TITLE:	**Adjuvant paclitaxel and trastuzumab for node-negative HER2- positive breast cancer**
STUDY DRUGS:	TRASTUZUMAB (HERCEPTIN®) PACLITAXEL (TAXOL®)
COORDINATING CENTER:	**Dana Farber Cancer Institute**
PRINCIPAL INVESTIGATORS:	SARA TOLANEY, MD, MPH [redacted]
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PROTOCOL VERSION/DATE:	November 18, 2021

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1.0 SCHEMA

HER2+ Breast Cancer:
Tumor ≤3cm
Lymph node negative* → Surgery** → Registration → Paclitaxel qwk + Trastuzumab qwk x 12 → Trastuzumab qwk or q3wks x 40 wks†
HER2-positive***

*Patients with a micrometastasis are eligible

**Surgery with either lumpectomy or mastectomy, as clinically indicated, with sentinel lymph node biopsy or axillary node dissection.

*** HER2 3+ by IHC or FISH ≥2

†Trastuzumab may be given 6 mg/kg every 3 weeks or 2 mg/kg weekly for 40 weeks after completion of paclitaxel
(Total of 13 doses if given every 3 weeks)

Adjuvant endocrine therapy as per institutional standard. Tamoxifen should not be given during treatment with paclitaxel

2.0 OBJECTIVES

2.1 Primary Objective

2.1.1 Evaluate disease free survival (DFS) in patients with node-negative HER2-positive breast cancer with tumors ≤ 3cm treated with adjuvant trastuzumab and paclitaxel

2.2 Secondary Objectives

2.2.1 Describe DFS in patient groups defined by tumor size (≤1 cm or > 1 cm) and hormone receptor status

2.2.2 Evaluate the incidence of grade III/IV cardiac left ventricular dysfunction from adjuvant trastuzumab and paclitaxel

2.2.3 Evaluate the incidence of grade III/IV neurotoxicity associated with adjuvant paclitaxel

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2.2.5 Investigate the percentage of patients with amenorrhea at various times after start of treatment in premenopausal women treatment with paclitaxel and trastuzumab for early stage breast cancer

2.2.6 Explore predictors of amenorrhea that persist to the time period 6 to 12 months after the start of treatment with paclitaxel and trastuzumab for early stage breast cancer.

3.0 BACKGROUND

3.1 Rationale

Five randomized trials have established trastuzumab-based therapy as standard of care for patients with HER2-positive, early stage breast cancer. Despite these compelling results, there remains little data on outcomes for patients with lower-risk, stage1 breast cancers which are HER2-positive. Most of the clinical trials excluded patients with small lymph node negative, while others accrued few patients with node-negative disease (see Table below).

Trial	Eligibility Requirements
NSABP B-31	Node-positive disease
N-9831	Node-positive disease OR High-risk node-negative disease: tumor ≥2 cm and ER- or PR-positive, or tumor ≥1cm and ER- and PR-negative
HERA	Node-positive disease OR Node-negative disease with tumor ≥1cm
BCIRG 006	Node-positive disease OR Node-negative disease AND one of the following risk factors: tumor >2cm, ER- and PR-negative, grade 2-3, or age <35
FinHER	Node-positive disease OR Node-negative disease with tumor ≥2 cm and PR-negative

To date, none of the reported data for adjuvant trastuzumab has reported outcomes for node-negative patients in direct, absolute terms, though subset analyses suggest similar relative risk reduction for these tumors (approximately 50%) as seen in the overall study population. Because the absolute benefit of trastuzumab for low-risk HER2-positive tumors remains unknown, the role of trastuzumab in patients with small, node-negative tumors remains controversial. Current consensus guidelines from the NCCN do not recommend adjuvant trastuzumab for tumors <1 cm in size, owing to the lack of data for safety and efficacy in this patient population (see www.nccn.org). Based on historical experience, it is reasonable to expect that the absolute benefit of chemotherapy and trastuzumab is more modest in node-negative breast cancer, as these tumors have in general a better prognosis and thus lower gains with adjuvant therapy. Thus, the decisions to use adjuvant chemotherapy with agents such as anthracyclines and taxanes, and the use of adjuvant trastuzumab, may be informed by different considerations of risk and benefit for this lower risk group than for higher risk early stage breast cancer. In clinical practice, oncologists have taken many different approaches in this group of women. While some have held back on treatment entirely, others have given chemotherapy alone, adriamycin and cytoxan (AC) followed by trastuzumab (H), AC followed by paclitaxel and trastuzumab (AC-TH), docetaxel, carboplatin, and trastuzumab (T_2CH), or paclitaxel and trastuzumab (TH).

This trial is designed to characterize the clinical course of women with small, node negative breast cancer who are treated with a uniform trastuzumab-containing regimen. The inclusion criteria specifically target women who were generally not eligible for

previous trials with HER2-directed therapy. Ideally, a randomized controlled trial would be conducted in this population to compare the utility of treatment with trastuzumab in combination with paclitaxel compared to paclitaxel alone, but such a trial would require many thousands of patients, and it is not believed feasible to enroll a sufficient number of patients in a reasonable time period in order to accomplish such a trial. For that matter, based on discussions with oncologists and breast cancer advocates, it seems unlikely that physicians and patients would be comfortable with the randomization.

Most adjuvant trastuzumab trials administered trastuzumab for 52 weeks of treatment. The small but provocative FinHER trial included only 232 patients, but administered only 9 weeks of trastuzumab paired with taxane therapy. This study demonstrated relative risk reduction of >50% for the use of trastuzumab, suggesting indirectly that concurrent taxane-trastuzumab therapy can be effective treatment for early stage breast cancer and is superior to taxane-anthracycline chemotherapy given in the absence of trastuzumab. Preliminary analyses of NCCTG N9831 suggest that concurrent use of taxane-trastuzumab therapy is superior to sequential use, further justifying a combination treatment program.

The most worrisome toxicity of adjuvant trastuzumab is congestive heart failure and cardiomyopathy, which arises in 2-4% of patients receiving adjuvant trastuzumab and anthracyclines (see Table below). Limited clinical experience suggests a substantially lower risk of cardiomyopathy (on the order of 1%) for patients receiving non-anthracycline chemotherapy and trastuzumab. In an attempt to potentially reduce toxicity, while maintaining efficacy, weekly paclitaxel therapy was selected as the agent of choice to be administered with trastuzumab therapy. Paclitaxel has been shown to be efficacious in standard combination chemotherapy regimens in the neoadjuvant and metastatic settings. While anthracyclines have been shown to be effective in the treatment of HER2-positive breast cancer, we believe that the potential cardiac toxicity that may result from administration of both an anthracycline and trastuzumab would outweigh the benefits in this lower-risk population.

Trial	Class III or IV CHF
NSABP B-31	
AC→T	0.8%
AC→TH	4.1%
N-9831	
AC→T	0.3%
AC→TH	2.5%
HERA	
Chemotherapy	0.06%
Chemotherapy + Trastuzumab x 1 year	1.7%
BCIRG 006	
AC→T	0.29%
AC→TH	1.59%
TCH	0.38%
FinHER	
Docetaxel or Vinorelbine + FEC	2.59%
Docetaxel or Vinorelbine + FEC + Trastuzumab	0.27%
AC, adriamycin/cytoxan; TH, paclitaxel/trastuzumab; TCH, docetaxel/carboplatin/trastuzumab	

For these reasons, we have developed an exploratory study to evaluate the safety and efficacy of paclitaxel and trastuzumab therapy as adjuvant treatment for patients with small, node-negative HER2-positive breast cancer.

3.2 HER2 Overexpression and Breast Cancer

Overexpression of HER2 is observed in 20-25% of all breast cancers.[1] HER2-positive breast cancers are associated with earlier recurrence and shorter overall survival and are associated with other adverse prognostic markers, such as high tumor grade, high rates of cell proliferation, increased nodal metastases, and relative resistance to certain types of chemotherapy.[2,3] Based on retrospective studies, anthracycline-based chemotherapy is particularly beneficial in HER2-positive tumors.[4] In addition, recent evidence suggests that paclitaxel may also be a particularly active agent in the adjuvant treatment of HER2 positive disease.[5] While about half of HER2-positive breast cancers express hormone receptors for estrogen and/or progesterone, they may be relatively resistant to tamoxifen.[6]

Trastuzumab is a recombinant humanized monoclonal antibody that targets the extracellular domain of HER2. While trastuzumab has been shown to improve outcomes in women with node-positive HER2-positive breast cancer, its mechanism of action is not clear. It is thought that trastuzumab binds to the HER2 protein, resulting in apoptosis, down-regulation of surface HER2 expression, and alteration of downstream signaling.

3.3 Testing for HER2 Overexpression

Initial clinical trials were performed using immunohistochemistry (IHC) to determine which patients had HER2-positive breast cancer. This test measures HER2 protein levels on the cell surface, to determine HER2-positivity in breast cancer specimens. IHC scoring is performed by a pathologist on a subjective basis. HER2 immunostaining is graded as 0, 1+, 2+, or 3+ based on the percentage of malignant cells stained and the degree of membrane staining present in these malignant cells.[7] Two types of IHC tests are approved by the FDA: HerceptTest (Dako, Carpinteria, CA) and PATHWAY (Ventana Medical Systems, Inc, Tucson, AZ).

More recently, fluorescence in situ hybridization (FISH) has been developed to identify patients with HER2-positive tumors. This test directly measures HER2 gene amplification. Three types of FISH tests are approved by the FDA: PathyVysion (Vysis Inc, Downers Grove, IL; ≥2.0 ratio HER2:CEP17 considered amplified), INFORM (Ventana Medical Systems Inc; ; ≥5.0 gene copies of HER2 considered amplified), and HER2 FISH pharmDx (Dako; ≥2.0 ratio HER2:CEN-17 considered amplified).

A strongly HER2-positive tumor is associated with the most clinical benefit from trastuzumab. Subgroup analysis in a large phase III trial showed that patients with IHC 3+ tumors experienced significant improvement in all clinical outcomes with trastuzumab therapy, however, the benefit in patients with IHC 2+ tumors was minimal.[8] Because patients with false-positive HER2 results may receive little benefit from trastuzumab, excluding them from clinical trials will better help evaluate the true effect of trastuzumab therapy. When local and central evaluation used the same methodology, concordance was 88.1% for FISH and 81.6% for IHC.[7] Furthermore, the IHC 2+ category is the most likely to be discordant with FISH HER2 status. In comparing FISH with HercepTest in central laboratories, agreement rates between FISH and IHC were 97% for IHC 0, 93% for IHC 1+, 24% for IHC 2+, and 89% for IHC3+.[9]

3.4 Trastuzumab in the Adjuvant Setting

Five important trials validate the use of trastuzumab in the adjuvant treatment of breast cancer.

The NSABP B-31 and N-9831 trials compared adjuvant chemotherapy with or without concurrent trastuzumab in women with surgically resected HER2-positive breast cancer.[10] Both trials enrolled patients with node-positive disease. N-9831 also enrolled patients with high-risk node-negative disease, defined as ER- or PR-positive with tumor size >2cm or ER- and PR-negative with tumor size >1cm. Node-negative patients represented 0.1% of all patients on this study. Due to similar eligibility and treatment protocols, the decision was made to perform a combined analysis. The NSABP B-31 compared doxorubicin 60 mg/m^2, cyclophosphamide 600 mg/m^2 every 3 weeks for 4 cycles followed by paclitaxel 175 mg/m^2 every 3 weeks for 4 cycles with the same regimen plus 52 weeks of weekly trastuzumab (loading dose 4 mg/m^2 followed by 2 mg/m^2 thereafter). The N-9831 compared 3 arms: doxorubin and cyclophosphamide every 3 weeks for 4 cycles followed by weekly paclitaxel 80 mg/m2 for 12 cycles, the same regimen followed by 52 weeks of

trastuzumab, and the same regimen plus 52 weeks of trastuzumab initiated concomitantly with paclitaxel. Because trastuzumab was not given concurrently in the second arm, this arm was not included in the overall analysis. Overall, there were 1,736 patients in the B-31 trial and 1,615 patients in N-9831, and at the time of the combined analysis, the median follow-up was 2.0 years. Compared with the control arm, the trastuzumab arm showed a statistically signficant increase in 3-year disease-free survival (DFS), 75.4% vs 87.1% respectively, $p<0.0001$; and a significant increase in overall survival (OS), 91.7% vs 94.3%. The cumulative incidence of class III or IV heart failure was 0.8% in the control group and 4.1% in the trastuzumab group.

The HERA (Herceptin Adjuvant) trial focused on determining the optimal duration of trastuzumab for early-stage breast cancer.[11] Patients were randomized to one of three arms: observation, trastuzumab every 3 weeks for 1 year, or trastuzumab every 3 weeks for 2 years. Trastuzumab was dosed with 8 mg/kg IV followed by 6 mg/kg IV every 3 weeks thereafter. Patients had either node-negative disease if tumor size >1cm (32.1%) or node-positive disease, and all patients had HER2-positive tumors. At a median follow-up of 2 years, the addition of trastuzumab to neoadjuvant and adjuvant chemotherapy resulted in a statistically signficant improvement in DFS (86.1% vs 78%) and overall survival (96.9% vs 93.6%). Further analysis is expected to determine if 2 years is superior to a year of treatment.

The BCIRG 006 trial aimed at maximizing the efficacy of trastuzumab while minimizing cardiac toxicity.[12] The trial enrolled 3,222 patients with node-positive or high-risk lymph node-negative HER2-positive tumors to 1 of 3 arms: doxorubicin-cyclophosphamide every 3 weeks for 4 cycles followed by docetaxel every 3 weeks for 4 cycles (ACT); or the same regimen plus 52 weeks of trastuzumab,weekly during chemotherapy then every 3 weeks during follow-up (ACTH); or docetaxel-carboplatin every 3 weeks for 6 cycles plus 52 weeks of trastuzumab with the same schedule as given in arm 2 (TCH). At a 36 month follow-up, presented at the 2006 San Antonio Breast Conference, the 2 trastuzumab arms showed a statistically significant improvement in DFS compared with the control arm. Symptomatic cardiac events and an LVEF decline >15% were statistically significantly greater in the ACTH group compared with the ACT. There was no statistically significant difference between the ACT and TCH groups in terms of cardiac side-effects. This trial shows that fewer cardiac events are observed when trastuzumab is administered without prior anthracycline-based therapy. There was no statistically significant difference in terms of disease free survival across the two trastuzumab-containing arms. A recent trial in the metastatic setting revealed that carboplatin does not improve response rates, time to progression, or overall response rate when added to docetaxel and trastuzumab.[13] Indirectly, this study suggests that a taxane-trastuzumab adjuvant regimen may be almost as effective as a regimen that contains both an anthracycline and a taxane and is almost certainly associated with less cardiotoxicity. In patients at lower risk of recurrence, toxicity associated with adjuvant therapy becomes a major concern.

The FinHer trial involved 1,010 patients randomized to docetaxel every 3 weeks for three doses versus 9 weeks of vinorelbine followed, in both groups, by three weeks of cyclophosphamide, epirubicin, and fluoruracil (CEF).[14] The 232 patients found to be HER2 positive were randomized to receive weekly trastuzumab for 9 weeks with docetaxel

or vinorelbine. This trial enrolled women with axillary node-positive disease or women with node-negative breast cancer with tumors >2cm and progesterone-receptor negative. After a median follow-up of 3 years, they found that recurrence was less frequent amongst women receiving docetaxel/CEF with 42/502 recurrences compared with 71/507 recurrences in the vinorelbine/CEF arm at 3 years. They also found that trastuzumab for 9 weeks was effective in preventing breast cancer recurrence, with 12/115 events in the trastuzumab arm compared with 27/116 events in the arm without trastuzumab (p= 0.01).

In November 2006 the FDA approved trastuzumab for use as part of a treatment regimen containing doxrubicin, cyclophosphamide and paclitaxel for the treatment of patients with HER2 overexpressing, node positive breast cancer.

3.5 Node-negative HER2-positive patients

There have been a few studies done to look at the prognosis of node-negative breast cancers. Andrulis et al found that a patients with a HER2-positive tumor had a two-fold increase in the risk of recurrence compared to those with HER2-negative tumors.[15] The 3-year DFS was 80% for those with HER2-positive tumors, and 90% for HER2-negative tumors. Press et al also found a two-fold increase risk of recurrence for HER-positive tumors relative to HER-negative tumors.[16] The 4-year DFS was approximately 90% for HER-2 negative tumors, and 70% for HER-2 positive tumors. They also found that patients with breast cancers ≤1cm in diameter who had evidence of HER2-amplification had a 5.7 times higher risk of recurrence compared to HER2-negative patients with small tumors. A recent retrospective study reviewed records of 164 patients with node-negative HER2-positive breast cancer, and found a 19% risk of recurrence in T1a tumors, 15% in T1c, and 23% in T2 tumors at 5 years. This reveals that small node-negative HER2-positive breast cancer have a moderate risk of locoregional and distant recurrence.[17]

3.6 Interactions between HER2 expression and chemotherapy

Among node-negative patients, HER2-overexpressing tumors have been found to be relatively refractory to benefit from CMF (cyclophosphamide, methotrexate, fluorouracil)-based chemotherapy, and studies suggest that dose-intensification with an anthracycline-containing chemotherapy such as CAF (cyclophosphamide, adriamycin, and fluorouracil) may be beneficial to patients with HER2-positive, lymph-node positive early breast cancers.[18]

The combination of paclitaxel and trastuzumab has shown good efficacy, and favorable toxicity in the metastatic setting. In a phase II study in women with metastatic breast cancer, weekly paclitaxel and trastuzumab resulted in a 67-81% response rate in HER2-positive patients, with a 6% incidence of grade 3/4 neutropenia.[19] Similarly, docetaxel and trastuzumab have had high response rates and favorable toxicity in metastatic HER2-positive patients.[20,21]

3.7 Paclitaxel as a Single-Agent in Adjuvant Therapy of Breast Cancer

Paclitaxel has been compared to FAC (5-FU, doxorubicin, cyclophosphamide) in the neoadjuvant setting.[22] Patients with T1-3, N0-1 disease were randomized to receive either paclitaxel (250 mg/m2) as a 24-hour infusion or FAC every 3 weeks. Each patient received 4 cycles of preoperative therapy, and the extent of residual disease at the time of surgery was similar between the two arms.

