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PROTOCOL

STUDY TITLE SINGLE ARM, NEOADJUVANT, PHASE II TRIAL OF PERTUZUMAB AND TRASTUZUMAB ADMINISTERED CONCOMITANTLY WITH WEEKLY PACLITAXEL AND FEC FOR CLINICAL STAGE I-III HER2-POSITIVE BREAST CANCER

STUDY DRUGS:

HERCEPTIN (trastuzumab)

PERJETA (pertuzumab)

PACLITAXEL (Taxol),5-FLUOROURACIL (5FU), EPIRUBICIN (Ellence), CYCLOPHOSPHAMIDE (Cytoxan)

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1. INTRODUCTION

1.1 DISEASE BACKGROUND

Each year approximately 40,000 women are diagnosed with clinical stage I-III HER2-positive breast cancer. Neoadjuvant chemotherapy is often administered to these patients because clinical tumor response is very common, is associated with smaller tumor resection and higher rates of breast conservation, and the extent of residual cancer after chemotherapy provides important prognostic information (Wolff et al 2008, Symmans et al, 2007). Patients with pathologic complete response (pCR) have excellent overall survival and therefore an important clinical research direction is to develop regimens that maximize pCR. The US Food and Drug Administration (FDA) has recently recognized this strong association between pCR and long-term survival in this disease subset and expressed interest in accepting this endpoint for accelerated drug approval (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM305501.pdf).

The purpose of this Phase II study is to estimate the pathologic complete response rate when pertuzumab is added to the regimen that currently produces the highest pCR rates in HER-2 positive breast cancers, weekly trastuzumab/paclitaxel followed by trastuzumab/FEC (5-fluorouracil/epirubicin/ cyclophosphamide), in a single-arm phase II neoadjuvant study. Pathologic complete response is defined as complete absence of any viable invasive cancer cells in the resected breast and lymph nodes. Because pCR rates differ between estrogen receptor (ER) positive and ER negative breast cancers, we will determine the sample size and analyze pCR rates separately for ER positive and negative cancers. However, this is conducted as a single clinical trial, although accrual may finish sooner for one cohort than for the other (see statistical section). The goal is to estimate if pCR rates could be improved from 50% to 70% in ER positive breast cancers and from 70% to 90% in ER negative breast cancers by adding pertuzumab to the weekly trastuzumab/paclitaxel followed by trastuzumab/FEC treatment regimen.

1.2 HER2 AND BREAST CANCER

Growth factors and their receptors play critical roles in development, cell growth, differentiation, and apoptosis (Cross and Dexter 1991). Such receptors span the cell membrane, with the extracellular domain binding specific growth factors and the intracellular domain transmitting growth signals. Interaction of the extracellular domain with its cognate ligand typically results in activation of intracellular tyrosine kinase activity. Overexpression of human epidermal growth factor receptor 2 (HER2, also known as *erb*B2, neu, and p185HER2) is observed in approximately 25-30% of human breast cancers (Slamon et al. 1987). HER2 overexpression has been reported to only rarely occur in the

absence of gene amplification (Kallioniemie et al. 1992; Pauletti et al. 1996). High level of HER2 expression has been correlated with poor clinical outcome (Slamon et al. 1987).

Several lines of evidence support a direct role for HER2 overexpression in the pathogenesis and poor clinical course of human tumors (Hynes 1994). When the mutated gene is transfected into murine fibroblast (NIH 3T3) cells, it causes transformation, and the resulting cells are tumorigenic in nude mice (Di Fiore et al. 1987; Hudziak et al. 1987). Additionally, transgenic mice that overexpress the rodent homolog of the human HER2 gene develop mammary cancer (Guy et al. 1992). Finally, specific antibodies to the extracellular domain of HER2 inhibit the experimental growth of tumors that overexpress the gene (Drebin et al. 1985, 1988; Fendly et al. 1990). These data suggest a direct role for HER2 in both malignant transformation and enhanced tumorigenicity. Therefore a strategy to antagonize the abnormal function of overexpressed HER2 was developed to improve the course of patients with HER2-overexpressing tumors. Monoclonal antibodies directed against the HER2 protein were developed and humanized to minimize the likelihood of immunogenicity. One of these antibodies (trastuzumab) was very effective in inhibiting both in vitro and in vivo proliferation of human breast cancer tumor cells overexpressing the HER2 protein and in mediating antibody-dependent cellular cytotoxicity in the presence of human effector cells (Jurianz et al. 1999).

There is substantial preclinical evidence that inhibition of signal transduction pathways can potentiate the cytotoxic activity of chemotherapeutic drugs. Indeed, trastuzumab has been shown to have synergy, in vitro and in vivo, with several chemotherapeutic drugs including cisplatin, doxorubicin, thiotepa, etoposide, vinorelbine, and taxanes (Pegram et al. 2000; Pietras et al. 1994; Arteaga et al. 1994; Hancock et al. 1991; Baselga et al. 1998; Pegram et al. 1997). Given this promising preclinical data, trastuzumab was tested in the clinic both as a single agent and in combination with chemotherapy.

