAEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB Adverse Event Reporting Policy.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures

3.9.2 Guidelines for Processing IND Safety Reports

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

3.10 Data Management

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

3.11 Data Forms Review

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

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC ODQ Data Analyst, Coordinating Center or designee. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

Missing Forms

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

4. REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol Section 8.

5. MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the ODQ provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions may be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring will occur during protocol performance and through study completion. 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 files, protocol departures, pharmacy records, response assessments, and data management.

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

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants' complete medical record and source

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

5.2 Monitoring Reports

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

5.3 Accrual Monitoring

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

A minimum of 3 participants per site annually is typically recommended for Phase II protocols. However, given the rarity of inflammatory breast cancer, this protocol will have an accrual expectation of 0-1 accruals annually per site.

6. AUDITING: QUALITY ASSURANCE

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

6.1 Audit Plan: DF/HCC Sponsored Trials

The DF/HCC Sponsor will trigger an audit if significant issues with non-compliance are noted at participating sites. This study will be monitored as required by the federal regulations. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Audit Notification

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

6.3 Audit Reports

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

6.4 Participating Institution Performance

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

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation. A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.

APPENDIX D 12-497 DFCI SPECIMEN REQUISITION FORM

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: DF/HCC Core Blood and Tissue Bank, DFCI, Smith Bldg- SM 956, 450 Brookline Ave, Boston, MA 02215

Specimen Information

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

Date specimen(s) shipped: _____ Time Point: ☐ Pre-treatment (week 1) ☐ Week 2 ☐ Surgery

Site of tumor: ☐ Right breast ☐ Left breast

Specimen Type *(indicate inclusion in shipment by checking box)*	**Pathology Number(s) or Serial Coding**	**Quantity submitted**	**Date specimen obtained**	**Time from resection to fixative immersion**
☐ One 10 ml Whole Blood Lavender (EDTA) Top Tube				
☐ Two 10 ml Streck Tubes				
☐ Core biopsies in OCT				Minutes
☐ Other, specify:				

Responsible contact: _____

Email: _____

Phone Number: _____

APPENDIX E 12-497 MICHIGAN SPECIMEN REQUISITION FORM

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: Dr ZhiFen Wu, c/o Merajver Lab, 7121 CC, 1500 East Medical Center Drive, Ann Arbor, MI 48109

Specimen Information

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

Date specimen(s) shipped: _____ Time Point: ☐ Pre-treatment (week 1) ☐ Week 2 ☐ Surgery ☐ Post Mastectomy

Site of tumor: ☐ Right breast ☐ Left breast

Specimen Type *(indicate inclusion in shipment by checking box)*	**Pathology Number(s) or Serial Coding**	**Quantity submitted**	**Date specimen obtained**	**Time from resection to fixative immersion**
☐ Core biopsies in liquid nitrogen				Minutes
☐ Other, specify:				

Responsible contact: _____

Email: _____

Phone Number: _____