A recent trial compared nonanthracycline adjuvant docetaxel and cyclophosphamide (TC) to doxorubicine and cyclophosphamide (AC) as adjuvant chemotherapy for women with operable breast cancer.[23] In this study, 1016 patients were randomized to AC or TC given every 3 weeks for 4 cycles. At a median follow-up of 66 months, patients treated with TC had significantly improved disease-free survival compared with AC-treated patients (86% vs 80%, p=0.015) and a favorable overall survival (90% vs 87%, =0.13). There was significantly more grade 3 or 4 febrile neutropenia in the TC arm and more grade 3 or 4 nausea and vomiting in the AC arm. This study adds to the evidence that taxanes should be a part of adjuvant therapy, and suggests that omission of anthracycline therapy is possible.

When studied in the metastatic setting, paclitaxel resulted in similar response rates to patients receiving CMFP (cyclophosphamide, methotrexate, 5-FU, and prednisone).[24] In addition, many aspects of quality of life were better in patients receiving paclitaxel compared to CMFP. Paclitaxel resulted in significantly less bone marrow suppression, documented infection, mucositis, and nausea and vomiting. Alopecia, peripheral neuropathy, and myalgias and arthralgias were greater with paclitaxel. Overall quality of life appeared better for patients receiving paclitaxel.

While it is difficult to extrapolate this information to the adjuvant setting, it appears that single agent paclitaxel is at least equivalent to FAC in the neoadjuvant setting, and to CMFP in the metastatic setting. Also, CMF has been shown to be equivalent to AC as an adjuvant therapy in NSABP B-15, suggesting that paclitaxel will likely be equivalent to AC in the adjuvant setting. There is an ongoing study, CALGB 40101 which is comparing 4-6 cycles of AC to 4-6 cycles of paclitaxel, and this will help further compare paclitaxel to an anthracycline-based regimen.

Based on the little that is known about the failure rate of the patients eligible for this study, we want a large chance of stopping accrual to the study early if the true 3 year failure percent is as large as 14%. We want a small chance of declaring the regimen worthy of further research if the true 3 year failure percent is around 9%, and want a large chance of declaring the regimen worthy of further research if the true 3 year failure percent is as small as 5%.

3.8 Scheduling of Paclitaxel

The optimal schedule for paclitaxel was studied in metastatic breast cancer in CALGB 9840. Patients were randomized to paclitaxel 80 mg/m2 over 1 hour weekly or a standard 3-hour infusion of 175 mg/m2 every 3 weeks. Patients with HER2 overexpression received trastuzumab. Women in the weekly paclitaxel arm showed a significantly increased TTP (9 vs. 5 months) and a trend toward increased OS (24 vs. 16 months, p=0.17.[25] Weekly paclitaxel caused less neutropenia (5% vs. 15%) but worsened neurosensory toxicity (23% vs. 12%).

In the adjuvant setting, E1199 compared adriamcyin and cyclophosphamide (AC) followed by paclitaxel or docetaxel, given either weekly, or every 3 weeks.[26] Disease-free survival (DFS) was not significantly different between docetaxel and paclitaxel or weekly and every 3 weekly therapy. However there was an important and unanticipated interaction between the taxane and dose. DFS was significantly improved in the weekly paclitaxel and every 3 week docetaxel arms compared with the every 3 week paclitaxel arm.

3.9 Amenorrhea During and After Treatment with Paclitaxel and Trastuzumab

Many premenopausal women undergoing adjuvant chemotherapy for breast cancer are concerned about the risk of ovarian damage due to the treatment. Standard adjuvant breast cancer chemotherapy regimens are associated with chemotherapy-related amenorrhea (CRA) as well as with a risk of infertility. Menses may cease temporarily or permanently during or shortly after chemotherapy. Women may also continue menstruating normally (or resume menses after a period of amenorrhea) only to experience premature ovarian failure later on. The risk of CRA with paclitaxel-trastuzumab therapy is unknown.

Risk of CRA is known to increase with older age and when greater cumulative dose of alkylating agents are used.[27] In the International Breast Cancer Study Group (IBCSG) Trial V, 31% of the 188 premenopausal women with node-positive disease who received only one cycle of perioperative cyclophosphamide-methotrexate-fluorouracil (CMF) reported at least three months of amenorrhea within the nine months after surgery, compared with 68% of the 387 similar patients who received 6-7 cycles of CMF.[28] A recent retrospective evaluation of long-term follow-up data from IBCSG Trials V and VI (which also administered variable numbers of CMF cycles) showed that the 227 women who remained premenopausal after 6 cycles of adjuvant CMF had high probabilities of menopause at five years after start of CMF, even in younger age cohorts.[29,30] In IBCSG V or VI, a woman who was 30 years old at time of diagnosis with continued menstruation after six cycles of CMF had a 37% risk of menopause at age 35 and an 84% risk at age 40. Thus, alkylating agents can induce both immediate amenorrhea and eventual premature ovarian failure. However, the occurrence of not immediate, but nevertheless premature menopause following adjuvant chemotherapy is not well studied, as most studies of amenorrhea have focused on earlier endpoints.

For example, a prospective study of 25-40 year old women with breast cancer undergoing either doxorubicin-cyclophosphamide (AC, 120 pts), doxorubicin-cyclophosphamide-

paclitaxel (ACT, 168 pts), CMF (83 pts), 5-FU-doxorubicin-cyclophosphamide (FAC, 38 pts), doxorubicin-cyclophosphamide-docetaxel (ACD, 19 pts), or another regimen (58 pts) found that menstrual cycles were more likely to persist after the regimens that contained a lower cumulative dose of cyclophosphamide (AC, ACT, or ACD rather than FAC or CMF).[31] While women who were on CMF were more likely than those on AC, ACT, or ACD to bleed during the one month following chemotherapy (approx 50% vs. 20%, odds ratio 2.9, 95% CI 1.7-5), one year later the likelihood of menses was less in the CMF group (OR .37, 95% CI .37-.67).

A meta-analysis of twelve studies confirmed that amenorrhea occurred after CMF chemotherapy in approximately 40% of women younger than 40, and in 76% of women older than 40.[32] Anthracycline-containing regimens including cyclophosphamide-epirubicin-5-FU (CEF) may be even more gonadotoxic than CMF. In one study, premenopausal women who received CEF had a 51% risk of amenorrhea compared to a 42.6% risk in women who received CMF at the 6 month follow-up (an 8% difference).[33] However, in this study, percents of amenorrhea were slightly closer in the two groups at 12 months (76% in CEF group, 71% in CMF group, a 5% difference), highlighting the importance of longer term follow-up.

Because trastuzumab is a monoclonal antibody against Her2-expressing cells, it does not carry a risk of ovarian damage. However, because paclitaxel is a mitotic inhibitor, it does carry at least a theoretical risk of amenorrhea, and the gonadotoxic effect of taxanes remains uncertain. A small prospective evaluation of 50 premenopausal women revealed hormonal changes in follow-up suggesting that regimens containing a taxane were more gonadotoxic than those that did not.[34] Another retrospective survey of 195 breast cancer patients under age 50 found that it was only among women aged 40 or younger that adding a taxane to AC increased the likelihood of six months of CRA beginning within a year of the start of chemotherapy (40% vs 61%, p=.04 in the $\leq$40 group; 81% vs 84%, p=.35 in the >40 group).[35] In this study, however, many resumed menses in the $\leq$40 group after this period of CRA (33% in the AC alone group, 43% in the AC$\rightarrow$T group). In contrast, a retrospective evaluation of 235 premenopausal women younger than 40 treated with AC followed by a taxane showed only 17% had amenorrhea, which was no higher than historic controls treated with AC alone. [36] Similarly, a retrospective study of 403 patients evaluated after treatment with AC or AC followed by paclitaxel every 14 or 21 days revealed no significant increase in amenorrhea at six months or later with the addition of paclitaxel (OR 1.45, 95% CI .78-2.69), when controlling for age and other variables.[37]

Notably, none of these trials have evaluated percents of amenorrhea using paclitaxel without an anthracycline. Study 07-199 will investigate the incidence of amenorrhea during and after therapy with paclitaxel-trastuzumab. It is important that data on menstrual history is collected before start of treatment in order to establish a baseline, then again at the completion of chemotherapy to investigate how regular menses were during exposure to these agents, and then at each follow-up visit (every six months) in order to assess for duration of amenorrhea and also premature ovarian failure. This information will be valuable to physicians and premenopausal patients making decisions regarding adjuvant therapy, as well as in their follow-up care and concerns.

3.9.1 Research Design and Methods

In premenopausal patients (those with at least one menstrual period in the 6 months prior to registration) who are registered on 07-199 and who agree to take part in this sub-study on amenorrhea, this will be a prospective assessment of menstrual functioning and percents of amenorrhea over time in premenopausal women enrolled in 07-199. A patient who was registered on 07-199 before the activation of the amenorrhea amendment will be assessed for eligibility at the time of her first study visit after the amendment is activated, and if she is deemed to have been premenopausal at the time of study registration and agrees to participate in this sub-study, she will be asked to fill out the menstrual forms for past scheduled assessments retrospectively and the menstrual forms for future scheduled assessments prospectively. Those who agree to participate in the sub-study (whether in a retrospective or prospective manner) will fill out a questionnaire regarding baseline menstrual status and date of last menstrual period at enrollment onto the trial (using the Baseline Menstrual Status Form, Appendix 1). Prospective follow-up assessments will occur as close to the following specified timepoints as possible: on the final day of chemotherapy using the 12-week Menstrual Status Form (Appendix 2); at the office visit three months later using the Menstrual Follow-up Form (Appendix 3); and every six months to 4 years past enrollment, and annually at 5 and 6 years past enrollment, using the Menstrual Follow-Up Form (Appendix 3). The surveys will be given to patients in clinic or mailed to them to complete at home. If mailed, patients will both complete and mail back the survey or they will be contacted for collection of the information. These surveys will ask about use of hormonal agents before diagnosis, at baseline, and during follow-up. Completion of the menses assessment survey by the patient is waived at the specified time points if the required information is documented in a physician's note.

Outcomes: The primary endpoint of this sub-study will be to estimate the proportion of women who have menstruated at least once in the period of 6 to 12 months after enrollment on 07-199 (Trastuzumab therapy ends at the 12 month point.) Although some women may lose or recover menses soon after this time point, the one-year mark should allow time for most women who will resume ovarian cycling to do so. The secondary endpoints will be to estimate the proportion of women who report at least one menstrual period over the six month period that encompasses the 12 weeks of chemotherapy (so including 3 months of menstrual history before starting 07-199), and the proportion of women who report at least one period in the prior six months at each of the follow-up points (every six months to 4 years past enrollment, and annually at 5 and 6 years past enrollment). An exploratory secondary endpoint will be to examine possible predictors of menstruating at least once in the period 6 to 12 months after enrollment on 07-199.

3.10 Correlative Studies Background

Identification of molecular markers that predict clinical outcome after trastuzumab based therapy will allow more optimal tailoring of therapy to a particular individual's cancer. In addition, such markers may provide insight into the mechanisms of resistance to trastuzumab based therapy as well as provide potential approaches to overcoming this resistance.

3.10.1 Molecular Alterations of Small HER2+ cancer

Determining molecular alterations within cancer can help provide predictive and prognostic information. Since there is currently no standard of care treatment for early stage HER2-positive breast cancer, developing a better understanding of this subtype may provide deeper insights into therapeutic approaches for this disease.

We propose performing sequencing on all archival tumor which yields adequate DNA from to better characterize molecular alterations within this subset of HER2-positive breast cancer. We will also obtain tumor samples from biopsies performed at the time of relapse and perform next generation sequencing on these samples as well.

We anticipate that we will be able to get adequate DNA from approximately 50% of samples (n=200). All slides will initially be reviewed to localize invasive cancer. These areas will be extracted for DNA by the Center for Molecular Oncologic Pathology at Dana-Farber Cancer Institute. Nikhil Wagle at the Broad Institute will be responsible for performing generation sequencing, and for the analysis.

We will analyze all baseline samples paired with the recurrence samples for sequencing, assuming adequate DNA can be obtained from samples. We will explore results to see if there are particular baseline molecular alterations that predict recurrence, and will explore how frequent of an alteration this is within this molecular subtype. Additionally we will explore what alterations occur at time of recurrence to help define what escape mechanism may be responsible for development of recurrent disease. Defining these molecular alterations may help us develop better therapies for this particular subgroup of breast cancer.

3.10.2 Predictors of Paclitaxel-induced neuropathy

It is possible that inherited variation in the activity of drug-metabolizing enzymes can result in differences in the pharmacokinetics of paclitaxel between different patients. Thus, some patients may be susceptible to a paclitaxel-induced neuropathy. The purpose of this investigation is to correlate genetic polymorphisms in enzymes involved in paclitaxel metabolism and elimination with paclitaxel-induced neuropathy. We plan to compare the genetic profiles of patients with paclitaxel-induced neuropathy to patients who do not manifest this side effect. The drug metabolizing enzymes that will be evaluated include the MDR1, CYP2C8, CYP3A4, CYP3A5, NR1I2.

Allele	Frequency
CYP2C8*3	0.130
CYP2C9*2	0.125
CYP2C9*3	0.085
CYP3A4*1B	0.100
CYP3A5*3	0.900
MDR1 $C_{3435}T$	0.539
PXR/NR1I2	0.620

Paclitaxel is a novel chemotherapy agent derived from the bark of the yew tree (Taxus Brevifolia) that promotes microtubule assembly and stabilizes tubulin formation.[58] Paclitaxel inhibits proliferation by inducing mitotic block. However, this drug may have many other effects that have not been entirely elucidated. Paclitaxel is metabolized to a minor metabolite by CYP3A4 and to a major but inactive metabolite 6-α-hydroxytaxol by CYP2C8. Recently a polymorphism in CYP2C8 has been discovered and characterized. In vitro studies have shown the polymorphic CYP2C8 has diminished catalytic activity for converting paclitaxel into 6-α-hydroxytaxol.[59] The effects of this polymorphism on paclitaxel's metabolism in vivo have not been determined and it is therefore a goal of this study to examine its correlation with increased paclitaxel's toxicity.

Methods:
DNA extraction and SNP analysis: The goal of this study is to identify SNPs that are associated with paclitaxel-induced neuropathy. SNPs can be isolated from DNA from multiple tissue sources including whole blood, buccal swabs and formalin-fixed, paraffin-embedded tissue.[60-62] DNA will be extracted from whole blood as described by previously using the DNeasy® Tissue kit. DNA samples will be assayed for genetic polymorphisms as described previously using the Applied Biosystems' Taqman® Allelic Discrimination Assay (Foster City, CA) according to the manufacturer's instructions. Briefly, 10 ng DNA will be added to a 25 ul reaction containing forward and reverse primers along with 2 allele specific labeled probes (one wild-type and one variant allele specific). The PCR and fluorescence measurements will be performed using the ABI Prism 770 sequence detection system.

We propose using the ABI Taqman assays for our SNP analysis because it has proven to be a robust and high-throughput approach. However, development of alternative assays will also be considered based on the anticipated need for future genotyping. If we encounter any SNPs that we cannot be reliably assayed from whole genome amplified DNA, we will use our expertise in other assays to design assays using alternative methods. We currently run multiple different SNP assays for several other types of assays such as TaqMan OpenArray Genotyping System, restriction fragment length polymorphisms (RFLP),

SNPstream, and Illumina platforms. Baseline information will be collected to assess if patients have a known diagnosis of diabetes, an important risk factor for the development of neuropathy.

3.11 Rationale for banking tissue and serum

Banking of blood samples at the time of enrollment, every 6 months for two years, and upon disease recurrence will allow for future analyses that may identify predictors of recurrence.

3.11.1 Tissue and Serum Bank Summary

Banking of tissue and serum will take place during this course of this study at the protocol specified timepoints. Specimens will be banked for use in accordance with the research as outlined in this protocol.

A tissue block or 15 uncharged slides will be stored and will become property of the DF/HCC SPORE Tissue and Pathology Core. In addition, a de-identified copy of the associated pathology report will accompany the tissue specimen.

Serum will be collected and analyzed for the biomarkers as outlined in the protocol. Any remaining serum will be stored in the DF/HCC Clinical Trials Core Laboratory until the supply has been exhausted. Only DF/HCC researchers as deemed appropriate by the PI and DF/HCC SPORE CORE Committee will have access to these specimens.