1.3 TRASTUZUMAB AND PERTUZUMAB CLINICAL EXPERIENCE

Trastuzumab Clinical Experience in Metastatic Breast Cancer

The clinical benefit of trastuzumab in women with metastatic breast cancer has been demonstrated in two pivotal studies. A large Phase II trial (H0649g) assessed the activity of trastuzumab as a single agent in 222 women with HER2 overexpressing metastatic breast cancer with progressive disease after one or more chemotherapy regimens (Cobleigh et al. 1999). A blinded, independent response evaluation committee identified 8 complete and 26 partial responses, for an objective response rate of 15% in the intent-to-treat population (95% confidence interval, 11% to 21%). The median duration of response was 9.1 months, and the median survival was 13 months. The most common adverse events, which occurred in approximately 40% of patients, were mild to moderate infusion-associated fever and/or chills. These symptoms usually occurred only

during the first infusion. The most clinically significant event was cardiac dysfunction, which occurred in 4.7% of patients.

A large, open-label, randomized Phase III study (H0648g) in 469 patients with HER2-positive metastatic breast cancer was conducted to evaluate the efficacy of trastuzumab in combination with chemotherapy as first-line treatment. Patients who were anthracycline-naïve were randomized to receive either anthracycline plus cyclophosphamide (AC) or trastuzumab plus AC. Patients who had received prior anthracyclines in the adjuvant setting were randomized to receive either paclitaxel or trastuzumab plus paclitaxel. Patients randomized to trastuzumab and chemotherapy measurably benefited in comparison to patients treated with chemotherapy alone in terms of time to disease progression, overall response rate, median duration of response, and survival. As determined by an independent Response Evaluation Committee (REC), trastuzumab prolonged median time to disease progression from 4.6 months to 7.4 months ($p<0.001$), improved the overall response rate (complete and partial responses) from 32% to 50% ($p<0.001$), and increased median duration of response from 6.1 to 9.1 months ($p<0.001$). Compared to chemotherapy alone, the addition of trastuzumab significantly lowered the incidence of death at one year from 33% to 22% ($p=0.008$) and increased median overall survival 24% from 20.3 months to 25.1 months ($p=0.046$). The observed survival advantage remained despite crossover of 66% of patients initially randomized to chemotherapy alone who elected to receive trastuzumab upon disease progression (Tripathy et al. 2000). Fever/chills were observed with the initial trastuzumab infusion in approximately 25% of patients. Class III or IV cardiac dysfunction was observed in 16% of the trastuzumab + AC subgroup; increasing age was an associated risk factor for the development of cardiotoxicity in this treatment cohort.

Based on these data, trastuzumab was approved by the U.S. Food and Drug Administration (FDA) for use in HER2-overexpressing metastatic breast cancer in combination with paclitaxel for first-line treatment and as a single agent for patients failing prior chemotherapy for metastatic disease. However, current usage patterns of trastuzumab indicate that the drug is now being used in a broader array of circumstances than in the pivotal clinical trials. Since initiation of the pivotal clinical trials, docetaxel has become a commonly used taxane in the treatment of metastatic breast cancer (Chevallier et al. 1995) and data have emerged on the weekly use of paclitaxel (Akerley et al. 1997). Trastuzumab has been studied in combination with paclitaxel and docetaxel using a variety of doses and schedules with promising results (Seidman et al. 1999; Nicholson et al. 2000; Kuzur et al. 2000). In addition, the combination of trastuzumab with vinorelbine has been studied (Burstein et al. 2001). In this study, 30 of 40 women treated with trastuzumab (4 mg/kg x 1, 2 mg/kg weekly thereafter) and vinorelbine (25 mg/m^2 weekly, with dose adjusted each week for neutrophil count) responded to therapy, for an overall response rate of 75% (95% confidence interval 57% to 89%). Neutropenia was the only grade IV toxicity. No patients had symptomatic heart failure. Grade 2 cardiotoxicity was observed in 3

patients; prior cumulative doxorubicin dose in excess of 240 mg/m^2 and borderline pre-existing cardiac function were associated with this toxicity.

Trastuzumab Clinical Experience in Adjuvant Breast Cancer

Four large, randomized, phase III trials showed significant reduction in risk of disease recurrence with the addition of 1 year of trastuzumab to adjuvant therapy in patients with HER2-positive, early breast cancer. The 3-year planned joint interim analysis of the National Surgical Adjuvant Breast and Bowel Project (NSABP B-31) and the North Central Cancer Treatment Group (NCCTG N-9831) trials demonstrated significant improvements in disease-free survival (DFS) (hazard ratio [HR] 0.48, p<0.0001) and overall survival (OS) (HR 0.67, p=0.015) when 1-year of trastuzumab is added to adjuvant chemotherapy in patients with HER2-positive breast cancer (Romond et al 2005). At the 4-year follow-up, DFS and OS results were consistent (Perez et al 2011). In the HERA trial, single agent trastuzumab given sequentially after adjuvant chemotherapy demonstrated significant improvements in DFS (HR 0.64, p<0.0001) and OS (HR 0.66, p=0.0115) compared with observation alone at a median follow-up of 23.5 months (Smith et al 2007). At a median follow-up of 48.4 months, a DFS benefit was observed (HR 0.66, p=0.0115) with 1 year of trastuzumab, however, the OS benefit was not statistically significant at 4 years (HR 0.85, p=0.11). At the time of analysis, over 50% of patients in the observation arm had crossed-over to receive trastuzumab (Gianni et al 2011) The third protocol-specified analysis of the Breast Cancer International Research Group (BCIRG) 006 study continued to show that the addition of 52 weeks of trastuzumab to docetaxel-based adjuvant regimens significantly improved DFS. At a median follow-up of 65 months, 5-year DFS rates were 84% (HR 0.64, p<0.001) and 81% (HR 0.75, p=0.04) in the doxorubicin-containing trastuzumab and non-anthracycline-containing trastuzumab arms, respectively (Slamon et al 2011).