Confidentiality of each patient will be maintained to the best of the study team's ability. All study participants will be assigned a unique subject number at study registration. Once the specimen has been received at either the SPORE Tissue and Pathology Core or the DF/HCC Clinical Trials Core Laboratory, the specimens will be assigned a separate specimen ID number that will be tracked by the researchers associated at the specified laboratory. Patient-derived material will be linked to patient clinical information using identifiers which will be coded using DFCI hospital number and internal banking identifying code numbers. Any material shared with collaborators, in or outside of DFCI, will be supplied with code number identifiers only, and without patient name or other patient identifiers. All study information will be stored in locked file cabinets and in password-protected computer files. Only authorized study personnel will have access to these files.

Any remaining specimens after these analyses are completed will be banked until the specimen has been fully exhausted. Any research done on any remaining tissue or serum will be vetted by the PI and members of the SPORE CORE Committee in writing. The committee will evaluate the proposed research for its scientific merit and need. Patient information will not be disclosed for any non-protocol related research.

3.11.2 Patient Withdrawal of Consent to Use Research Samples

Study participants retain the right to change their minds at a later date regarding the authorization that they have granted for the storage and use of their biological samples. In order to withdraw consent for the storage and use of their translational research samples, study participants must submit a request, in writing, to the Principal Investigator:

[redacted]

The request must indicate whether it is the participant's desire to temporarily or permanently halt use of their samples, and whether they wish to have the biological samples extant in the repository destroyed. They will have the option of allowing their samples to remain in the repository in an irrevocably anonymized fashion, if they are willing. Their wishes will be honored and such individuals will be notified by registered mail that their request has been fulfilled. A copy of the participants' withdrawal letter will be maintained in their research file at Dana-Farber.

4.0 STUDY DESIGN

4.1 Description of the study

This is a single-arm exploratory study for women with node-negative HER2-positive invasive breast cancer with tumors ≤ 3cm. Patients will undergo appropriate surgical resection (mastectomy or lumpectomy) with sentinel node biopsy or axillary dissection, and will then undergo treatment with paclitaxel 80 mg/m^2 administered weekly for 12 weeks in combination with trastuzumab 4 mg/kg IV loading dose on day 1 followed by 2 mg/kg IV dose weekly, for a total of 12 doses. After completion of 12 weeks of trastuzumab, the dosing of trastuzumab may be changed to 6 mg/kg IV every 3 weeks for 40 weeks (total of 13 doses), or continued at 2 mg/kg dosed weekly for 40 weeks. After completion of 12 weeks of paclitaxel and trastuzumab combination therapy, trastuzumab monotherapy dosing may occur at a non-participating institution. The referring site is responsible for initiating contact with the provider at the non-participating institution and ensuring the provider has all protocol-related instructions regarding administration of trastuzumab per protocol. The participating site is responsible for obtaining and reviewing all source documentation related to trastuzumab monotherapy administration. This information should be placed in the participant's research file. Patients will receive additional adjuvant radiation therapy and/or endocrine therapy according to standard institutional practice. This study also requires submission of tumor blocks or slides in order to perform biomarker analysis. In addition, surveys will be administered to women who are premenopausal at study entry in order to investigate the percentage of patients with amenorrhea that results from the combination of paclitaxel and trastuzumab.

4.2 Demographic and Baseline Assessments

A signed, written, informed consent form must be obtained prior to screening assessments, and before any study-specific assessments are initiated, including obtaining archived tumor tissue for biomarker analysis.

The following will be obtained at screening:

- Demographic data: date of birth, race, gender, height in centimeters, body weight in kilograms, and ECOG Performance Status.
- Medical history including details of malignancy: date of diagnosis, primary tumor type, histology, ER and PR status, HER2 status, stage of cancer, relevant prior surgical procedures, and prior and concomitant medications within 2 weeks of first dose of investigational agents.

4.2.1 Screening Assessments

Assessments prior to registration

- Subject to sign the informed consent form
- Initiate completion of study eligibility form
- Ascertain availability of archived tumor tissue for biomarker analysis
- Documentation of HER2 positivity (IHC 3+ or FISH ≥ 2)

Assessments within 21 days prior to first dose (unless otherwise noted)

- Physical examination
- Medical history, ECOG Performance Status
- Electrocardiogram (12-lead ECG). This test must be performed within 12 weeks prior to first dose of investigational products.
- Vital signs (blood pressure, pulse rate, body temperature, height, and body weight)
- Hematology and blood chemistry
- Serum pregnancy test for women of childbearing potential. Test must be performed within 14 days prior to first dose of investigational products, but preferably as close to the first dose as possible. The results must be reviewed prior to initiating treatment.
- Echocardiogram (ECHO) or MUGA scan. This test must be performed within 12 weeks prior to first dose of investigational products.
- Baseline Assessment of Menses survey (Appendix 1) to women who have had a period in the past 6 months (deemed premenopausal at baseline)

Note: After all baseline and screening evaluations have been completed, and the data is obtained, determine if the subject is eligible for the study by reviewing the inclusion and exclusion criteria.

Assessments for Weeks 2-12

- Hematology (CBC with differential) weekly
- Blood chemistry (Sodium, potassium, chloride, bicarbonate, BUN, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, SGOT(AST), SGPT(ALT)) every 3 weeks (+/- 1 week)
- Review all laboratory results.
- Physical examination to be done every 3 weeks (+/- 1 week)
- Record any non-serious and serious adverse events (AE) and assign appropriate toxicity grade (NCI CTCAE version 3, published March 31, 2003) weekly.
- Echocardiogram (ECHO) or MUGA scan at 12 weeks (even if treatment has been delayed, cardiac evaluation should still occur at 12 weeks)

Assessments every 9 weeks (+/- 3 weeks) during trastuzumab monotherapy

- Hematology (CBC with differential)
- Blood chemistry (Sodium, potassium, chloride, bicarbonate, BUN, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, SGOT(AST), SGPT(ALT))
- Physical examination
- Vital signs (blood pressure, pulse rate, body temperature, and body weight)
- Record any non-serious and serious adverse events (AE) and assign appropriate toxicity grade (NCI CTCAE version 3, published March 31, 2003)

Assessment after 12 weeks, 6 months, and at one year

- Echocardiogram (ECHO) or MUGA scan (+/- 4 weeks)
- Blood sample for translational research at 6 months and 1 year (+/- 8 weeks)
- Follow-up Menses Assessment survey (1 year: Appendix 2) to women deemed premenopausal at baseline (+/- 4 weeks)

Assessment every 6 months for years 2, 3, and 4 or until endpoint reached (as defined by 4.3.1) (+/- 8 weeks)

- Physical Follow-up Menses Assessment (Appendix 2) to women deemed premenopausal at baseline
- Vital signs (blood pressure, pulse rate, body temperature, and body weight)
- Blood sample for translational research at 18 months, 2 years, and at progression
- Record any non-serious and serious adverse events (AE) and assign appropriate toxicity grade (NCI CTCAE version 3, published March 31, 2003)

- Appropriate screening of residual breast tissue performed at least yearly (+/- 3 months)

Assessment annually for years 5, 6, 7, 8, 9, and 10or until endpoint reached (+/- 3 months)

- Physical examination
- Follow-up Menses Assessment survey (Appendix 2) to women deemed premenopausal at baseline (+/- 3 months) only for years 5 and 6
- Vital signs (blood pressure, pulse rate, body temperature, and body weight)
- Blood sample for translational research at progression (+/- 3 months)
- Record any non-serious and serious adverse events (AE) and assign appropriate toxicity grade (NCI CTCAE version 4).
- Appropriate screening of residual breast tissue performed at least yearly (+/- 3 months)
- Patients may be contacted by phone after completion of annual follow-up visits in order to obtain long-term follow-up data.
- All patients will be followed for survival until withdrawal of consent or death, whichever occurs first.

4.3 Outcome Measures

4.3.1 Primary Outcome Measure

The primary end-point of this study is disease-free survival (local invasive breast cancer recurrence, a new local invasive breast cancer, regional recurrence, distant recurrence, contralateral invasive breast cancer, and death before recurrence are all counted as "events that end disease-free survival). The study is designed to have a large chance of declaring the regimen worthy of further research if the true 3 year failure percent is as small as 5% and a small chance of declaring the regimen worthy of further research if the true 3 year failure percent is as large as 9.2%.

4.4 Outcome Assessment Schedule

Assessment prior to the first dose

- Documentation of tumor status: dimension of tumor, HER2 status (IHC 3+ or FISH ≥ 2), lymph node evaluation (sentinel node or axillary dissection)
- Bone Scan only if clinically indicated
- Blood samples for translational research

Assessment at 6 months

- Blood sample for translational research

Assessment at 12 months

- Blood sample for translational research

Assessment at 18 months

- Blood sample for translational research

Assessment at 24 months

- Blood sample for translational research

Assessment at disease recurrence

- Blood sample for translational research
- Biopsy of metastatic disease for ER, PR, HER2 and optional tissue collection for translational research

4.5 Biomarkers

4.5.1 Tumor Tissue

Paraffin-embedded tissue blocks (or 15 sections of paraffin-embedded tissue on uncharged slides) from archived tumor tissue samples (from time of biopsy or surgical resection) will be tested using gene sequencing to define molecular alterations that characterize these small tumors. Fifteen (15) sections of paraffin-embedded tissue on uncharged slides (or a tissue block of sufficient size to make 15 slides) should be sent to DFCI for testing.

4.5.2 Serum

We will use blood tests to examine the serum SNPs for predictors of neurotoxicity from paclitaxel. These research blood draws can be performed within +/- 8 weeks of the designated time points. Sites will be sent a separate translational research specimen and tumor tissue procedures and shipping manual for further instructions concerning research blood and tumor tissue requirements.

4.6 Ancillary Safety Outcomes

4.6.1 Safety Plan

Patients will be evaluated at each study visit for the duration of their participation in the study.

Specific potential safety issues for this trial are outlined below.

4.6.2 Pregnancy

4.6.2.1 Pregnancy Testing

A screening β-hCG (human chorionic gonadotrophin) pregnancy test is mandatory for all women of childbearing potential within 2 weeks prior to the first dose of investigational product. Thereafter, the serum pregnancy test need only be repeated if clinically indicated or as required by local regulation.

4.6.2.2 Action to be taken if pregnancy occurs

The investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Patients, who become pregnant on study, will be taken off study treatment. The investigator will record information and submit it within 2 weeks of learning of a subject's pregnancy. The subject will also be followed to determine the outcome of the pregnancy. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported. While pregnancy itself is not considered an AE or SAE, any pregnancy complication or elective termination of pregnancy for medical reasons will be recorded as an AE or SAE. A spontaneous abortion is considered an SAE, and will be recorded as such. All pregnancies must be reported to Genentech, Inc.

Genentech Drug Safety

Herceptin Safety Monitor

Tele: 1-888-835-2555

Fax: (650) 225-4682

4.6.3 Cardiac Dysfunction

Signs and symptoms of cardiac dysfunction were observed in a number of women who received trastuzumab alone or in combination with chemotherapy, most often anthracycline-based treatment.

The nature of the observed cardiac dysfunction was similar to the syndrome of anthracycline-induced cardiomyopathy. The signs and symptoms of cardiac dysfunction usually responded to treatment. Complete and partial responses were observed among patients with cardiac dysfunction. The risk appears to be independent of tumor response to therapy. Analysis of the clinical database for predictors of cardiac dysfunction revealed only advanced age and exposure to an anthracycline as possible risk factors. In the clinical trials, most patients with cardiac dysfunction responded to appropriate medical therapy, often including discontinuation of trastuzumab. In many cases, patients were able to resume treatment with trastuzumab. In a subsequent study using weekly paclitaxel and trastuzumab as first-line treatment for metastatic breast cancer, the observed incidence of serious cardiac dysfunction was 3% (N=95) (Seidmen et al. 2001). Since the occurrence of cardiac dysfunction in the trastuzumab plus chemotherapy trial was an unexpected observation, no information is available regarding the most appropriate method for monitoring cardiac function in patients receiving trastuzumab. We will be using Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 to evaluate left ventricular systolic dysfunction. Significant advances in the understanding and treatment of CHF have been made in the past several years, with many of the new drugs demonstrating the ability to normalize cardiac function.

4.6.4 Management of Cardiac Safety

All patients must have a MUGA scan or echocardiogram (ECHO) at baseline, at 12 weeks, 6 months, and at one year. Investigators are strongly urged to schedule MUGA scans or ECHOs at the same radiology facility where the patient's baseline scan was done whenever possible. MUGA scans or ECHOs are required at protocol-specified time points and after any patient has any of the following: discontinuation of protocol therapy, congestive heart failure, or breast cancer recurrence. When a cardiac event occurs, this should be reported in the electronic data capture system (eDC) as an adverse event.

5.0 PATENT SELECTION

5.1 Eligibility criteria

5.1.1 Patients must have histologically confirmed invasive carcinoma of the breast

5.1.2 Tumors must be ≤ 3 cm in greatest dimension

5.1.3 Patients must have node-negative breast cancer according to the AJCC 7th edition. This includes:
Definition of node-negative disease: If the patient has had a negative sentinel node biopsy, then no further axillary dissection is required, and the patient is determined to be node-negative. If an axillary dissection, without sentinel lymph node biopsy, is performed to determine nodal status, at least

6 axillary lymph nodes must be removed and analyzed and negative for the patient to be considered node-negative. Axillary nodes with single cells or tumor clusters ≤ 0.2 mm by either H&E or immunohistochemistry (IHC) will be considered node-negative. Any axillary lymph node with tumor clusters between 0.02 and 0.2cm is considered a micrometastasis. Patients with a micrometastasis are eligible if an axillary dissection has been performed and no further lymph node involvement is seen. In cases where the specific pathologic size of lymph node involvement is subject to interpretation, the principle investigator will make the final determination as to eligibility. The investigator must document approval in the patient medical record.

5.1.4 ER/PR determination is required. ER- and PR-assays should be performed by immunohistochemical methods according to the local institution standard protocol.

5.1.5 HER-2 positive: IHC 3+ or FISH ≥2
NOTE: DCIS components should not be counted in the determination of HER2 status

5.1.6 Bilateral breast cancers that individually meet eligibility criteria are allowed.

5.1.7 Patients should have tumor tissue available, and a tissue block of sufficient size to make 15 slides, which must be sent to DFCI for testing. If a tissue block is unavailable, sites may send 15 sections of paraffin-embedded tissue on uncharged slides. If tumor is not available, the investigator must document why tissue is not available in the patient medical record, and that efforts have been made to obtain tissue.

5.1.8 ≤ 90 days from the patient's most recent breast surgery for this breast cancer

5.1.9 All tumor should be removed by either a modified radical mastectomy or a segmental mastectomy (lumpectomy), with either a sentinel node biopsy or axillary dissection

5.1.9.1 All margins should be clear of invasive cancer or DCIS. The local pathologist must document negative margins of resection in the pathology report. If all other margins are clear, a positive posterior (deep) margin is permitted, provided the surgeon documents that the excision was performed down to the pectoral fascia and all tumor has been removed. Likewise, if all other margins are clear, a positive anterior (superficial; abutting skin) margin is permitted provided the surgeon documents that all tumor has been removed. Radiation therapy to the conserved breast is required.

5.1.10 Endocrine Therapy

5.1.10.1 May have received up to 4 weeks of tamoxifen therapy, or other hormonal therapy, for adjuvant therapy for this malignancy but must temporarily stop such therapy at time of entry to study.

5.1.10.2 Prior oophorectomy for cancer prevention is allowed.

5.1.11 ≥ 18 years of age with any menopausal status

5.1.12 ECOG Performance Status 0 or 1 (see Appendix 3)

5.1.13 Adequate bone marrow function: ANC ≥ 1000/mm^3, hemoglobin ≥ 9 g/dl, and platelets ≥ 100,000/mm^3

5.1.14 Adequate hepatic function: Total bilirubin ≤ 1.5xULN, AST ≤ 1.5x ULN (Total bilirubin must be <2x ULN if it is determined to be a result of Gilbert's syndrome)
5.1.15 Left ventricular ejection fraction (LVEF) ≥ 50%
5.1.16 Willingness to discontinue any hormonal agent prior to registration and while on study
5.1.17 Patients who have participated in a window study (treatment with an investigational agent prior to surgery for ≤2 weeks) are eligible. Patients must have discontinued the investigational agent at least 14 days before participation
5.1.18 Willingness to discontinue sex hormonal therapy, e.g. birth control pills, prior to registration and while on study
5.1.19 Patients who have undergone partial breast radiation (duration ≤ 7 days) prior to registration are eligible
5.1.20 Patients with a history of ipsilateral DCIS are eligible if they were treated with wide-excision alone, without radiation therapy.
5.1.21 Patients undergoing breast conservation therapy (i.e. lumpectomy) must not have any contraindications to radiation therapy.
5.1.22 Willing and able to sign informed consent
5.1.23 Enrollment on Other Investigational Studies
5.1.23.1 Adjuvant Hormonal Studies: Patients may be enrolled on adjuvant hormonal studies, such as the SOFT and TEXT trials. Hormonal therapy will begin as outlined in the specific hormonal trial. Specifically, patients enrolled on the TEXT trial should begin ovarian suppression concurrently with chemotherapy.
5.1.23.2 Adjuvant Bisphosphonate Studies: Patients may be enrolled on adjuvant bisphosphonate studies, such as the adjuvant SWOG study (S0307). Bisphosphonate therapy will begin as outlined in the specific trial.
5.1.23.3 Patients may be enrolled in any other investigational studies that do not use HER2-directed therapies or chemotherapy.

5.2 Ineligibility criteria

5.2.1 Any of the following due to teratogenic potential of chemotherapy:
- Pregnant women
- Nursing women
- Women of childbearing potential who are unwilling to employ adequate contraception (condoms, diaphragms, IUDS, surgical sterilization, abstinence, etc). Hormonal birth control methods are not permitted.

5.2.2 Locally advanced tumors at diagnosis, including tumors fixed to the chest wall, peau d'orange, skin ulcerations/nodules, or clinical inflammatory changes (diffuse brawny cutaneous induration with an erysipeloid edge)
5.2.3 Patients with a history of previous invasive breast cancer.
5.2.4 History of prior chemotherapy in the past 5 years.
5.2.5 History of prior trastuzumab therapy

5.2.6 Active, unresolved infection
5.2.7 Prior history of any other malignancy in the past 5 years, except for early stage tumors of the skin or cervix treated with curative intent
5.2.8 Sensitivity to benzyl alcohol
5.2.9 ≥ grade 2 neuropathy per NCI's Common Toxicity Criteria Version 3.0
EXCEPTION: Any chronic neurologic disorder will be looked at on a case-by-case basis by the study chair
5.2.10 Active cardiac disease
- Any prior myocardial infarction (asymptomatic changes on EKG suggestive of old MI is not an exclusion)
- Documented congestive heart failure (CHF)
- Current use of any therapy specifically for CHF
- Current uncontrolled hypertension (diastolic >100 mmHg or systolic > 200 mmHg)
- Clinically significant pericardial effusion

5.3 Patient Registration

Prior to the initiation of treatment, all protocol subjects must be registered in the Dana-Farber/Harvard Cancer Center QACT Protocol Registration System. The registration system is a computerized registry of all patients on DF/HCC oncology protocols. When an investigator identifies a potential study candidate, he/she must confirm in detail if the candidate is appropriate to participate in the study. The QACT eligibility checklist assures that all inclusion/exclusion criteria are met prior to protocol enrollment.

5.3.1 Patient Registration Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of treatment. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

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

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible using the treatment completion/off-study form supplied in Appendix 6.

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

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. If a participant must be registered during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

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

- Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

- Fax the eligibility checklist and all pages of the consent form to the QACT at 617-632-2295.

- The QACT Registrar will (a) validate eligibility, and (b) register the participant on the study.

- The QACT Registrar will send an email confirmation of the registration to the person initiating the registration immediately following the registration.

If you have any questions regarding this process you can contact the study coordinator and/or a QACT registrar at 617-632-3761.

5.3.3 Registration Process for Other Participating Institutions

To register a participant, the following documents should be completed by the participating site's research nurse or research coordinator and faxed to the DFCI study coordinator or designee at 617-632-3550:

- Copy of required laboratory tests, pathology report(s), EKG results, ECHO/MUGA results, and any other source documentation
- Signed study consent form
- HIPAA authorization form (if separate from the main informed consent document)
- DF/HCC Eligibility Checklist (supplied by DFCI)

The research nurse or data manager at DFCI will then call or email the study coordinator at the participating site to verify eligibility. To complete the registration process, the research nurse or study coordinator will:

- Register the participant on the study with QACT
- Fax or e-mail the confirmation of registration including the participant subject number to the participating site

Once the participating site has received confirmation of registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed

with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible using the treatment completion/off-study form supplied in Appendix 3.

If you have any questions regarding this process you can contact the study coordinator and/or a QACT registrar at 617-632-3761.

6.0 STUDY CALENDAR

Tests and procedures	≤21 days prior to registration	Q6 months x 1 year, then q6months for year 2 and at progression	At 12 weeks, 6 months and at 1 year	Every week during weekly paclitaxel	Every 3 weeks during weekly paclitaxel (+/- 1 week)	Every 9 weeks during trastuzumab monotherapy (+/- 3 weeks)	Observation years 2-10 or until progression[14]	Yearly (+/- 3 months)
History + exam, ECOG PS (ECOG at baseline only)	X				X	X	X	
Vital signs, weight, height (height at baseline only)	X					X[15]	X	
Pelvic Exam[1]	X							
Hematology (CBC with diff)	X			X		X		
Chemistry[7] (Cr, T.bili, AST, ALT, Alk Phos)	X				X	X		
Bone Scan[2]	X							
Blood samples for translational research[9]	X[7]	X						
EKG[4]	X							
Mammogram or breast US	X[6]							X
MUGA or echocardiogram[3]	X		X					
Pregnancy test[5]	X							
Submission of tumor block[8]	X							
Menses Assessment survey[10]	X[11]		X[12]				X[13]	
Non-serious & serious AE + Toxicity grade	→							

[1] Pelvic exam within 12 months of study entry is recommended. Yearly pelvic exams are recommended for those receiving tamoxifen, unless s/p hysterectomy.
[2] If clinically indicated
[3] Use the same method for each evaluation at the same facility where the baseline was done whenever possible. Baseline MUGA or ECHO must be within 12 weeks of beginning study treatment. Cardiac evaluation should still occur at these time points even if there has been a treatment delay.
[4] EKG should be performed within 12 weeks of beginning study treatment.
[5] For women of childbearing potential only. Must be done within 14 days of first dose of treatment.
[6] Mammograms obtained as part of the initial diagnosis, biopsy, and surgery will suffice (do not need to be repeated).
[7] Sodium, potassium, chloride, bicarbonate, BUN, creatinine, , total protein, albumin, total bilirubin, SGOT(AST), SGPT(ALT), Alkaline Phosphatase

8A tumor sample is obtained at the time of core biopsy (or surgical resection if there is inadequate tumor sample from the initial core biopsy), as well as the time of recurrence biopsy if applicable. (Note: An additional biopsy is not being collected under this protocol; however if a patient had a biopsy of tissue at time of recurrence, and if this patient consented to allow us access to this tissue for research purposes at the time of the original consent, then this tissue will be requested for research purposes. The tumor block or 15 sections of paraffin-embedded tissue on uncharged slides should be labeled with the DFCI MRN, subject number, site of collection, date of collection, and protocol number. The paraffin block or slides along with the requisition form found in the separate translational research specimen and tumor tissue procedures and shipping manual should be sent to:

[redacted]

[9]Translational Research Blood samples being collected: whole blood should be collected at baseline in 1- 10mL Red top tube (Fischer # 367820) and 1- 8 mL Cell Processing Tube (Becton Dickinson #362761). 1- 10 mL Red top tube only should be collected every 6 months for 2 years. Specimens should be labeled with the DFCI MRN, date of collection, timepoint of collection, and protocol number (please send with requisition form found in separate translational research specimen and tumor tissue procedures and shipping manual)
and sent Monday-Thursday only by either FedEx or UPS overnight to:

[redacted]

[10] Menses assessment survey to be filled out by patients deemed premenopausal at the time of study registration (i.e. having at least one menstrual period in 6 months prior to registration). Completion of the menses assessment survey by the patient is waived if the required information is documented in a physician's note.
[11] Appendix 1: Baseline Assessment of Menses is to be filled out prior to initiation of chemotherapy
[12] Appendix 2: Follow-up Menses Assessment is to be filled out at 1 year (+/- 1 month)
[13] Follow-up Menses Assessment (Appendix 2) is to be filled every 6 months for years 2, 3, and 4 (+/- 8 weeks), and then annually for years 5 and 6 (+/- 3 months)
[14] Observation for years 2, 3, 4 requires visits every 6 months (+/- 8 weeks). Observation for years 5-10 requires annual visits (+/- 3 months). Participants may be followed at a local facility if unable to make annual visit at participating site. A medical record release is required prior to local facility follow-up in order for source documents to be obtained.
[15] Vital signs are recommended to be obtained every week or 3 weeks during monotherapy trastuzumab treatment (depending on treatment schedule - qweekly vs q3week infusions). Patients can receive trastuzumab monotherapy treatment in between the every 9 week required study visits at a non-participating institution. The referring site is responsible for initiating contact with the provider at the non-participating institution and ensuring the local provider has all protocol-related instructions regarding administration of trastuzumab per protocol. The participating site is responsible for obtaining and reviewing all source documentation related to trastuzumab monotherapy administration. This information should be placed in the participant's research file.

7.0 INCLUSION OF WOMEN AND MINORITIES

This study will be open to patients of all ethnic backgrounds who meet eligibility criteria. Accrual targets will not be specific for ethnic groups.

8.0 TREATMENT PLAN

8.1 Chemotherapy Administration and Supportive Measures

8.1.1 Trastuzumab

8.1.1.1 Concurrent Phase with Paclitaxel: Patients will receive a 4 mg/kg IV loading dose on day 1 followed by 2 mg/kg IV dose weekly (+/- 2 days), for a total of 12 doses. When trastuzumab is being administered concomitantly with paclitaxel, trastuzumab administration may occur prior to, or after, chemotherapy administration.

8.1.1.2 Maintenance Phase after Paclitaxel: After completion of 12 weeks of trastuzumab, trastuzumab dosing may be on either of two schedules:
Weekly schedule: 2 mg/kg IV weekly (+/- 2 days) (over 30 minutes) x 40 weeks
Every 3 week schedule: 6 mg/kg IV every 3 weeks (+/- 7 days) (over 30 minutes or per local standard operating procedures) x 40 weeks. A total of 13 doses should be administered, if there are no missed doses, or treatment delays during therapy.

Trastuzumab monotherapy should begin no sooner than 1 week after the completion of combination therapy and no later than 3 weeks after the completion of combination therapy. It is permissible to switch from weekly to every 3 week dosing and vice versa. When switching from weekly to every 3 weeks there is no need for the 8 mg/kg loading dose. When switching from every 3 week to weekly, there is no need for the 4 mg/kg loading dose.

Trastuzumab monotherapy may be administered at a non-participating institution. The referring site is responsible for initiating contact with the provider at the non-participating institution and ensuring the local provider has all protocol-related instructions regarding administration of trastuzumab per protocol. The lead site is responsible for obtaining and reviewing all source documentation related to trastuzumab monotherapy administration. This information should be placed in the participant's research file.

8.1.1.3 The initial dose of trastuzumab will be administered over 90 minutes. If this first dose is well tolerated, subsequent infusion times may be shortened to 30 minutes or given per participating site's institutional SOP for trastuzumab administration. If the initial or a subsequent dose is not well tolerated (i.e. fevers, chills, or rigors), subsequent infusion times may be shortened only after a dose is well tolerated.

8.1.1.4 If during the 12 weeks of paclitaxel and trastuzumab a dose of paclitaxel is missed, this dose should be made up. Trastuzumab should be administered if paclitaxel is being held. Up to 3 doses of paclitaxel can be made up, and all paclitaxel must be completed within 16 weeks from starting therapy.

8.1.1.5 If during the maintenance phase with trastuzumab monotherapy a dose of trastuzumab is delayed due to patient scheduling, patients may continue on study, and 40 weeks of trastuzumab should be done, and the dose of trastuzumab is not made up. Up to 9 missed doses of weekly trastuzumab or up to 3 missed doses of every 3 week trastuzumab can be missed. If greater than 9 missed doses of weekly trastuzumab or greater than 3 missed doses of every 3 week of trastuzumab are missed, permanently discontinue the patient from study treatment, and notify the study chair. These patients permanently discontinued from the study treatment will still be followed for study endpoints. If a patient has been without a dose of trastuzumab for ≥ 28 days, they will require a reloading dose. The reloading dose is 8 mg/kg IV if given on the every 3 week schedule, or 4 mg/kg IV if given on the weekly schedule. This reloading dose should be infused over 90 minutes.

8.1.1.6 During the maintenance phase, trastuzumab should be administered either every 3 weeks (+/- 7days), or weekly (+/- 2 days). It is encouraged that patients stay on schedule +/- 1-2 days. Patients can receive maintenance trastuzumab at non-participating sites in between their scheduled every 9 week evaluations.

8.1.1.7 Treatment is permitted in an asymptomatic patient if:

- The LVEF increased or stayed the same OR
- The LVEF decreased by ≤15 percentage points, but is still at or above the radiology facility's lower limit of normal (LLN)

8.1.1.8 Treatment is prohibited in an asymptomatic patient if:

- The LVEF decreased by ≤15 percentage points and is below the radiology facility's lower limit of normal OR
- The LVEF decreased by 16 percentage point or more, regardless of the radiology facility's LLN
- See Table below

Asymptomatic Decrease in LVEF: Percentage Points from Baseline			
Relationship of LVEF to radiology facility's LLN	**Decrease of <10 percentage points**	**Decrease of 10 to 15 percentage points**	**Decrease of ≥16 percentage points**
Within normal limits	Continue	Continue	Hold and repeat MUGA/echocardiogram after 4 weeks
1-5 percentage points below LLN	Continue and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks
≥ 6 percentage points below LLN	Continue and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks

8.1.2 Paclitaxel

8.1.2.1 Paclitaxel 80 mg/m^2 will be administered over 30 – 180 minutes per local standard operating procedures. It will be administered weekly (+/- 2 days) for 12 weeks.

8.1.2.2 The following premedication regimen is recommended for the initial dose of paclitaxel. If the patient does not experience an allergic reaction, the premedication regimen may be altered at the discretion of the treating physician.

- Benadryl 12.5-50 mg IV, 30-60 minutes pre-paclitaxel
- Ranitidine 50 mg IV, 30-60 minutes pre-paclitaxel (can be replaced with cimetidine 300 mg, or famotidine 20 mg)
- Dexamethasone 10 mg IV, <60 minutes pre-paclitaxel OR Dexamethasone 20 mg po 6 hours and 12 hrs pre-paclitaxel. Methylprednisolone (60 mg IV) may be used instead of dexamethasone.

8.1.2.3 In order to initiate each weekly treatment, the following is needed:

ANC ≥ 800/mm^3
Platelets ≥ 100,000/mm^3

If these criteria are not met, delay treatment with paclitaxel until counts recover to this level. Trastuzumab should be administered if paclitaxel is being held. Paclitaxel should be held for all instances of febrile neutropenia. Please refer to section 9.1.6 for more information. Missed doses of paclitaxel should be made-up. If a delay of >21 days is required, permanently discontinue patient from study treatment, and notify study chair. These patients permanently discontinued from the study treatment will still be followed for study endpoints.

8.2 Radiation Therapy

8.2.1 Patients who undergo lumpectomy (breast conserving surgery) must receive breast radiation therapy. This may be performed according to local institutional standards. Patients may be treated with conventional, post-chemotherapy, whole breast radiation, or partial breast radiation, administered by external beam or brachytherapy.

8.2.2 Radiation therapy will begin after the conclusion of all study paclitaxel.

8.2.3 Patients undergoing mastectomy may receive chest wall and nodal radiation according to local institutional standards.

8.2.4 Patients may receive adjuvant trastuzumab as per protocol during radiotherapy.

8.3 Endocrine Therapy

8.3.1 Patients may have received up to 4 weeks of tamoxifen therapy, or other hormonal therapy, for adjuvant therapy for this malignancy but must temporarily stop such therapy at time of entry to study. Patients may initiate adjuvant hormonal therapy per institutional standards after completion of treatment with paclitaxel.

9 DOSE MODIFICATIONS

9.1 Paclitaxel

- Dose modifications shall be considered optional when toxicities are deemed unrelated to treatment with Paclitaxel and do not adversely impact study drug administration.

- These patients permanently discontinued from the study treatment will still be followed for study endpoints

9.1.1 Anaphylaxis/Hypersensitivity

9.1.1.1 Mild symptoms (Grade 1): mild flushing, rash, pruritis
- **9.1.1.1.1** Complete infusion, observation in treatment area. No treatment required.

9.1.1.2 Moderate symptoms (Grade 2): moderate rash, flushing, mild dyspnea, chest discomfort
- **9.1.1.2.1** Stop infusion.
- **9.1.1.2.2** Give intravenous diphenhydramine 25 mg and intravenous dexamethasone 10 mg. Methylprednisolone (60mg IV) may be used instead of dexamethasone.
- **9.1.1.2.3** Resume paclitaxel infusion after recovery of symptoms, at a slower rate. 10 ml/hour for 15 minutes, then 25 ml/hour for 15 minutes, then, if no further symptoms, at full dose rate until infusion is complete.
- **9.1.1.2.4** If moderate or severe symptoms recur after rechallenge, stop paclitaxel infusion, and report as an adverse event.
- **9.1.1.2.5** Patient may be rechallenged after premedication with dexamethasone 8 mg po or IV q6hrs x 4 doses (moderate symptoms) or 20 mg po or IV q6hrs x 4 doses (severe symptoms) and diphenhydramine 25 mg po or IV q6hrs x 4 doses (moderate or severe symptoms).Methylprednisolone (60mg IV) may be used instead of dexamethasone.
- **9.1.1.2.6** The paclitaxel should be administered at a slower rate. 10 ml/hour for 15 minutes, then 25 ml/hour for 15 minutes, then, if no further symptoms, at full dose rate until infusion is complete.

9.1.1.3 Severe life-threatening symptoms (Grade 3): hypotension requiring pressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria.
- **9.1.1.3.1** Stop paclitaxel infusion.
- **9.1.1.3.2** Give intravenous diphenhydramine 25 mg and intravenous dexamethasone 10 mg. Methylprednisolone (60mg IV) may be used instead of dexamethasone.
- **9.1.1.3.3** Add epinephrine or bronchodilators if indicated.
- **9.1.13.4** Report episode as an adverse event.
- **9.1.1.3.5** Patient may be rechallenged after premedication with dexamethasone 20 mg po or IV q6hrs x 4 doses and diphenhydramine 25 mg po or IV q6hrs x4 doses. Methylprednisolone (60mg IV) may be used instead of dexamethasone.