Trastuzumab Clinical Experience in Neoadjuvant-Adjuvant Treatment of Early Breast Cancer

Study MO16432/NOAH investigated the effect of adding trastuzumab to neoadjuvant chemotherapy using doxorubicin plus paclitaxel, then paclitaxel, then cyclophosphamide plus methotrexate plus fluorouracil (CMF) in patients with HER2-positive locally advanced breast cancer. Patients who were randomized to trastuzumab received it for a total of one year before and after surgery. The primary endpoint was event-free survival; secondary endpoints included pathological complete response (pCR) rate, and safety. At the time of the primary analysis, 118 HER2-positive patients were enrolled in the chemotherapy-alone arm with 116 HER2-positive patients enrolled in the chemotherapy plus trastuzumab arm. Baseline characteristics were well-balanced across treatment arms. Thirty-six percent of HER2-positive tumors were hormone-receptor positive. In the HER2-positive population, the 3-year EFS was 71% (95% CI, 61%-78%) in the Herceptin-containing combination arm compared with 56% (95% CI, 46%-65%) in the chemotherapy-alone arm. The unadjusted HR were 0.59 (p=0.0123) for EFS and 0.62 (p=0.114) for overall

survival (OS) in the HER2-positive population. In the HER2-positive population, both ORR and pCR in breast tissue were significantly higher in the Herceptin with chemotherapy arm compared with chemotherapy alone: 87% vs 74% for ORR (p=0.009); 43% vs 22% for pCR in breast tissue (p=0.0007), respectively. Overall, treatment was well-tolerated with acceptable cardiac safety (Gianni et al 2010).

A Phase III neoadjuvant study compared four cycles of paclitaxel followed by four cycles of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) with and without weekly trastuzumab for 24 weeks. The study reported significantly higher pCR rate in the group receiving trastuzumab (66.7% vs 25%, p = 0.02) (Buzdar et al 2005). A three year follow up study showed 100% disease-free survival (p = 0.041) with 0% recurrence, with no cardiac dysfunction and no deaths from cardiac reasons (Buzdar et al 2007). Similar results were obtained by another group in Spain which reported 61% pCR with the same regimen (Pernas et al, 2012). A retrospective single institution study examining anthracycline and non-anthracycline based trastuzumab-containing neoadjuvant chemotherapies in HER2 positive breast cancer confirmed increased pCR rate in the anthracycline group (60.6% vs. 43.3%, p = 0.016) (Bayraktar et al, 2011). These pCR rates represent the highest rates reported in any clinical trial for the HER2 positive patient population.

Pertuzumab Clinical Experience

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

Trastuzumab and pertuzumab monoclonal antibodies bind to distinct epitopes on HER2 without competing with each other, resulting in distinctive mechanisms for disrupting HER2 signaling. These mechanisms are complementary and result in augmented therapeutic efficacy when pertuzumab and trastuzumab are given in combination. Preclinical data indicate at least additive efficacy when the two agents are administered together, resulting in significantly reduced tumor volume compared with either agent alone.

Clinically, pertuzumab may have optimal therapeutic effects when given in combination with trastuzumab to patients with HER2-positive cancers, evidenced by data generated in a Phase II study of patients with previously treated HER2-positive MBC (Baselga et al. 2010). A recently published meta-analysis of pertuzumab phase II trials concluded that pertuzumab has a low cardiac risk and there is no notable increase in cardiac events when pertuzumab is used in combination with other anticancer agents (Lenihan et al. 2011).

Trastuzumab and Pertuzumab Combination Therapy in Patients with HER2-positive Tumors

Metastatic Breast Cancer: In the Phase III, pivotal study WO20698/TOC4129g (CLEOPATRA; N=808) in patients with previously-untreated HER2-positive MBC, a statistically significant and clinically meaningful improvement in progression-free survival (PFS) was observed in patients treated with pertuzumab, trastuzumab and docetaxel (N=406) compared to those receiving placebo, trastuzumab and docetaxel (N=402). PFS was prolonged at the median by 6.1 months and the risk of disease progression or death was reduced by 38% (Hazard ratio [HR] = 0.62; 95% CI = 0.51, 0.75; p < 0.0001) with an improvement in median PFS from 12.4 months to 18.5 months (Baselga et al 2011). There were no significant differences in toxicity in the two treatment arms. In a Phase II, single-arm study (BO17929; N= 66) in patients with previously-treated HER2-positive MBC, four complete responses and 12 partial responses (24% objective response rate) were observed following combined treatment with pertuzumab and trastuzumab (Baselga et al 2007). The combination resulted in minimal cardiotoxicity, with no changes in overall mean LVEF.

Early Breast Cancer: In the Phase II study WO20697 (NEOSPHERE; N=417) patients with HER2-positive early breast cancer (EBC) receiving combination neoadjuvant therapy with pertuzumab, trastuzumab and docetaxel (N=107) had a pathological complete response (pCR) rate of 46%, compared with 29% in patients receiving trastuzumab plus docetaxel (N=107) (P=0.0141, 95% CI 21-39) (Ginani et al 2011). The Phase II study BO22280 (TRYPHAENA; N=223) investigated neoadjuvant pertuzumab and trastuzumab a) concomitantly with anthracycline-based treatment (N=72); b) following anthracycline-based treatment (N=75) or c) concomitantly with a carboplatin-based regimen in patients with HER2-positive EBC (N=76). All three treatment regimens were efficacious, with 57%-66% of patients achieving a pCR (Schneeweiss et al. 2011). The primary end point of this study was cardiac safety and it reported transient, reversible decline in left ventricular ejection fraction (defined as drop in LVEF > 10% or to below 50%) in 3.9% to 5.3% of patients.