9.1.1.3.6 The paclitaxel should be administered at a slower rate, 10 ml/hour for 15 minutes, then 25 ml/hr for 15 minutes, then, if no further symptoms, at full dose rate until infusion is complete.

9.1.2 Cardiac Arrhythmias

9.1.2.1 Asymptomatic, EKG-documented arrhythmias

9.1.2.1.1 Stop paclitaxel, and manage arrhythmia according to standard practice.

9.1.2.1.2 Protocol treatment will be discontinued and the episode reported as an adverse event.

9.1.2.2 Asymptomatic sinus bradycardia or tachycardia

9.1.2.2.1 No intervention is necessary.

9.1.2.3 Sinus bradycardia or tachycardia associated with hypersensitivity reaction

9.1.2.3.1 Please see above.

9.1.3 Hematologic Toxicity

9.1.3.1 Requirements to initiate any cycle of paclitaxel:

9.1.3.1.1 ANC $\geq 800/mm^3$

9.1.3.1.2 Platelets $\geq 100{,}000/mm^3$

9.1.3.2 If ANC<800

- Delay paclitaxel. Continue trastuzumab
- Check counts weekly until ANC $\geq 800\ mm^3$
- If a delay of greater than 21 days is required, permanently discontinue protocol therapy and notify study chair.

9.1.3.3 If platelets <100,000

9.1.3.3.1 Delay paclitaxel. Continue trastuzumab.

9.1.3.3.2 Check counts weekly until platelets $\geq 100{,}000/mm^3$

9.1.3.3.3 If a delay of >21 days is required, permanently discontinue protocol therapy and notify study chair.

9.1.3.4 There will be no dose reductions for hematologic toxicity. Doses will not be reduced based on low nadir counts. Nadir blood counts are not required.

9.1.4 Neurologic Toxicity: Neuropathy (motor and sensory)

9.1.4.1 Grade 1 or 2 Neurotoxicity

9.1.4.1.1 There will be no dose modifications for grade 1 or 2 neurotoxicity.

9.1.4.1.2 Treatment does not need to be delayed and previously administered doses can be continued.

9.1.4.1.3 If the patient is experiencing significant distress from grade 2 toxicity or the treating physician is uncomfortable with continuing the same doses, a dose reduction of 10 mg/m^2 in the paclitaxel dose is acceptable.

9.1.4.2 Grade 3 Neurotoxicity

9.1.4.2.1 Patient should not receive additional treatment until the toxicity has resolved to ≤ grade 2.

9.1.4.2.2 The next infusion may be delayed up to 2 weeks to allow for neurologic toxicity to improve.

9.1.4.2.3 If it does not resolve to ≤ grade 2 after 2 weeks, the patient will be permanently discontinued from protocol therapy.

9.1.4.2.4 Re-treatment should be initiated with a dose of paclitaxel 10 mg/m^2 below the previous dose level when the toxicity resolves to grade 2 or less. All subsequent infusions will be administered using the reduced dose.

9.1.4.2.5 If grade 3 neurotoxicity develops with additional infusions, further dose reductions may be made in increments of 10 mg/m^2 to a maximum of 4 dose level reductions.

9.1.4.2.6 Patients whose toxicity does not improve after 4 dose level reductions will be permanently discontinued from protocol therapy.

9.1.4.2.7 Patients who develop worsening neurotoxicity with each infusion, even if it remains grade 2, should be carefully evaluated to determine if a dose reduction would be appropriate.

9.1.4.2.8 Dose reductions for neurotoxicity:
Full dose: 80 mg/m^2
1st dose reduction: 70 mg/m^2
2nd dose reduction: 60 mg/m^2
3rd dose reduction: 50 mg/m^2
4th dose reduction: 40 mg/m^2

9.1.5 Gastrointestinal Toxicity

9.1.5.1 Nausea/Vomiting

9.1.5.1.1 Grade 0-2 Nausea/Vomiting: No change

9.1.5.1.2 ≥ Grade 3 despite maximal anti-emetic therapy: Hold paclitaxel until ≤ grade 2, then restart with 20% dose reduction in subsequent cycles. Continue trastuzumab.

9.1.5.1.3 If nausea/vomiting toxicity causes a dosing delay of >21 days, or if ≥ Grade 3 nausea/vomiting recurs despite dose

reduction, permanently discontinue protocol therapy and notify study chair.

9.1.5.1.4 Prophylactic antiemetics should be used at the discretion of the investigator. The specific regimen must be recorded in the patient's medical record.

9.1.5.2 Mucositis

9.1.5.2.3 Grade 2 Mucositis

9.1.5.2.3.1 If grade 2 mucositis is present on the day of any treatment, the treatment should be delayed until the mucositis has resolved to a grade 1 or 0, and then resume paclitaxel at 80 mg/m^2.

9.1.5.2.4 Grade 3 or 4 Mucositis

9.1.5.2.4.1 Delay treatment until mucositis has resolved to grade 1 or 0, then the dose of paclitaxel should be reduced to 70 mg/m^2.

9.1.5.2.4.2 If grade 3 or 4 mucositis recurs, treatment should be delayed until mucositis has resolved to grade 1 or 0, and the dose of paclitaxel should be reduced to 60 mg/m^2.

9.1.5.2.4.3 If mucositis causes a delay of >21 days, the patient should be permanently discontinued from protocol therapy.

9.1.5.2.4.4 Once the paclitaxel dose has been decreased, it should not be re-escalated.

9.1.5.3 Diarrhea

9.1.5.3.1 Grade 2

9.1.5.3.1.1 If grade 2 diarrhea is present on the day of any treatment, the treatment should be delayed until the diarrhea has resolved to grade 1 or 0, and then resume paclitaxel at 80 mg/m^2

9.1.5.3.1.2 If diarrhea causes a delay of >21 days, the patient should be permanently discontinued from protocol therapy.

9.1.5.3.1.3 Optimal use of anti-diarrheal agents is encouraged.

9.1.5.3.3 Grade 3 or 4

9.1.5.3.3.1 If grade 3 or 4 diarrhea occurs, delay treatment until the diarrhea has resolved to grade 1 or 0, then the dose of paclitaxel should be reduced to 70 mg/m^2.

9.1.5.3.3.2 If grade 3 or 4 diarrhea recurs, treatment should be delayed until diarrhea has resolved to grade 1 or 0,

and the dose of paclitaxel should be reduced to 60 mg/m^2.

9.1.5.3.3.3 If diarrhea causes a delay of >21 days, the patient should be permanently discontinued from protocol therapy.

9.1.5.3.3.4 Once the paclitaxel dose has been decreased, it should not be re-escalated.

9.1.5.3.3.5 Optimal use of anti-diarrheal agents is encouraged.

9.1.5.4 Hepatic Dysfunction

Because the plasma clearance of paclitaxel is reduced in patients with hepatic impairment, careful evaluation of liver enzymes in necessary before the administration of each new cycle of paclitaxel (i.e. once every 3 weeks). For elevations in total bilirubin, SGOT(AST), SGPT(ALT), the following dose modifications will be applied:

9.1.5.4.1 Grade 1

9.1.5.4.1.1 No dose modifications

9.1.5.4.2 Grade 2

9.1.5.4.2.1 Hold paclitaxel for one week.

9.1.5.4.2.2 If abnormal tests return to grade 0 or 1, paclitaxel should be continued at full dose.

9.1.5.4.2.3 If the abnormal test does not return to grade 0 or 1 in one week, but remains at grade 2, continue paclitaxel at 70 mg/m^2. If the abnormal test result returns to grade 0 or 1, return to full dose, 80 mg/m^2.

9.1.5.4.3 Grade 3 or 4

9.1.5.4.3.1 Patient should be permanently discontinued from protocol therapy.

9.1.6 Febrile Neutropenia

9.1.6.1 Fever ≥38°C (101.3°F) in the presence of neutropenia (ANC <1000) Paclitaxel should be held for all instances of febrile neutropenia.

9.1.6.1.1 First episode: G-CSF or GM-CSF may be used for subsequent cycles at the discretion of the treating physician, but it is not required. There will be no dose reduction for the first episode of febrile neutropenia.

9.1.6.1.2 Second episode: Remaining doses will be reduced to paclitaxel 70 mg/m^2 given with G-CSF or GM-CSF.

9.1.6.1.3 Three episodes: Remaining doses will be reduced to paclitaxel 60 mg/m^2 given with G-CSF or GM-CSF.

9.1.6.1.4 More than three episodes: Discontinue protocol therapy.

9.1.7 Infection with/without neutropenia

9.1.7.1 Grade 3

Paclitaxel should be held for all instances of Grade 3 infection with/without neutropenia.

9.1.7.1.1 First episode: G-CSF may be used for subsequent cycles at the discretion of the treating physician, but it is not required. There will be no dose reduction for the first episode of febrile neutropenia.

9.1.7.1.2 Second episode: Remaining doses will be reduced to paclitaxel 70 mg/m^2. This should be given with G-CSF if infection occurred with neutropenia.

9.1.7.1.3 Three episodes: Remaining doses will be reduced to paclitaxel 60 mg/m^2. This should be given with G-CSF if infection occurred with neutropenia.

9.1.7.1.4 More than three episodes: Discontinue protocol therapy.

9.1.7.2 Grade 4

9.1.7.2.1 Discontinue protocol therapy.

9.1.8 Other Grade 3 or 4 Toxicities

9.1.8.1 Any patient experiencing grade 3 or 4 toxicity, other than those described above must be discussed with the study chair.

9.1.9 Dose Modifications for Obese Patients

9.1.9.1 There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight.

9.1.9.2 All dosing is to be determined solely by the patient's BSA as calculated from actual weight, OR actual weight without any modification unless explicitly described in the protocol.

9.1.9.3 This will eliminate the risk of calculation error and the possible introduction of variability in dose administration.

9.1.9.4 Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.

9.1.9.5 Physicians who are uncomfortable with administering chemotherapy dose based on actual body weight should not enroll obese patients on this protocol.

9.2 Trastuzumab

- No cardioprotective drugs are permitted. There are no data for the use of cardioprotective agents such as dexrazoxane (Zinecard®)

- No dose modifications are permitted.

- Must be discontinued if more than 8 weeks of therapy have been missed and permanently discontinue patient from study treatment, and notify study chair. These patients permanently discontinued from the study treatment will still be followed for study endpoints.

- Trastuzumab may be administered at non-participating sites during the maintenance phase in between the required every 9 week visits. The every 9 week evaluations must be conducted at a participating site.

9.2.1 Infusion-associated symptoms

9.2.1.1 During the first infusion, a symptom complex of fever and/or chills may occur. These are usually mild-to-moderate and may be accompanied by nausea, vomiting, headache, dizziness, rigors, pain, hypotension, rash, and asthenia. These symptoms occur infrequently during subsequent infusions.

9.2.2 Trastuzumab when paclitaxel is delayed or discontinued

9.2.2.1 If paclitaxel is delayed for any reason other than cardiotoxicity or severe hypersensitivity reactions that occurred when both paclitaxel and trastuzumab were administered, trastuzumab may be continued.

9.2.3 Cardiac Dysfunction

9.2.3.1 Initiation of trastuzumab will depend on review of the initial MUGA scan/echocardiogram results.
9.2.3.2 If trastuzumab is initiated, a decision about whether to continue or discontinue must occur after completion of all chemotherapy and while the patient is on trastuzumab alone.
9.2.3.3 Individual patients should have their MUGA scans/echocardiograms performed at the same radiology facility to eliminate variability between facilities.
9.2.3.4 Asymptomatic decrease in LVEF:
9.2.3.4.1 Decision to continue or stop is based on the measured ejection fraction as it relates to the radiology facility's

LLN and change in ejection fraction from baseline. Guidelines for performing MUGA scan/echocardiogram and management of patients who have an asymptomatic decrease in LVEF from baseline are in the table below:

Asymptomatic Decrease in LVEF: Percentage Points from Baseline			
Relationship of LVEF to radiology facility's LLN	**Decrease of <10 percentage points**	**Decrease of 10 to 15 percentage points**	**Decrease of ≥16 percentage points**
Within normal limits	Continue	Continue	Hold and repeat MUGA/echocardiogram after 4 weeks
1-5 percentage points below LLN	Continue and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks
≥ 6 percentage points below LLN	Continue and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks	Hold and repeat MUGA/echocardiogram after 4 weeks

9.2.3.4.2 If trastuzumab is held or discontinued during therapy with paclitaxel, paclitaxel may be continued at the discretion of the investigator.

9.2.3.4.3 If trastuzumab is not started or discontinued during therapy, MUGA scan/echocardiogram still needs to be done at 12 weeks and at 1 year.

9.2.3.4.4 Trastuzumab must be permanently discontinued when two consecutive "hold" categories occur.

9.2.7.1.1 Trastuzumab must be permanently discontinued when three intermittent "hold" categories occur.

9.2.3.4.6 At the discretion of the investigator, trastuzumab may also be permanently discontinued prior to the occurrence of three intermittent "hold" categories.

9.2.3.4.7 If LVEF is maintained at a "continue and repeat MUGA/echocardiogram" or improves from a "hold" to a "continue and repeat MUGA/echocardiogram" category, additional MUGA scans/echocardiogram prior to the next scheduled MUGA scan/echocardiogram will be at the discretion of the investigator.

9.2.3.5 Symptomatic decrease in LVEF

9.2.3.5.1 Grade 3 CHF

9.2.3.5.1.1 Monitor for signs and symptoms of CHF (i.e. dyspnea, tachycardia, cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, etc)

9.2.3.5.1.2 If patient develop these signs and symptoms, hold treatment.

9.2.3.5.1.3 If CHF occurs while on paclitaxel plus trastuzumab, resumption of paclitaxel is at the discretion of the investigator.

9.2.3.5.1.4 Confirm diagnosis of CHF with either a MUGA scan/echocardiogram. A chest x-ray is also required. Once a diagnosis of CHF is confirmed, trastuzumab must be permanently discontinued and reported as an adverse event.

9.2.3.5.1.5 Follow-up at 3, 6, and 12 months from time of CHF diagnosis with MUGA scan/echocardiogram.

9.2.3.5.2 Grade 4 CHF (severe refractory CHF or requiring intubation)

9.2.3.5.2.1 Discontinue treatment, and report as an adverse event.

9.2.3.5.2.2 Follow-up at 3, 6, and 12 months with MUGA/echocardiogram.

9.2.4 Ischemia

9.2.4.1 Grade 1

9.2.4.1.1 Continue treatment with frequent monitoring.

9.2.4.2 Grade 2

9.2.4.2.1 Hold treatment and conduct cardiac evaluation.

9.2.4.2.2 Based on this evaluation, treatment may be continued at the discretion of the investigator.

9.2.4.3 Grade 3 or 4

9.2.4.3.1 Discontinue treatment.

9.2.5 Arrhythmia

9.2.5.1 Grade 1

9.2.5.1.1 Continue treatment with careful monitoring OR hold treatment (and paclitaxel if patient is receiving paclitaxel) and conduct cardiac evaluation.

9.2.5.1.2 Based on cardiac evaluation, treatment with trastuzumab and paclitaxel or trastuzumab alone may continue or discontinue at the discretion of the investigator.

9.2.5.1.3 If trastuzumab is discontinued, paclitaxel may also be discontinued at the discretion of the investigator, and patient removed from protocol therapy.

9.2.5.2 Grade 2

9.2.5.2.1 Hold treatment (and paclitaxel if patient is receiving paclitaxel) and conduct cardiac evaluation.

9.2.5.2.2 Based on cardiac evaluation, treatment with trastuzumab and paclitaxel or trastuzumab alone may continue or discontinue at the discretion of the investigator.

9.2.5.3 Grade 3 or 4

9.2.5.3.1 Discontinue trastuzumab.

9.2.5.3.2 Paclitaxel is not permitted.

9.2.5.3.3 Patient should be removed from protocol therapy.

9.2.6 Myocardial Infarction

- Discontinue Treatment

9.2.7 Fever

9.2.7.1 Grade 1 (38°C - 39°C [100.4° - 102.2°F] OR Grade 2 (39.1°C - 40°C [102.3° - 104°F]

9.2.7.1.1 Stop infusion and give antipyretics. Once temperature is <38°C, resume infusion at a slower rate.

9.2.7.2 Grade 3 (>40°C [104°])

9.2.7.2.1 Stop infusion immediately and give antipyretics

9.2.7.2.2 Monitor patient for a minimum of one hour

9.2.7.2.3 If temperature drops to <38°C within 3 hours, resume infusion at a slower rate.

9.2.7.2.4 If fever does not resolve within 3 hours, inpatient monitoring is strongly recommended.

9.2.7.2.5 If temperature drops to <38°C within 3 days, re-challenge at a slower rate.

9.2.7.2.6 If temperature remains >38°C after 3 days, abandon this administration and subsequent administration is at the discretion of the investigator

9.2.7.3 Grade 4 (40°C [104°F] for 24 hours)

9.2.7.3.1 Stop infusion immediately and give antipyretics

9.2.7.3.2 Monitor patient for a minimum of one hour

9.2.7.3.3 If fever does not resolve within 3 hours, inpatient monitoring is strongly recommended.

9.2.7.3.4 If temperature drops to <38°C within 3 days, re-challenge at a slower rate.

9.2.7.3.5 If temperature remains >38°C after 3 days, abandon this administration and subsequent administration is at the discretion of the investigator

9.2.8 Chills

- Treat with acetaminophen and/or diphenhydramine hydrochloride.
- Meperidine may be given at the investigator's discretion.