Together, these findings suggest that pertuzumab confers minimal cardiotoxicity when added to trastuzumab, and that the pertuzumab/trastuzumab combination is more effective than either treatment alone.

1.4 SAFETY

TRASTUZUMAB SAFETY

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

containing regimen in metastatic breast cancer. Much of the cardiac dysfunction was reversible on discontinuation of trastuzumab.

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

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

PERTUZUMAB SAFETY

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

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

months after completion of treatment.

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

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

Hematologic Toxicity and Neutropenic Infections with Trastuzumab. In clinical trials, an increased incidence of anemia was observed in patients receiving trastuzumab plus chemotherapy compared with patients receiving chemotherapy alone. The majority of these anemia events were mild or moderate in intensity and reversible; none resulted in discontinuation of trastuzumab therapy. In clinical trials, the per-patient incidences of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving trastuzumab in combination with myelosuppressive chemotherapy as compared to those who received chemotherapy alone. In the post marketing setting, deaths due to sepsis in patients with severe neutropenia have been reported in patients receiving trastuzumab and myelosuppressive chemotherapy, although in controlled clinical trials (pre- and post-marketing), the incidence of septic deaths was not significantly increased. The pathophysiologic basis for exacerbation of of neutropenia has not been determined; the effect of trastuzumab on the pharmacokinetics of chemotherapeutic agents has not been fully evaluated.

Risk of Neutropenia with Pertuzumab and Pertuzumab/Trastuzumab combination. Neutropenic events are virtually absent with chemotherapy-free Pertuzumab regimens and with single-agent pertuzumab. In the pivotal study WO20698/TOC4129g incidence of neutropenic events was increased in patients receiving pertuzumab, trastuzumab and docetaxel, compared to patients in the placebo-controlled arm. This was largely driven by an increase in Grades 3 and

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

Secondary acute leukemia or myelodysplastic syndrome has been reported in 4 of approximately 1200 patients who participated in trastuzumab clinical trials. Patients treated with chemotherapeutic agents are known to be at increased risk for secondary leukemia. The observed incidence of leukemia among trastuzumab-treated patients appears to be consistent with the expected incidence of leukemia among patients treated with chemotherapy for metastatic breast cancer. Therefore, the contribution of trastuzumab to the etiology of acute leukemia or myelodysplastic syndrome in these cases is unclear.

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

Risk of EGFR-related toxicities. Although pertuzumab targets the HER2 receptor, it inhibits heterodimerization with other members of the HER family (eg, EGFR [HER1]). Accordingly, it may cause toxicities associated with the use of EGFR inhibitors such as diarrhea, rash and other dermatologic toxicities (eg, dry skin, pruritus, nail disorders, mucositis). Diarrhea**.** In the 7-week IV and 26-week toxicity studies in cynomolgus monkeys, there was a treatment-related increase in the incidence of diarrhea. Diarrhea has been observed in

approximately 60% of patients (treatment-related diarrhea in 50% of patients) being treated with pertuzumab in phase II single-agent studies, and up to approximately 70% of patients in combination therapy studies. Diarrhea was CTC Grade 1 or 2 in the majority of cases. To prevent dehydration, early treatment of diarrhea with anti-diarrheal medication should be considered and patients treated with fluids and electrolyte replacement, as clinically indicated. Rash has also been observed with EGFR inhibitors, mostly of mild to moderate intensity. Rash has been observed in approximately 17% of patients receiving pertuzumab in Phase II single-agent studies and up to 73% of patients in combination studies. The rash was generally of CTC Grade 1 or 2 in severity. Treatment recommendations for EGFR associated rash include topical or oral antibiotics, topical pimecrolimus, topical or (for severe reactions) systemic steroids. These agents may be used in patients experiencing pertuzumab-related rash, as clinically indicated, although they have not been studied in this context.

Risk of left ventricular dysfunction. Decreases in LVEF have been reported with drugs that block HER2 activity. Trastuzumab and pertuzumab both target HER2, thus there is a risk of cardiac dysfunction with these agents. In the CLEOPATRA pivotal trial WO20698/TOC4129g, pertuzumab in combination with trastuzumab and docetaxel was not associated with increases in the incidence of symptomatic LVSD or decreases in LVEF compared with placebo in combination with trastuzumab and docetaxel. Pertuzumab combined with trastuzumab and chemotherapy also did not result in any significantly greater incidence of symptomatic LVSD or decreases in LVEF than trastuzumab and chemotherapy in patients with EBC (Study WO20697). However, in the pivotal MBC trial (CLEOPATRA Study WO20698/TOC4129g) a greater proportion of patients who developed symptomatic LVSD had received prior anthracyclines and/or radiotherapy compared to the proportion of patients receiving prior anthracyclines and/or radiotherapy in the overall pertuzumab-treated population. Therefore patients who have received prior anthracyclines or prior radiotherapy to the chest area may be at higher risk of decreased LVEF.

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

1.5 TRASTUZUMAB AND PERTUZUMAB PHARMACOKINETICS

Trastuzumab

A Phase I single dose study (H0407g) of intravenous trastuzumab infusions ranging from 10-500 mg resulted in dose-dependent pharmacokinetics (PK) with

serum clearance of trastuzumab decreasing with an increasing dose at doses <250 mg. PK modeling of trastuzumab concentration-time data from 7 patients that were administered doses of 250 mg and 500 mg had a mean half-life of 5.8 days (range 1-32 days). Additionally, PK modeling showed that weekly trastuzumab doses ≥250 mg resulted in serum trough levels of >20 μg/mL that was above the minimum effective concentration observed in preclinical xenograft studies in tumor-bearing mice. The Phase I data supported the weekly dosing schedule that was implemented in all subsequent Phase II and Phase III clinical trials. A weight-based dose schedule was adopted after two Phase II trials (H0551g and H0552g) suggested that inter-subject variability in trastuzumab PK was related to body weight. These findings resulted in a trastuzumab dose schedule of a 4 mg/kg loading dose followed by a weekly 2 mg/kg maintenance dose utilized in the two pivotal Phase III trials (H0648g and H0649g) that were the basis of the BLA filing and subsequent FDA approval of trastuzumab for HER2+ metastatic breast cancer.

The trastuzumab PK data from studies H0407g (Phase I), H0551g (Phase II), and H0649 (pivotal) have been subsequently reanalyzed by a population PK approach using nonlinear mixed effect modeling (NONMEM). A linear two-compartment model best described the concentration-time data, and accounted for the accumulation of trastuzumab serum concentrations seen in the Phase II and Phase III clinical studies. A covariate analysis was conducted using the subjects from these single agent studies to evaluate the effect of pathophysiologic covariates (e.g. age, weight, shed antigen) on the PK parameter estimates. The covariates that significantly influenced clearance were the level of shed antigen and the number of metastatic sites. Volume of distribution was significantly influenced by weight and shed antigen level. Additionally, data from the Phase III study, H0648g, were added to assess the influence of concomitant chemotherapy on trastuzumab PK. Importantly, chemotherapy (AC or paclitaxel) did not significantly alter trastuzumab PK. The estimated half-life of trastuzumab based on the final model was 28.5 days.

Analysis of data obtained from two Phase II studies which utilized a loading dose of 8 mg/kg trastuzumab followed by a 6 mg/kg maintenance dose administered every 3 weeks (q3 week) as a single-agent, and in combination with paclitaxel (175 mg/m^2), confirmed that a two-compartment model best describes the PK of trastuzumab. Model-independent analysis of the of the data obtained in these studies gives comparable PK parameter estimates to those obtained by the population PK model, thus confirming the validity of the population PK model. In addition, the population PK model adequately predicted trastuzumab serum concentrations obtained independently in these studies. After two treatment cycles, trastuzumab exposures were similar to those measured in the once weekly dosing regimen used in the pivotal trials. Trough levels were in excess of the targeted serum concentrations established from preclinical xenograft models, and as expected, peak levels were greater than those observed upon weekly administration. The apparent half-life of trastuzumab in these studies was determined to be approximately 21 days, and the PK was supportive of a q3 week dosing schedule.

The efficacy and safety results from these Phase II studies with q3 week dosing do not appear to be different from those with weekly dose-schedules {ref Slamon, Cobleigh, Vogel}. In the trastuzumab q3 weekly monotherapy study, 105 patients with HER2+ metastatic breast cancer were treated, with an objective response rate of 19% (23% in patients with measurable centrally confirmed HER2+ disease). The median baseline LVEF was 63%, which did not significantly change during the course of the study. One patient experienced symptomatic CHF, which resolved with medical treatment for CHF and discontinuation of trastuzumab. In the study of q3 weekly trastuzumab and paclitaxel, 32 patients were treated with an investigator-assessed response rate of 59%. Ten patients had a decrease in LVEF of 15% or greater. One patient experienced symptomatic CHF, which improved symptomatically after medical therapy for CHF and discontinuation of trastuzumab.

Pertuzumab

Similar pharmacokinetics (PK) have been observed across clinical studies, with no dose dependent changes in clearance at doses ranging from 2.0 to 25.0 mg/kg (equivalent to 140 mg to 1750 mg for a 70 kg patient). A two-compartment model adequately described the concentration-time data with a systemic serum clearance of 0.239 L/day and a median terminal half-life of 17.2 days for a typical patient. Population PK analysis and simulations based on data from Phase Ib/II studies, with pertuzumab administered as a single agent, predicted that > 90% of patients receiving the fixed, non-weight-based dosing regimen (840 mg loading dose with a 420 mg maintenance dose every three weeks [q3w]) would have steady-state serum concentrations that were higher than the target serum concentration (> 20 µg/mL) identified in nonclinical xenograft models. This flat dosing regimen was used in subsequent studies. Population PK modeling of data from Phase I, II, and III studies supports the use of fixed, non-weight based dosing based on the rationale described above, regardless of sex and race (Japanese vs. Non-Japanese). Results from studies where pertuzumab was administered in combination with various small molecule chemotherapeutic agents (gemcitabine, capecitabine, erlotinib or docetaxel), indicate that pertuzumab does not alter the PK of these agents and the PK of pertuzumab is similar to that observed in single-agent pertuzumab studies. In addition, data from the Phase III trial WO20698/TOC4129g demonstrate that pertuzumab administration did not change the PK of trastuzumab, and there was no evidence of drug-drug interactions (DDI) when docetaxel was combined with pertuzumab plus trastuzumab.