9.2.9 Gastrointestinal

9.2.9.1 Diarrhea

9.2.9.1.1 Any grade

Any antidiarrheal medication may be given at the investigator's discretion.

9.2.10 Allergy/Immunology

9.2.10.1 Allergic reaction/hypersensitivity (including drug fever)

9.2.10.1.1 Stop the infusion and give diphenhydramine hydrochloride

9.2.10.1.2 If toxicity resolves within 3 hours, treatment in next dose is allowed at a slower rate and under close observation.

9.2.10.1.3 If toxicity does not resolve in 3 hours, overnight observation is recommended and treatment in the next dose under close observation is at the discretion of the investigator.

9.2.11 Pulmonary

9.2.11.1 Any (e.g. Adult Respiratory Distress Syndrome[ARDS], pneumonitis/pulmonary infiltrates, etc)

9.2.11.1.1 Delay trastuzumab until case is known.

9.2.11.1.2 If pneumonitis/fibrosis, or pulmonary infiltrate is confirmed, and the relationship to trastuzumab cannot be excluded, trastuzumab must be permanently discontinued.

10 DRUG FORMULATION, AVAILABILITY, AND PREPARATION

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

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

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

10.1 Trastuzumab (Trastuzumab)

10.1.1 Availability

Trastuzumab is a commercially available sterile, white to pale yellow, preservative-free- lyophilized powder for intravenous (IV) administration. Each vial of trastuzumab contains 440 mg of trastuzumab, 9.9 mg of L-histadine HCl, 6.4 mg of L-histadine, 400 mg of α,α-trehalose dihydrate, and 1.8 mg of polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI) USP, containing 1.1% benzyl alcohol as a preservative, yields 21 mL of a multidose solution containing 21 mg/mL trastuzumab, at a pH of ~6.

10.1.2 Preparation

10.1.2.1 Use appropriate aseptic technique. Each 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.

10.1.2.2 If the patient has known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with Sterile Water for Injection. Trastuzumab which has been reconstituted with SWFI must be used immediately and any unused portion discarded. Use of other reconstitution diluents should be avoided.

10.1.2.3 Determine the dose of trastuzumab needed, based on a loading dose of 4 mg trastuzumab/kg body weight for weekly dosing schedules or a maintenance dose of 2 mg/kg trastuzumab/kg body weight for weekly dosing schedules (OR 6 mg trastuzumab/kg body weight during q3wk dosing). 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.

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

10.1.3 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 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. 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).

10.1.4 Administration

Treatment may be administered in an outpatient setting by administration of a 4 mg/kg trastuzumab loading dose for weekly dosing schedules by intravenous (IV) infusion over 90 minutes. **DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.** When trastuzumab is being administered concomitantly with paclitaxel, trastuzumab administration should precede

chemotherapy administration. Patients should be observed for fever and chills or other infusion-associated symptoms. If prior infusions are well tolerated subsequent doses of 2 mg/kg Trastuzumab weekly may be administered over 30 minutes.

10.1.5 Toxicities

10.1.5.1 Cardiac Toxicity

Trastuzumab may result in clinically manageable left ventricular systolic dysfunction, and occasionally advanced congestive heart failure (CHF) in a small proportion of patients. The incidence and severity of cardiac dysfunction has been greatest in patients who received trastuzumab in combination with an anthracycline. In the pooled NSABP/NCCTG experience, the risk of cardiac events was 4% among those treated with chemotherapy and trastuzumab, as compared with .6% for those given chemotherapy alone. There are data suggesting that the risk is lower when sequential chemotherapy followed by trastuzumab is given. Furthermore, older patients and those patients with borderline normal LVEF at baseline may be at greater risk for cardiac events. The incidence of class III or IV cardiac dysfunction was 2% for those receiving concurrent paclitaxel plus trastuzumab.[66] The risk of cardiac toxicity is substantially lower in patients who received trastuzumab alone. Cardiac dysfunction appears to improve in most patients who receive supportive medical treatment.

10.1.5.2 Infusion-Associated symptoms

10.1.5.2.1 During the first infusion of trastuzumab, symptoms consisting of chills and/or fever are observed in about 40% of patients. Other symptoms may include nausea, vomiting, pain, rigors, headache, cough, dizziness, rash, and asthenia. These symptoms are usually mild to moderate in severity, and occur infrequently with subsequent trastuzumab infusions.

10.1.5.2.2 Serious adverse reactions to trastuzumab infusion include 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 fatal outcome.

10.1.5.3 Hematologic Toxicity

10.1.5.3.1 Anemia

An increased incidence of anemia has been 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.

10.1.5.3.2 Neutropenia

In randomized controlled clinical trials designed to assess the impact of the addition of trastuzumab to chemotherapy, the incidence of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving trastuzumab in combination with chemotherapy as compared to those who received chemotherapy alone. The pathophysiologic basis for exacerbation of neutropenia has not been determined.

10.1.5.3.3 Secondary acute leukemia or myelodysplastic syndrome

Incidence of approximately 4 in 1200 patients who participated in trastuzumab clinical trials. Patients treated with chemotherapeutic agents are known to be at increased risk of 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.[67] The contribution of trastuzumab to the etiology of acute leukemia or myelodysplastic syndrome in these cases is unclear.

10.2 Paclitaxel

10.2.1 Availability

Paclitaxel is commercially available in 20 mg/5mL, 100 mg/16.7mL, 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 paclitaxel, 527 mg of purified Cremophor® EL (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP. Please refer to FDA-approved package insert for complete product information.

10.2.2 Preparation

Paclitaxel must be diluted prior to administration with 0.9% sodium chloride Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP; or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL.

Paclitaxel should be prepared and stored in glass, polypropylene, or polyolefin containers due to leaching of DHEP [di-(2ethylhexyl)phthalate] plasticizer from polyvinyl chloride (PVC) containers. Non-PVC containing tubing and connectors such as the IV administration sets (polyethylene or polyolefin) used to infuse parenteral nitroglycerin should be used.

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 or 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.

10.2.3 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.

10.2.4 Administration

Paclitaxel will be administered as an IV infusion using an in-line 0.22 micron filter.

10.2.5 Toxicities

Myelosuppression, liver function test abnormalities (elevated SGOT, SGPT, bilirubin, alkaline phosphatase), nausea, vomiting, diarrhea, mucositis, peripheral neuropathy, transient asymptomatic bradycardia, and with much less frequency, arrhythmias, hypotension, hypersensitivity/anaphylaxis reactions (dyspnea, tachycardia, rash, urticaria, hypotension, or hypertension), myalgias, arthralgias, and alopecia.

10.3 Ancillary Therapy

10.3.1 Patients should receive full supportive care, including transfusions of blood or blood products, antibiotics, antiemetics, etc., when appropriate.

10.3.2 Treatment with hormones or other chemotherapeutic agents or biologic agents may not be administered during therapy except for steroids given for adrenal failure: hormones administered for non-disease-related conditions (insulin for diabetes, synthroid for hypothyroidism); and intermittent use of

dexamethasone as an antiemetic and premedication for paclitaxel. Patients may have received up to 4 weeks of tamoxifen therapy, or other hormonal therapy, for adjuvant therapy for this malignancy but must temporarily stop such therapy at time of entry to study. Patients may initiate adjuvant hormonal therapy per institutional standards after completion of treatment with paclitaxel.

10.3.3 Use of Growth Factors

10.3.3.1 Erythropoetin (EPO) and Related Agents

10.3.3.1.1 The use of EPO is permitted at the discretion of the treating physician.

10.3.3.2 Filgrastim (G-CSF) and Related Agents

10.3.3.2.1 The use of filgrastim is permitted during treatment with weekly paclitaxel. Please see section 9.1.6 and 9.1.7for specific recommendations about the use of these agents.

11.0 DEFINITION OF PRIMARY ENDPOINT AND REMOVAL OF PATIENTS FROM THERAPY

11.1 Primary Endpoint: Disease-Free Survival (DFS)

Disease-free survival (DFS) is defined to end at the time of the first of the following events: local/regional ipsilateral invasive recurrence (or ipsilateral invasive new primary), contralateral invasive breast cancer, distant recurrence, or death from any cause. Local/regional recurrence is defined as an invasive ipsilateral breast cancer invasive breast cancer in the axilla, regional lymph nodes, chest wall, or skin of the ipsilateral breast. A distant recurrence is metastatic disease that has either been biopsy confirmed or clinically diagnosed as recurrent invasive breast cancer. A single new lesion on a bone scan without evidence of lytic disease on x-ray and without symptoms does not in and of itself constitute distant recurrence, but multiple new bone lesions, or increased isotope uptake associated with new bone symptoms are more likely due to metastases. Bone metastases must be documented with x-rays and clinical description. Contralateral invasive breast cancer will be included as an event. Death attributable to any cause, including breast cancer, non-breast cancer, or unknown cause will be included as an event. In situ cancer will not be included as an event that ends DFS. If a patients has in situ breast cancer (on the ipsilateral or contralateral side) diagnosed during follow-up before any of the events which are defined to end DFS, then the patient should continue to be followed for DFS on study (even if she is given hormonal therapy after the in situ diagnosis). The primary endpoint is to estimate disease-free survival (DFS). Yearly mammograms will not be used to determine DFS. Any abnormality on imaging alone is not used towards the DFS endpoint. A disease recurrence, or a new primary breast cancer can only

be determined by a biopsy. There is therefore no need for central review of mammography.

11.1.1 If a patient is diagnosed with a cancer of another site (i.e. other than a new breast cancer, a breast cancer recurrence, or breast cancer metastases) that is NOT a non-melanoma skin cancer or a cervical/vaginal carcinoma in situ, then the patient will be removed from study treatment. These patients will still be followed for survival. If a patient is diagnosed with a non-melanoma skin cancer or a cervical/vaginal carcinoma in situ, she will continue on this study and continue to be followed for DFS.

11.2 Removal of Patients from Protocol Therapy (patients should still be followed for study endpoints)

11.2.1 Patients will be removed from protocol therapy if they experience toxicities per the guidelines in section 9 of the protocol. Patients should still be followed for study endpoints after removal from protocol therapy.

11.2.2 Disease Progression

11.2.2.1 If a patient has a DFS event (see section 11.1) treatment should be permanently discontinued.

11.2.2.2 Extraordinary Medical Circumstances

11.2.2.3 If at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue on protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify study chair
- Document the reasons for discontinuation of therapy

At the time of removal of patients from protocol therapy, participating sites are responsible for completing the treatment ending part of the treatment completion/off-study form found in appendix 6 and faxing it to the QACT at 617-632-2295 and inputing this information in the eDC system. This patient will continue in follow-up on study (for DFS and survival if the reason for discontinuing treatment is something other than a DFS event, and for survival if the reason for discontinuing treatment is a DFS event). This form must be submitted again when the patient goes off study.

12.0 STATISTICAL CONSIDERATIONS

12.1 Study Design

This is a single arm, three stage study evaluating the efficacy and toxicity of weekly paclitaxel chemotherapy x 12 with weekly trastuzumab x 12 followed by trastuzumab given every 3 weeks or weekly, for a total of 52 weeks in node-negative HER2-positive patients with tumors ≤ 3cm.

12.2 End Points

12.2.1 The primary end point is duration of disease-free survival. Events that determine the end of disease-free survival are listed in 11.1.

12.2.2 In the rest of this section (section 12), a patient with any one of these events is said to have a "failure". At any particular calendar time after the study opens for accrual, each patient who has entered the study and who has already had a failure has a time (from entry) to failure. Each patient who has entered the study and has not yet had a failure has a censored time (which is either the time between study entry and the diagnosis of a non-breast cancer other than those listed in section 11.1, or else the time between entry on study and the most recent date that patient is known to not have a failure, which may be zero for a patient who has recently entered and has no follow-up yet). The phrase "patient-years of follow-up" refers to the sum of failure times for all patients who have failed plus the sum of censored times for all patients who have not yet failed.

12.2.3 The secondary endpoints are listed in section 2.2 (describe DFS in patients grouped by tumor size and hormone status, evaluate the incidence of serious cardiac toxicity, evaluate the incidence of serious neurotoxicity, ,characterize molecular alterations in small HER2+ breast cancers, investigate the percentage of patients with amenorrhea at various times after start of treatment in premenopausal and explore predictors of amenorrhea).

12.3 Sample Size Justification

The target accrual of this study is approximately 400 patients.

This is a single arm, three stage study. The study will terminate early if more than 7 failures are observed by the time there has been 225 patient-years of follow-up or if more than 24 failures are observed by the time there has been 800 patient-years of follow-up. If more than 40 failures are observed by the time there has been 1600 patient-years of follow-up, then the regimen will be declared not worthy of further study. If none of these boundaries are crossed, then the regimen will be declared worthy of further study.

Based on the little that is known about the failure rate of the patients eligible for this study, the investigators want a large chance of stopping the study at the first look if the true 3 year failure percent is as large as 14%, want a small chance of declaring the regimen worthy of further research if the true 3 year failure percent is around 9%, and want a large chance of declaring the regimen worthy of further research if the true 3 year failure percent is as small as 5%.

Using the exact Poisson distribution and the stopping rule above, the probability of stopping the study at the first look (225 patient-years of follow-up) is 0.88 if the true 3 year failure percent is 14%, is 0.74 if the true 3 year failure percent is 12%, and is 0.53 if the true 3 year failure percent is 10%. Using the exact conditional Poisson distribution, the probability that the regimen is declared worthy of further research is 0.05 if the true 3 year failure percent is 9.2% and is 0.95 if the true 3 year failure percent is 5%.

Among HER2-positive patients receiving bisphosphonate therapy, the 3 year failure percent is unknown. Given the lack of data in this population of patients, the investigators hypothesize that patients who do not receive bisphosphonate therapy may have a failure rate that is not much different from those who receive bisphosphonate therapy. In addition, it is expected that fewer than 20 (5%) of patients who are enrolled will be receiving adjuvant bisphosphonate therapy (such as in S0307). Therefore, it is hypothesized that the overall 3 year failure percent (combining patients who do not receive bisphosphonates with those receving bisphosphonates) will be the same as that of the original cohort.

It is hypothesized that the failure rate is higher among patients with micrometasis, however little is known about the 3 year failure percent in this group. With the inclusion of patients with micrometastasis, the overall 3 year failure percent (taking both cohorts together) is anticipated to be higher and therefore the probability that the regimen will be declared not worthy of further study will be higher. It is estimated that patients with micrometastasis will account for at most 10% of accrual. If the 3 year failure percent remains 9.2% among patients without micrometastasis (original cohort) and it is up to 50% higher among patients with micrometastasis (13.8%), then approximately the overall 3 year failure percent is hypothesized to be 9.7% and the probability that the regimen is declared not worthy of further study will be at most 3% higher. In an extreme case where 20% of patients have micrometastasis, the overall 3 year failure percent will be 10.1% and the probability will be at most 4% higher. These calculations are based on an overall 3 year percent that is a weighted average of the 3 year percents of the two cohorts.

The table below gives the probability of stopping the study at the first look and the probability of declaring the regimen worthy of further study for various true 3 year failure percents.

True 3 Year Failure Percent	Probability That Study Will Be Stopped At First Look	Probability That Regimen Will Be Declared NOT WORTHY Of Further Study	Probability That Regimen Will Be Declared WORTHY Of Further Study
10.1	0.55	0.99	0.01
9.7	0.50	0.98	0.02
9.2	0.44	0.95	0.05
8.7	0.37	0.90	0.10
8.2	0.31	0.81	0.19
6.1	0.11	0.20	0.80
5.5	0.07	0.10	0.90
5.3	0.06	0.07	0.93
5.0	0.04	0.05	0.95

12.4 Statistical Analysis

12.4.1 The primary analysis will include all patients enrolled to the study. Kaplan-Meier estimates of DFS will be plotted for the study as a whole and for subgroups of patients determined by tumor size (≤1 cm vs. >1 cm) and hormone receptor status (positive vs. negative). If the regimen is deemed to be worthy of further research, there will be at most 40 failures at the time of final analysis, and if the hypothesis of a true 5% 3 year failure percent is correct, it is expected that there will be only 26 failures, or about 6.5% of the 400 patients (at a median follow-up at about 4 years). So analysis of DFS will not be much more powerful than the analysis of the percentage of patients who have failed. If 25% of the patients entered have tumor size ≤ 1 cm, this study will have adequate (85%) power to declare that the two size groups differ significantly if the true percents of patients with failures (at a median follow-up of 4 years) are 1% and 8.3%. If 25% of the patients are ER positive, this study will have adequate (85%) power to declare the two hormone receptor status groups differ significantly if the true percents of patients with failures are 1% and 8.3%. These calculations assume that one-sided 0.05 Fisher exact tests are used and that there is no adjustment for multiple comparisons.

12.4.2 The incidence of grade 3 or worse cardiac toxicity will be estimated, with a 95% confidence interval. If the study accrues 400 patients (i.e., the study is not terminated before accrual is complete), then the 95% Binomial confidence intervals for grade 3 or worse cardiac toxicity will be 0 to 0.7% if no cases of serious or worse cardiac toxicity are observed, 0.3 to 2.5% if 4 cases are observed, 0.8 to 3.9% if 8 cases are observed, 1.5 to 5.2% if 12 cases are observed, 2.3 to 6.4% if 16 cases are observed, and 3.1 to 7.6% if 20 cases are observed.