1.6 OTHER STUDY DRUG(S) BACKGROUND

PACLITAXEL (TAXOL®)

Paclitaxel is a natural product with antitumor activity. Paclitaxel is obtained via a semi-synthetic process from *Taxus baccata.* The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-N-benzoyl-3-phenylisoserine. Paclitaxel is a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.91. It is highly lipophilic, insoluble in water, and melts at approximately 216°C to 217°C.Paclitaxel is an antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. Paclitaxel induces abnormal arrays of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Human Toxicology: Unless otherwise noted, the following discussion refers to the overall safety database of 812 patients with solid tumors treated with single-agent paclitaxel in clinical studies.

Hematologic: Bone marrow suppression was the major dose-limiting toxicity of paclitaxel. Neutropenia, the most important hematologic toxicity, was dose and schedule dependent and was generally rapidly reversible. Fever was frequent (12% of all treatment courses). Infectious episodes occurred in 30% of all patients and 9% of all courses; these episodes were fatal in 1% of all patients, and included sepsis, pneumonia and peritonitis. Thrombocytopenia was reported. Twenty percent of the patients experienced a drop in their platelet count below 100,000 cells/mm3 at least once while on treatment; 7% had a platelet count <50,000 cells/mm3 at the time of their worst nadir. Bleeding episodes were reported in 4% of all courses and by 14% of all patients, but most of the hemorrhagic episodes were localized and the frequency of these events was unrelated to the paclitaxel dose and schedule. In an adjuvant breast carcinoma trial, the incidence of severe thrombocytopenia and platelet transfusions increased with higher doses of doxorubicin. Anemia (Hb <11 g/dL) was observed in 78% of all patients and was severe (Hb <8 g/dL) in 16% of the cases. No consistent relationship between dose or schedule and the frequency of anemia was observed. Among all patients with normal baseline hemoglobin, 69% became anemic on study but only 7% had severe anemia. Red cell transfusions were required in 25% of all patients and in 12% of those with normal baseline hemoglobin levels.

Neurologic: In general, the frequency and severity of neurologic manifestations were dose-dependent in patients receiving single-agent paclitaxel. Peripheral neuropathy was observed in 60% of all patients (3% severe) and in 52% (2% severe) of the patients without pre-existing neuropathy. The frequency of peripheral neuropathy increased with cumulative dose. Paresthesia commonly occurs in the form of hyperesthesia. Neurologic symptoms were observed in 27% of the patients after the first course of treatment and in 34 to 51% from course 2 to 10. Peripheral neuropathy was the cause of paclitaxel discontinuation in 1% of all patients. Sensory symptoms have usually improved

or resolved within several months of paclitaxel discontinuation. Pre-existing neuropathies resulting from prior therapies are not a contraindication for paclitaxel therapy. Other than peripheral neuropathy, serious neurologic events following paclitaxel administration have been rare (<1%) and have included grand mal seizures, syncope, ataxia, and neuroencephalopathy.

Arthralgia/Myalgia: There was no consistent relationship between dose or schedule of paclitaxel and the frequency or severity of arthralgia/myalgia. Sixty percent of all patients treated experienced arthralgia/myalgia; 8% experienced severe symptoms. The symptoms were usually transient, occurred 2 or 3 days after paclitaxel administration, and resolved within a few days. The frequency and severity of musculoskeletal symptoms remained unchanged throughout the treatment period.

Gastrointestinal (GI): Nausea/vomiting, diarrhea, and mucositis were reported by 52%, 38%, and 31% of all patients, respectively. These manifestations were usually mild to moderate. Mucositis was schedule dependent and occurred more frequently with the 24-hour than with the 3-hour infusion.

Other Clinical Events: Alopecia was observed in almost all (87%) of the patients. Transient skin changes due to paclitaxel-related hypersensitivity reactions have been observed, but no other skin toxicities were significantly associated with paclitaxel administration.

Paclitaxel Pharmacology:

Kinetics: Following IV administration of TAXOL, paclitaxel plasma concentrations decline in a biphasic manner. The pharmacokinetics of paclitaxel have been shown to be nonlinear. On average, 89% of drug is bound to serum proteins; the presence of cimetidine, ranitidine, dexamethasone, or diphenhydramine does not affect protein binding of paclitaxel. Paclitaxel is extensively metabolized in the liver primarily to 6α-hydroxypaclitaxel by the cytochrome P450 isozyme CYP2C8; and to 2 minor metabolites, 3'-p-hydroxypaclitaxel and 6α, 3'-p-dihydroxypaclitaxel, by CYP3A4. In vitro, the metabolism of paclitaxel to 6α-hydroxypaclitaxel was inhibited by a number of agents (ketoconazole, verapamil, diazepam, quinidine, dexamethasone, cyclosporin, teniposide, etoposide, and vincristine). The pharmacokinetics of paclitaxel may also be altered in vivo as a result of interactions with compounds that are substrates, inducers, or inhibitors of CYP2C8 and/or CYP3A4. Patients with hepatic dysfunction had increased plasma paclitaxel exposure with no apparent increase in the frequency or severity of toxicity. The effect of renal dysfunction on the disposition of paclitaxel has not been investigated.

Formulation: Paclitaxel is available in 30 mg (5 mL), 100 mg (16.7 mL), and 300 mg (50 mL) multidose vials. 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.

Storage and Stability: Unopened vials of paclitaxel are stable until indicated date between 20°–25°C. Neither freezing nor refrigeration adversely affects the stability of the product. Solutions for infusion prepared as recommended are stable at ambient temperature (approximately 25° C) and lighting conditions for up to 27 hours.

5-FLUOROURACIL

5-fluoro-2,4-(1H,3H)-pyrimidinedione, 5-fluorouracil, is metabolized and blocks the methylation reaction of deoxyuridylic acid to thymidylic acid, interfering with DNA and RNA synthesis.

Human Toxicology:

Stomatitis and esophagopharyngitis (which may lead to sloughing and ulceration), diarrhea, anorexia, nausea and emesis are commonly seen during therapy. Leukopenia usually follows every course of adequate therapy with fluorouracil. The lowest white blood cell counts are commonly observed between the 9th and 14th days after the first course of treatment, although uncommonly the maximal depression may be delayed for as long as 20 days. By the 30th day the count has usually returned to the normal range.

Alopecia and dermatitis may be seen in a substantial number of cases. The dermatitis most often seen is a pruritic maculopapular rash usually appearing on the extremities and less frequently on the trunk. It is generally reversible and usually responsive to symptomatic treatment.

The administration of 5-fluorouracil has been associated with the occurrence of palmar-plantar erythrodysesthesia syndrome, also known as hand-foot syndrome. This syndrome has been characterized as a tingling sensation of hands and feet which progress over the next few days to pain when holding objects or walking. The palms and soles became symmetrically swollen and erythematous with tenderness of the distal phalanges, possibly accompanied by desquamation. Interruption of therapy is followed by gradual resolution over 5 to 7 days. Although pyridoxine has been reported to ameliorate the palmar-plantar erythrodysesthesia syndrome, its safety and effectiveness has not been established.

5-Fluorouracil Pharmacology:

Kinetics: Following IV injection, fluorouracil distributes into tumors, intestinal mucosa, bone marrow, liver and other tissues throughout the body. In spite of its limited lipid solubility, fluorouracil diffuses readily across the blood-brain barrier and distributes into cerebrospinal fluid and brain tissue. Seven percent to 20% of the parent drug is excreted unchanged in the urine in 6 hours; of this over 90% is excreted in the first hour. The remaining percentage of the administered dose is metabolized, primarily in the liver. The catabolic metabolism of

fluorouracil results in inactive degradation products, which are excreted in the urine. The mean half-life of 5-fluorouracil elimination from plasma is approximately 16 minutes and is dose dependent. No intact drug can be detected in the plasma 3 hours after an IV injection. 5-Fluorouracil should be used with extreme caution in patients with a history of impaired hepatic or renal function.

Formulation: 5-fluorouracil is supplied in 10 mL aliquots containing 500 mg fluorouracil, with pH is adjusted to approximately 9.2 with sodium hydroxide.

Storage and Stability: 5-fluorouracil should be stored at room temperature 15° to 30°C (59° to 86°F), protected from light, and retained in carton until time of use.

EPIRUBICIN (ELLENCE®)

Epirubicin hydrochloride is the 4-epimer of doxorubicin and is a semi-synthetic derivative of daunorubicin. The chemical name is (8*S*-*cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-arabinohexopyranosyl)oxy]-7,8,9,10-tetrahydro6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride. Epirubicin is an anthracycline cytotoxic agent that binds to DNA through intercalation, triggering DNA cleavage by topoisomerase II, and resulting in cell death.

Human Toxicology:

Hematologic: Dose-dependent, reversible leukopenia and/or neutropenia is the predominant manifestation of hematologic toxicity associated with epirubicin hydrochloride injection and represents the most common acute dose-limiting toxicity of this drug. In most cases, the white blood cell (WBC) nadir is reached 10 to 14 days from drug administration. Leukopenia/neutropenia is usually transient, with WBC and neutrophil counts generally returning to normal values by Day 21 after drug administration. Clinical consequences of severe myelosuppression include fever, infection, septicemia, septic shock, hemorrhage, tissue hypoxia, symptomatic anemia, or death.

Gastrointestinal: A dose-dependent mucositis (mainly oral stomatitis, less often esophagitis) may occur in patients treated with epirubicin hydrochloride injection. Clinical manifestations of mucositis may include a pain or burning sensation, erythema, erosions, ulcerations, bleeding, or infections. Mucositis generally appears early after drug administration and, if severe, may progress over a few days to mucosal ulcerations; most patients recover from this adverse event by the third week of therapy. Hyperpigmentation of the oral mucosa may also occur. Nausea, vomiting, and occasionally diarrhea and abdominal pain can also occur. Severe vomiting and diarrhea may produce dehydration. Antiemetics may reduce nausea and vomiting; prophylactic use of antiemetics before therapy should be considered.

Cardiovascular: In a retrospective survey, including 9,144 patients, mostly with solid tumors in advanced stages, the probability of developing CHF increased with increasing cumulative doses of epirubicin hydrochloride injection. The estimated risk of epirubicin hydrochloride injection-treated patients developing clinically evident CHF was 0.9% at a cumulative dose of 550 mg/m^2, 1.6% at 700 mg/m^2, and 3.3% at 900 mg/m^2. The risk of developing CHF in the absence of other cardiac risk factors increased steeply after an epirubicin hydrochloride injection cumulative dose of 900 mg/m^2. In another retrospective survey of 469 epirubicin hydrochloride injection-treated patients with metastatic or early breast cancer, the reported risk of CHF was comparable to that observed in the larger study of over 9,000 patients.

Cutaneous Reactions: Alopecia occurs frequently, but is usually reversible, with hair regrowth occurring within 2 to 3 months from the termination of therapy.