12.4.3 The incidence of grade III/IV neurotoxicity will be estimated, with a 95% confidence interval. If the study accrues 400 patients (i.e., the study is not terminated before accrual is complete), then the 95% Binomial confidence intervals for serious neurotoxicity will be 3.1 to 7.6% if 20 cases are observed, 5.5 to 11.1% if 32 cases are observed, and 7.2 to 13.4% if 40 cases are observed. The incidence of grade 2-4 neuropathy reported for prior studies of 12 weeks of paclitaxel (ECOG 1199) is approximately 27%. Assuming there are 50 patients who have the CYP2C8*3 polymorphism and 350 patients who do not have the polymorphism among the 400 patients, and the incidence of grade 2-4 neuropathy is 27% among those without the polymorphism, there is 80% power to detect an absolute difference of 20% (27% versus 47%) in the grade 2-4 neuropathy. This calculation assumes a 0.05 level 2-sided test for two binomial proportions. An exploratory analysis will be conducted for other possible SNPs that may predict neurotoxicity from paclitaxel.

12.4.4 For the correlative objective to characterizes the molecular alterations by gene sequencing on all archival tumor, descriptive statistics will be used to report the types and frequencies of molecular events seen using 95% Binomial exact confidence intervals for the observed proportions. Based on the few DFS events expected, no statistical inferences will be used to assess associations, and all point and interval estimates will be reported as exploratory and hypothesis generating.

12.4.4.1 Since approximately 25% of breast cancer patients are premenopausal, we expect there will be a 90% chance that there will be at least 89 premenopausal patients who agree to be on the amenorrhea sub-study and who will be available for the amenorrhea analysis. The primary endpoint will be the percent of patients who report at least one menstrual period in the time period 6 to 12 months after protocol entry (termed "percent still menstruating at 12 months" below). 12.4.4.1 We will first explore whether the proportions of patients still menstruating at 12 months differ between the patients assessed for menstrual history at the time of registration to 07-199 and patients assessed after registration (because the latter will be answering questions about menstruation further in the past). If these proportions differ by less than 15%, then the results of all patients reporting they were premenopausal at the time of enrollment on 07-199 will be combined. If these proportions differ by 15% or more, then the primary analysis will involve only those patients who were assessed for menstrual history at the time of enrollment on 07-199.

12.4.4.2 We will report the proportion of patients with at least one menstrual period in the past 6 months for the group as a whole (either all patients, or all patients who were assessed for menstrual history at the time of enrollment on 07-199) and also by age group (<40 years vs. >40 years) and by body mass index (BMI <30 or ≥30) at each time point. Exact binomial two-sided 95% confidence intervals for the percent of patients will menstruating at 12 months overall and in these 4 subgroups will be calculated; they will not be corrected for multiple comparisons. If there are at least 10 patients still menstruating at 12 months and at least 10 patients not still menstruating at 12 months (in the overall group), then factors associated with the probability of still menstruating at 12 months will also be evaluated in an exploratory manner using logistic regression, with one covariate at a time and using a step-up procedure with the logistic regression. Covariates that will be considered include age, (<40 years vs. ≥40), body mass index (<30 or ≥30), and whether the patient had some grade 3 toxicity (other than amenorrhea) on study. Exploratory descriptive statistics regarding the approximate frequency of recent (within the last 6 months) menses (once monthly, every two months, twice a month, every 2-6 months, or less than every six months) will also be presented for each time point.

If at least 89 patients who are menstruating at study entry are accrued and one year menstrual data is gathered on all of them and the patients assessed for menstrual history at the time of study registration can be combined with

the patients assessed for menstrual history after study registration, and if no corrections for multiple comparisons are made, then the largest width of the 95% confidence interval for the proportion still menstruating at 12 months will be 21% (i.e., 40% to 61% if 45 of the 89 are still menstruating). If approximately 75% of the patients are observed to be menstruating at 12 months, the 95% confidence interval will be 65%-84%.

The number of premenopausal patients accrued will be small, which means that the confidence intervals on percent still menstruating at any time point will be large and also means that only exploratory analysis can be done on factors affecting the proportion still menstruating. Also, if the study stops accrual early, there will be fewer patients available for estimating amenorrhea. In addition, there may be a difference in recall between patients first questioned about their menstrual history at protocol entry (before treatment starts) and patients first questioned later. However, data resulting from this assessment will provide preliminary estimates of percents of amenorrhea in the population receiving this regimen.

13.0 STUDY CONDUCT CONSIDERATIONS

13.1 Institutional Review Board or Ethics Committee Approval

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. The study will be conducted in accordance with U.S. FDA, applicable national and local health authorities, and IRB requirements.

The Principal Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case the IRB must be updated at least once a year. The Principal Investigator must also keep the IRB informed of any significant adverse events.

Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to serious adverse events that are not already identified in the Investigator Brochure and that are considered possibly or probably related to the molecule or study drug by the investigator. Some IRBs may have other specific adverse event requirements that investigators are expected to adhere. Investigators must immediately forward to their IRB any written safety report or update provided by Genentech (e.g., IND safety report, Investigator Brochure, safety amendments and updates, etc.).

13.2 Data Safety Monitoring Board (DSMB)

The Dana Farber DSMB will be utilized for this study. Interim analyses are planned to take place when a total of 225 and 800 patient years of follow-up have been collected, with a final analysis when a total of 1600 patient years of follow-up have been collected. If the assumptions of section 12.5 are correct, then these analyses will occur approximately at 1.5, 3, and 5 years after accrual to the study starts. The analyses will be done early if 8

failures are observed before 225 patient years of follow-up are collected, or if 25 failures are observed before 800 patients years of follow-up are collected, or if 41 failures are observed before 1600 patient years of follow-up are collected.

13.3 Study Monitoring Requirements

The coordinating site will collect and review data on an on going basis via an internet application using the Electronic Data Capture (eDC) system provided by the Quality Assurance for Clinical Trials (QACT). All appropriate participating site staff must complete the eDC system-training course prior to any data entry. The lead institution will supply each participating site with the appropriate information to register for training and to access the eDC system.

The coordinating site will conduct at least one visit at each participating site location within the first year of patient enrollment. QACT auditors or their designee (s) will require access to all patient medical records to verify source and ensure appropriate review of the data submitted on the eCRFs. Additional site visits and source documentation may be conducted at the discretion of the principal investigators. In addition, source documentation may be requested from participating sites periodically at the discretion of the principal investigators.

13.4 Disclosure of Data

Subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, national and local health authorities, Genentech, and the IRB for each study site, if appropriate.

13.5 Retention of Records

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the investigation is discontinued and the U.S. FDA and the applicable national and local health authorities are notified. Genentech will notify the Principal Investigator of these events.

14.0 ADVERSE EVENTS: REPORTING AND MONITORING

14.1 Adverse Event and Reporting Definitions

With the occurrence of an adverse event, the first concern will be for the safety of the subject. The investigator must report any serious adverse events, which occur during the clinical study or within 30 days of receiving the last dose of study medication, whether or

not related to the study drugs to the Principal Investigator, Genentech (if applicable per guidelines specified below), and per participating local IRB reporting requirements.

DF/HCC and DF/PCC institutions must report all SAEs directly to the DFCI IRB using the DFCI IRB-Serious Adverse Event Reporting Form found on the Oncology Protocol System website in addition to notifying the Principal Investigator. All other participating institutions SAE report notifications will be reviewed by the lead institution to determine if it meets DFCI IRB SAE reporting requirements. If the event does meet the DFCI IRB SAE reporting requirements, the study coordinator or designee at the lead institution will submit the event information to the DFCI IRB using the DFCI IRB IND Safety Reporting Form.

Investigators are required to report to Genentech Drug Safety any **serious adverse event**, whether **expected** or **unexpected**, and which is assessed by the investigator to be **reasonably or possibly related** to or caused by trastuzumab. All events meeting these criteria will be reported for the time period beginning with any amount of exposure to trastuzumab through the protocol-defined follow-up period. Serious criteria, definitions, and guidance for reporting follow.

An **adverse event (AE)** is any untoward medical occurrence in a subject participating in an investigational study or protocol regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product. During the course of this study, only grade 2 or higher adverse events will be followed and recorded in the electric data capture system (eDC). During the follow up period only Grade 2 neurotoxicity and cardiac events need to be reported.

Serious adverse events (SAE) are adverse events occurring at any dose which meet one or more of the following **serious criteria**:

Results in **death** (i.e. the AE caused or led to death)

Is **life-threatening** (i.e. the AE placed the subject at immediate risk of death; it does not apply to an AE which hypothetically might have caused the death if it were more severe)

Requires or prolongs inpatient **hospitalization** (i.e. the AE required at least a 24-hour inpatient hospitalization or prolonged a hospitalization beyond the expected length of stay; hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion)

Is **disabling** (i.e. the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions)

Is a **congenital anomaly/birth defect** (i.e., an adverse outcome in a child or fetus of a subject exposed to the study drug prior to conception or during pregnancy)

Does not meet any of the above serious criteria but **may jeopardize the subject** and **may require medical or surgical intervention** to prevent one of the outcomes listed above

Expected adverse events are those adverse events that are **listed** or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those **not listed** in the Package Insert (P.I.) or current Investigator Brochure (I.B.). This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

Reporting of Serious Adverse Events Associated with Trastuzumab

All SAEs that are serious and reasonably or probably related to the use of trastuzumab (this applies to both expected and unexpected events) should be recorded on a MedWatch 3500 Form and faxed to:

Genentech Drug Safety Contact Line

Tele: 1-888-835-2555

Fax: (650) 225-4682/ (650) 225-4683

AND:

Study Coordination Center/Principal Investigator: Sara Tolaney

Contact Information:

[redacted]

MedWatch 3500 Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500 form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics

- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500 report and submitting as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500 form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted). The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above.

Study Drug Relationship:

The investigator will determine which events are associated with the use of study drug. For reporting purposes, an AE should be regarded as possibly related to the use of trastuzumab if the investigator believes:

- There is a clinically plausible time sequence between onset of the AE and trastuzumab administration; and/or
- There is a biologically plausible mechanism for trastuzumab to cause or contribute to the AE; and

The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

15.0 STUDY ORGANIZATION

This study will be run by the Dana-Farber Cancer Institute as part of the Dana Farber/Harvard Cancer Center.

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APPENDICES

APPENDIX 1

Baseline Assessment of Menses

Patient Initials: _____Subject Number:

Date Today:_____

1) Have you menstruated in the past 6 months?

☐ Yes ☐ No

2) When was the month and year of your last menstrual period (approximately)?
____ / _____ (MM/YYYY)

3) Do you consider yourself pre-menopausal at this time?

☐ Yes ☐ No ☐ Unsure

4) Have you taken any hormonal agents (e.g. birth control pills or steroids or fertility drugs) in the past 6 months?

☐ No

If yes, please explain: _____

5) Approximately how often did you have a menstrual period in the 6 months prior to diagnosis?

☐ once every month ☐ once every 2 months ☐ 2 times per month
☐ once every 2-6 months ☐ less often than every 6 months ☐ none

Appendix 2

Follow-Up Menses Assessment
Patient Initials: _____ Subject Number:_____

Date Today:_____

1) Have you menstruated in the past 6 months?

☐ Yes ☐ No

2) When was the month and year of your last menstrual period (approximately)?
_____ / _____ (MM/YYYY)

3) Approximately how often have you had your menstrual period over the past six months?

☐ once every month ☐ once every 2 months ☐ 2 times per month
☐ once every 2-6 months ☐ less often than every 6 months ☐ none

4) Are you currently taking any of the following?

☐ Tamoxifen (date started :_____)

☐ Ovarian suppression (date started :_____)

☐ Birth control pill (date started :_____)

☐ Aromatase inhibitor (date started :_____)

☐ Other hormonal agent (_____date started :_____)

Appendix 3
National Cancer Institute Common Toxicity Criteria V4.0

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

Appendix 4

ECOG Performance Status*

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

***obtained from: http://www.ecog.org/general/perf_stat.html**

Appendix 5 **Treatment Completion/Off-Study Form**

QUALITY ASSURANCE OFFICE FOR CLINICAL TRIALS, OS-200
Dana-Farber/Harvard Cancer Center, [redacted]

TREATMENT ENDED/OFF STUDY FORM
INSTRUCTIONS: To be completed when a subject completes protocol treatment and/or when the subject comes off-study. Please fill out all the information requested below and email to the QACT registrars at [redacted]

STUDY SUBJECT INFORMATION

Subject Name (Last, First) _____

Hospital I.D. #

Protocol Number or Name

****

Date Treatment Ended
(Last day of protocol treatment; i.e., chemotherapy, radiotherapy)

Reason 1. Subject Canceled (never began treatment)

***If Reason 4* – Specify Toxicity Phase I DLT Y/N**

***If Reason 7* – Date of Death**

***If Reason 9* – Other (please describe)**

Will the subject continue on follow-up? (Y/N)

****

A. *Date Off Study*
(Last day patient was being followed on protocol) Leave blank if patient remains in follow-up.
B.
C. Reason 1. Subject Canceled (never began treatment)

***If Reason 4* – Specify Toxicity Phase I DLT Y/N**

***If Reason 7* – Date of Death**

***If Reason 9* – Other (please describe)**

****

Completed By Phone# Date

Definitions for the Treatment Ended/Off Study Form

DATE TREATMENT ENDED	This is when protocol treatment for a subject (i.e. chemotherapy, radiation, surgery etc) is stopped due to the various reasons listed below (1-10). The date treatment ended is linked to Chemo-order entry as applicable. Therefore, it is critical that as subjects complete protocol therapy that this is documented in the QACT registration system.
OFF STUDY	This is when the subject will no longer be followed on the protocol due to the reasons listed below (1-10). Subjects can come off treatment before they come off study if they are still being followed on protocol or, they may come off treatment at the same time. This often is protocol dependent**.**
1. Subject Canceled-	This is selected only when a subject never starts protocol treatment (i.e. chemotherapy, radiation, surgery etc). This should be selected for *both* off treatment and off study if the subject never started treatment.
2. Subject Ineligible-	**This is selected when a subject has already begun treatment (i.e. chemotherapy, radiation, surgery etc) and has been deemed ineligible. Some protocols require follow-up for ineligible subjects so the subject may continue on follow-up. This may be selected for off treatment or off study**. **NOTE:** The subject will be taken *Off Treatment* **AND** taken *Off Study* when selecting this number, unless 'Yes' is selected for Follow-up.
3. Subject Completed Protocol Requirements	**This is selected when a subject has finished the protocol requirements. (may mean treatment is finished or follow-up is finished). This may be selected for off treatment or off study.**
4. Unacceptable Toxicity	This is selected when a subject experiences toxicity that either the subject or physician find unacceptable. Please specify the toxicity and if a Phase I trial select Yes or No to indicate if the toxicity is a protocol DLT. This may be selected for off treatment or off study.
5. Progressive disease/Relapsed	This is selected when a subject's disease worsens and meets the criteria in the protocol for progression; i.e. the tumor is measured and evaluated according to the tumor evaluation criteria written in the protocol (RECIST, WHO, etc) and it is deemed "progressive" (the tumor has grown larger or the disease has spread to other parts of the body). This may be selected for off treatment or off study.
6. Subject Withdrawal of Consent and Follow-up	**This is selected when a subject, at any time during the study, decides to no longer participate in either the treatment portion of the protocol or the follow-up portion. Subject must be off treatment and off study.**
7. Subject Died-	If the subject dies while still receiving protocol treatment this option can be chosen for off treatment. If the subject has completed treatment but is in follow-up then this reason can be chosen for off-study but should not be selected for off-

	treatment. Please indicate the date of death when selecting this number. **NOTE:** The subject will be taken *Off Treatment* **AND** taken *Off Study* when selecting this number. **For DFCI only**: PI or study staff designee must notify the Medical Record Department/Health Information Services, dfcideceasedpatients@partners.org
8. Lost to Follow-up	This is selected when the subject cannot be located during the treatment or follow-up portion of the protocol and all communication is lost. Subject must be off treatment and off protocol.
9. Other	This is selected when the reason for stopping protocol treatment does not meet any of the other reasons listed on this form. A description of the reason MUST be written in. This may be selected for off treatment or off study.
10. Subject Withdrawal of Consent but Agrees to be Followed	This is selected when a subject, at any time during the study, decides to no longer participate in the treatment portion of the protocol *BUT* agrees to participate in the follow-up portion. Used for off treatment only.
11. Subject unable to proceed to treatment	This is selected when a subject meets eligibility requirements, is registered, and has study specific procedures performed but is unable to proceed to the study treatment (for example: transplant). This could be due to progression prior to the study intervention, subject no longer medically fit to receive transplant, donor unavailable, or in the case of vaccine studies, vaccine cannot be made. This should be selected for both off treatment and off study.

Appendix 6

Blood Collection Requisition Form

Blood Samples

1. Complete this requisition form and deliver with sample.
2. Label ALL tubes

Sample information

Patient Name: _____

Patient MRN: _____

Patient Case Number: _____

Protocol Number: _____

Date of Collection: _____

Comments: _____

Contact Information

Name: _____

Telephone Number: _____

Address: _____

_____

_____

APPENDIX 7
Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan

1.0 INTRODUCTION

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

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center (DF/HCC) Multi-center protocol will comply with Federal regulations (21 CFR Part 11); Good Clinical Practice (GCP) Guidelines; and Health Insurance Portability and Accountability Act (HIPAA) requirements in accordance with the CTEP Multi-center Guidelines.

1.2 Multi-Center Data and Safety Monitoring Plan Components

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

DF/HCC Multi-center Protocol: One or more outside institutions collaborating with Dana-Farber/Harvard Cancer Center on a research protocol where DF/HCC is the Lead Institution. *Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates are not viewed as outside sites in this definition.*

Lead Institution: Dana-Farber Cancer Institute will be the Lead Institution and will be responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (CTEP, FDA, OBA etc.).

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

Protocol Chair: Dr. Sara Tolaney is the Principal Investigator for the DF/HCC protocol submitted as the Lead Institution. For applicable protocols, the Protocol Chair will be the single liaison with any regulatory agencies (ie.CTEP Protocol and Information Office (PIO), FDA, OBA etc.).

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

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

2.0 GENERAL ROLES AND RESPONSIBILITIES

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

2.1 Protocol Chair (DF/HCC Principal Investigator)

The Protocol Chair will accept responsibility for all aspects of the Multi-Center Data and Safety Monitoring Plan to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Submit the Multi-Center Data and Safety Monitoring Plan as an inclusion to the protocol.
- Assure all participating institutions are using the correct version of the protocol.
- Monitor progress and overall conduct of the study at all participating institutions.
- Ensure all DFCI IRB, DF/HCC and other applicable (i.e. CTEP, FDA, OBA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with CTEP/PIO Office (CTEP trials) or FDA (sponsor-investigator IND trials) or OBA (gene therapy trials), as applicable.
- Identify participating institutions and obtain accrual commitments. The Protocol Chair will also submit a protocol attachment labeled as "Participating Investigators" to the DFCI IRB and if applicable CTEP, FDA or OBA that provides the names and contact information for all participating institutions that perform the function of recruiting, enrolling, and treating participants for the protocol. The Coordinating Center (Lead Institution or designee) must be designated on the title page. Revisions to the list will be submitted to the DFCI IRB and if applicable NCI/CTEP Protocol Information Office (PIO), FDA or OBA as an administrative protocol amendment to reflect changes in staff and assignment of responsibility as they occur.

2.2 Coordinating Center (Lead Institution)

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

- Assist in protocol review.
- Maintain copies of Institutional Review Board (IRB) approvals and Office for Human Research Protections (OHRP) form 310 from all participating institutions.
- Maintain CTEP, FDA or OBA correspondence, as applicable.
- Ensure all participating institutions' OHRP forms 310 have been submitted to CTEP/PIO, if applicable.
- Maintain updated roster of participants.

- Verify eligibility.
- Verify response.
- Collect data on protocol specific CRFs.
- Prepare all submitted data for review by the Protocol Chair.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to Protocol Chair for timely review.
- Distribute external Serious Adverse Event safety reports.
- Monitor and audit Participating Institutions either by on-site inspection of selected participant records and/or with submitted source documents and research records submitted to the Lead Institution.

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

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide Central Participant Registration.
- Confirm eligibility and consent.
- Provide auditing services.

2.3 Participating Institution

The Participating Institution(s) will be identified on the title page for each protocol. In addition, each participating institution will provide to the Lead Institution or designee a list of the key personnel assigned to the role for oversight of data management at their site. All sites must have office space, office equipment, and internet access that meet HIPAA standards.
The general responsibilities for each participating institution are as follows:

- Commit to accrual to the Lead Institution's (DF/HCC) protocol.
- Submit protocol and/or amendments to their local IRB.
- Update Coordinating Center (Lead Institution or designee) with research staff changes on a timely basis.
- Register participants through the QACT.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center (Lead Institution or designee).
- Submit Serious Adverse Event reports directly to the Coordinating Center (Lead Institution or designee). For CTEP trials, submit SAE reports directly to CTEP and provide copies to the Coordinating Center (Lead Institution or designee).
- Submit deviations and violations to the Coordinating Center (Lead Institution or designee).
- For protocols using investigational agents, the participating institution will order their own investigational agents regardless of the supplier (i.e. NCI, pharmaceutical company).

3.0 DF/HCC QUALITY ASSURANCE OFFICE FOR CLINICAL TRIALS

The DF/HCC QACT is a unit that has been developed to collect, manage, and monitor data for DF/HCC trials. The DF/HCC QACT is located administratively in the office of the Senior Vice

President for Clinical Research, at Dana-Farber Cancer Institute. The QACT uses DF/HCC computerized institutional databases for participant registrations and for the management of trial data as well as a set of quality assurance programs designed to monitor DF/HCC trials.

3.1 Organizational Structure

The DF/HCC Quality Assurance Office for Clinical Trials administrative structure consists of:

DF/HCC Quality Assurance Officer for Clinical Trials: Oversees the functions of the DF/HCC QACT.

QACT Assistant Director for Data: Provides direct oversight to the QACT Data Analysts assigned to CRF design, data collection and computerization for DF/HCC trials.

The DF/HCC QACT Data Analysts will be assigned on a protocol-by-protocol basis. Each protocol's data analyst is responsible for database management, data entry, data quality assurance, and protocol specific correspondence related to the collection and quality assurance of data.

QACT Assistant Director for Monitoring: Provides direct oversight to the QACT Protocol Registrars and Clinical Research Auditors.

The DF/HCC Protocol Registrars are responsible for the confirmation of each participant's eligibility and consent prior to protocol registration.

If funded and QACT approved, the DF/HCC Clinical Research Auditors may assist the Lead Institution in their auditing responsibilities for multi-center trials. The QACT auditor is responsible for systematically evaluating participant safety, protocol compliance, institutional SOPs, ICH GCP and Federal regulation compliance, data accuracy and investigational drug handling to assure a high standard of quality for DF/HCC trials.

4.0 PROTOCOL DEVELOPMENT

4.1 Activation of a Protocol

The Protocol Chair is responsible for the coordination, development, and approval of the protocol as well as its subsequent amendments, and reporting SAEs, violations and deviations per DFCI IRB guidelines and if applicable CTEP Multi-center, FDA or OBA Guidelines. Further, the Protocol Chair will be the single liaison with the CTEP/PIO, the FDA or OBA, as applicable.

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

- Inclusion of the DF/HCC Multi-Center Data and Safety Monitoring Plan in the protocol as an appendix.
- Identify participating institutions and obtain accrual commitments. The Protocol Chair will also submit a protocol attachment labeled as "Participating Investigators" to the DFCI IRB and if applicable CTEP, FDA or OBA, that provides the names and contact

information for all participating institutions that perform the function of recruiting, enrolling, and treating participants for the protocol. The Coordinating Center (Lead Institution or designee) must be designated on the title page.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the Protocol Chair.
- Ensure that there is only one version of the Protocol and that all Participating Institutions use the correct version.
- Oversee the development of data collection forms (case report forms) that are of common format for use at all the Participating Institutions.

4.2 Coordinating Center Support Function

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

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

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

5.0 PROTOCOL MANAGEMENT

The Coordinating Center (Lead Institution of designee) is responsible for assuring that each Participating Institution in the DF/HCC Multi-center Protocol has the appropriate assurance on file with the Office of Human Research Protection (OHRP). Additionally, the Lead Institution or designee must maintain copies of all IRB approvals, for each participating institution.

5.1 Protocol Distribution

The Coordinating Center (Lead Institution or designee) will distribute the final approved protocol and any subsequent amended protocols to all Participating Institutions.

5.2 Protocol Revisions and Closures

The participating institutions will receive phone, fax, mail or e-mail notification of protocol revisions from the Lead Institution or designee. It is the individual participating institution's responsibility to notify its IRB of these revisions.

Non life-threatening revisions: Participating institutions will receive written notification of protocol revisions regarding non life-threatening events from the Lead Institution or designee. Non-life-

threatening protocol revisions should be implemented within 90 business days from receipt of the notification.

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

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

5.3 NCI Physician Investigator Registration

All physicians registering participants on NCI funded investigational drug trials must have submitted a Form FDA 1572 to the NCI and possess an active investigator number. Each institution shall submit their FDA 1572 forms to the NCI and a copy of FDA 1572 form, Curriculum Vitae (CV), and NCI investigator number to the Lead Institution or designee annually. Physicians should not register participants to DF/HCC studies without an active FDA 1572, current CV, and NCI investigator number.

5.4 IRB Documentation

The following must be on file with the DF/HCC Lead Institution or designee prior to participant registration:

- Approval Letter of the institution's IRB **(**An Expedited IRB first approval is NOT acceptable)
- IRB approval for all amendments

It is the individual institution's responsibility to notify its IRB of protocol revisions. Participating institutions will have 90 days from receipt to provide the DH/HCC Lead Institution or designee their IRB approval for Major Amendments* to a protocol.

*** DF/HCC defines a Major Amendment** as: A substantive change in the study which may increase or decrease the risk to study participants. Major revisions require full IRB approval. The following criteria are examples of revisions to a protocol that are considered to be major amendments:

- Change of eligibility (inclusion/exclusion) criteria
- Change in design of protocol
- Change in statistical section
- Change in sample size/accrual (e.g., doubling the sample size)
- Change in informed consent
- Change of estimated dropout rate
- Change of treatment or intervention
- Change of device

- Change in primary objective evaluation process

5.5 IRB Re-Approval

Annual IRB re-approval from the Participating institution is required in order to register participants onto a protocol. There is no grace period for annual re-approvals.

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

5.6 Participant Confidentiality and Authorization Statement

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

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

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

5.7 DF/HCC Multi-center Protocol Case Number

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

5.8 DF/HCC Multi-center Protocol Registration Policy

5.8.1 Initiation of Therapy: Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the participant's Registration Confirmation memo from the DF/HCC QACT. Therapy must be initiated per protocol guidelines. The Protocol Chair and DFCI IRB must be notified of any exceptions to this policy**.**

5.8.2 Eligibility Exceptions: The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol. In addition, the Cancer Therapy Evaluation Program (CTEP) specifically prohibits registration of a participant on any NCI Sponsored protocol that does not fully and completely meet all eligibility requirements. The DF/HCC QACT requires each institution to fully comply with this requirement.

5.8.3 Verification of Registration, Dose Levels, and Arm Designation: A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one working day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

5.8.4 Confidentiality: All documents, investigative reports, or information relating to the participant are strictly confidential. Any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Lead Institution or designee must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number and protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification.

5.9 Schedule of Data Submission

The DF/HCC QACT develops a set of either paper or electronic case report forms, (CRF/eCRFs) for use with the DF/HCC Multi-Center Protocol. These forms are designed to collect data for each study. The schedule for submission of case report forms to the DF/HCC QACT is generally specified in each protocol.

5.10. Eligibility Checklist

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

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

If a time frame is not specified in the protocol, tests must be completed as follows:

- Lab tests required for eligibility must be completed within 14 days prior to study enrollment by the QACT.
- For protocols requiring measurable disease, lab baseline measurements must be completed within 14 days prior to study enrollment by the QACT. Examples: flow cytometry, HLA typing, fluid cytology, tumor markers and hormones (CEA, CA-27-29, CA-125).
- Non-lab tests required for eligibility must be performed within 30 days prior to study entry. Example: radiological scans

- **Schedule for Submission -** Completed prior to participant registration. The Informed Consent/ Participant Authorization for the Release of Personal

Health Information should be submitted with the Eligibility Checklist at the time of registration.

5.10.1 Off Treatment/Off Study Form

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

Schedule of Submission – Submitted within 14 days after completing treatment or taken off study for any reason.

5.10.2 Follow up / Survival Form

Purpose - Summarizes participant status at a given point in time after being removed from treatment.

Schedule of Submission – Submitted within 14 days after the protocol defined follow up visit date or call.

5.11 Data Form Review

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

Timeliness:
Did the form arrive on time as specified in the protocol?

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

Participant Eligibility:
Does the participant meet the eligibility requirements for the study based on the demographic data, lab values and measurements provided?

Stratification:
Are the stratification parameters consistent with what was given at the time of registration?

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

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

Notations concerning adverse events will address relationship to protocol treatment for each adverse event grade. All adverse events encountered during the study will be evaluated according

to the NCI Common Toxicity Criteria assigned to the protocol and all adverse events must be noted on the participant's Adverse Event (Toxicity) Forms.

5.12 Missing and Deficient Memorandum

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

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written query from the DF/HCC QACT Data Analyst. Responses to the query should be completed and returned within 14 days. Responses may be returned on the written query or on an amended case report form. In both instances the query must be attached to the specific data being re-submitted in response.

Missing Forms

If study forms are not submitted on schedule, the participating institution will receive a Missing Form Report from the DF/HCC QACT noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed a minimum of three times a year.

6.0 REQUISUITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is generally specified in the protocol.

Participating sites should order their own agent regardless of the supplier (i.e., NCI or a pharmaceutical company.)

If the agent is commercially available, check with the local Director of Pharmacy and/or the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB. If the agent is investigational, ensure that the pharmacy will be able to receive and store the agent. The local IRB should be kept informed of who will supply the agent (i.e., NCI or a pharmaceutical company) so that any regulatory responsibilities can be met in a timely fashion.

7.0 SAFETY ASSESSMENTS AND TOXICITY MONITORING

All participants receiving investigational agents will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported to the investigator by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria assigned to the protocol (CTCAE Version 3.0) and recorded prior to each course of therapy. Life-threatening toxicities should be reported immediately to the Protocol Chair and Institutional Review Board (IRB). Protocols using CTEP supplied agents should also report these toxicities via the AdEERS system.

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

7.1 Serious Adverse Events

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

Unless otherwise specified in the protocol, the study will utilize the Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 for Toxicity and Adverse Event reporting. A copy of the CTC or CTCAE Criteria can be downloaded from the CTEP home page (http://ctep.info.nih.gov).

7.2 Guidelines for Reporting Serious Adverse Events

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

Participating Institutions must report serious adverse events for CTEP trials online using AdEERS. This applies to any medical event equivalent to an unexpected grade 2 with a possible, probable or definite attribution, most grade 3 and all grade 4 and grade 5 (death) toxicities regardless of study phase or attribution.

In addition, the Participating Institutions must report the serious adverse events to the Protocol Chair and the Coordinating Center (Lead Institution) at the time SAEs are submitted.

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

7.3 Guidelines for Processing IND Safety Reports

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent. In compliance with these FDA regulations, CTEP will notify the Participating sites via the DF/HCC Lead Institution by the following methods:

IND Safety Reports: Investigators will be sent a copy of expedited adverse events which CTEP has sent to the FDA. CTEP will notify the Protocol Chair at the Lead Institution. Within 7 business days of receipt of the notification, the Lead Institution or designee will forward the letters to the Participating sites with protocol specific instructions for IRB submissions, participant notifications, etc. For routine IND Safety Reports, CTEP does not generally require an immediate revision to the

protocol and/or model informed consent documents. The investigators are to file a copy with their protocol file and send a copy to their IRB according to their local IRB's policies and procedures.

8.0 PROTOCOL VIOLATIONS AND DEVIATIONS

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

8.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol.

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

8.2 Reporting Procedures

The Protocol Chair: is responsible for ensuring that clear documentation is available in the medical record to describe all protocol deviations and violations.
The Protocol Chair will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

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

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

9.0 QUALITY ASSURANCE

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

9.1 Ongoing Monitoring of Protocol Compliance

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion. The Lead Institution or designee and if applicable QACT Data Analysts assigned to the Protocol will perform the ongoing protocol compliance monitoring with the support of the participating institution's Coordinators, the Principal Investigators, and the Protocol Chair.

9.2 Evaluation of Participating Institution Performance

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

9.2.5 Accrual of Eligible Participants: Annual accrual rates for eligible participants enrolled onto therapeutic clinical trials is calculated for each institution. Institutions are expected to maintain the minimum annual average accrual as defined by the protocol grant or contract.

9.3 On-Site Auditing

9.3.1 DF/HCC Sponsored Trials

For all DF/HCC sponsored protocols:
The participating institutions may be required to submit subject source documents to the DF/HCC Lead Institution or designee for monitoring. Also, the participating institution may be subject to on-site monitoring conducted by the DF/HCC Lead Institution or designee.

After a participating site has accrued 4-5 participants, the QACT will be conducting an on-site audit. This is anticipated to occur within the first year of enrollment as specified in the protocol, section 13.3. One visit to each site will be planned during the 18 month expected enrollment period of the trial. If significant violations are uncovered during the audit, a follow-up audit may be requested. The final audit reports for the external sites will be reported to the Overall PI, as well as the DF/HCC Audit Subcommittee, as is standard practice.

9.3.2 Participating Institution

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

9.3.3 Coordinating Center (Lead Institution or designee)

The Protocol Chair will review all DF/HCC Multi-Center Protocol Final Audit reports and corrective action plans if applicable. The Lead Institution or designee must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Subcommittee. Based upon the audit assessments the DF/HCC Audit Subcommittee could accept or conditionally accept the audit rating and final report. Conditional approval could require the Protocol Chair to implement recommendations or require further follow-up. For unacceptable audits, the Audit Subcommittee would forward the final audit report and corrective action plan to the Clinical Investigations Policy and Oversight Committee and the DFCI IRB as applicable.

9.4 Sub-Standard Performance

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

9.4.1 Corrective Actions: Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, and adherence to protocol requirements will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by

the improved performance measures. Institutions that fail to demonstrate significant improvement will be considered by the Protocol Chair for revocation of participation.