Secondary Leukemia: An analysis of 7,110 patients who received adjuvant treatment with epirubicin hydrochloride injection in controlled clinical trials as a component of poly-chemotherapy regimens for early breast cancer showed a cumulative risk of secondary acute myelogenous leukemia or myelodysplastic syndrome (AML/MDS) of about 0.27% (approximate 95% CI, 0.14 to 0.40) at 3 years, 0.46% (approximate 95% CI, 0.28 to 0.65) at 5 years, and 0.55% (approximate 95% CI, 0.33 to 0.78) at 8 years. The risk of developing AML/MDS increased with increasing epirubicin hydrochloride injection cumulative doses. The cumulative probability of developing AML/MDS was found to be particularly increased in patients who received more than the maximum recommended cumulative dose of epirubicin hydrochloride injection (720 mg/m^2) or cyclophosphamide (6,300 mg/m^2).

Epirubicin Pharmacology:

Epirubicin displays linear pharmacokinetics and its plasma concentration declines in a triphasic manner. Following IV administration, epirubicin is rapidly and widely distributed into the tissues. About 77% is bound to plasma proteins and is not affected by drug concentration. It also concentrates in red blood cells. Epirubicin is extensively and rapidly metabolized by the liver and red blood cells. Four main metabolic routes have been identified: (1) reduction of the C-13 keto-group with the formation of the 13(*S*)-dihydro derivative, epirubicinol; (2) conjugation of both the unchanged drug and epirubicinol with glucuronic acid; (3) loss of the amino sugar moiety through a hydrolytic process with the formation of the doxorubicin and doxorubicinol aglycones; and (4) loss of the amino sugar moiety through a redox process with the formation of the 7-deoxydoxorubicin aglycone and 7-deoxy-doxorubicinol aglycone. Epirubicinol has ~10% activity of epirubicin; no significant activity or toxicity has been reported for the other metabolites. Epirubicin and its major metabolites are eliminated primarily through biliary excretion and, to a lesser extent, by urinary excretion. Plasma clearance of epirubicin is reduced in elderly women, patients with hepatic dysfunction, and patients with significant kidney injury (creatinine >

5 mg/dL). Preadministration of paclitaxel (but not docetaxel), or coadministration of cimetidine causes variable increases in the mean AUC of epirubicin.

Formulation: Epirubicin hydrochloride injection is supplied as a sterile, clear, red solution in glass vials containing 50 or 200 mg of epirubicin hydrochloride, at a concentration of 2 mg/mL epirubicin hydrochloride. Inactive ingredients include sodium chloride and water for injection. The pH of the solution is adjusted to 3.0 with hydrochloric acid.

Storage and Stability: Epirubicin should be refrigerated between 2°-8°C. Refrigeration can result in the formation of a gel. The gel will return to a slightly viscous to mobile solution after 2-4 hours equilibration at room temperature. The solution should be used within 24 hours after removal from refrigeration.

CYCLOPHOSPHAMIDE (CYTOXAN®)

2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxidemonohydrate, Cyclophosphamide, is biotransformed principally in the liver to active alkylating metabolites which cross-link DNA.

Human Toxicology:

Toxicity from cyclophosphamide includes bone marrow suppression which usually occurs 10 to 12 days after administration, nausea, vomiting, anorexia, abdominal discomfort, diarrhea, stomatitis, hemorrhagic colitis, jaundice, reversible alopecia, hemorrhagic cystitis which can frequently be prevented with increased hydration, hematuria, ureteritis, tubular necrosis, fibrosis of the bladder, cardiac toxicity which may potentiate doxorubicin-induced cardiotoxicity, rare anaphylactic reaction, skin rash, hyperpigmentation of the skin and nails, interstitial pulmonary fibrosis, and cross sensitivity with other aklylating agents. Treatment with cyclophosphamide may cause significant suppression of the immune system.

Second malignancies, most frequently of the urinary bladder and hematologic systems, have been reported when cyclophosphamide is used alone or with other anti-neoplastic drugs. It may occur several years after treatment has been discontinued. It interferes with oogenesis and spermatogenesis and may cause sterility in both sexes, which is dose and duration related. It has been found to be teratogenic, and women of childbearing potential should be advised to avoid becoming pregnant. Increased myelosuppression may be seen with chronic administration of high doses of phenobarbital. Cyclophosphamide inhibits cholinesterase activity and potentiates the effect of succinylcholine chloride. If a patient requires general anesthesia within 10 days after cyclophosphamide administration, the anesthesiologist should be alerted. Adrenal insufficiency may be worsened with cyclophosphamide. Cyclophosphamide is excreted in breast milk, and it is advised that mothers discontinue nursing during cyclophosphamide administration. The occurrence of acute leukemia has been

reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.

Cyclophosphamide Pharmacology:

Kinetics: Cyclophosphamide is activated principally in the liver by a mixed function microsomal oxidase system. PO administration is well absorbed, with bioavailability greater than 75%. Five to twenty-five percent of unchanged drug is excreted in the urine. Several active and inactive metabolites have been identified with variable plasma protein binding. There appears to be no evidence of clinical toxicity in patients with renal failure, although elevated levels of metabolites have been observed.

Formulation: Cyclophosphamide is supplied in 100 mg, 200 mg, 500 mg, 1 gram and 2 gram vials as a white powder. The drug should be reconstituted with Sterile Water for Injection, USP, and may be diluted in either normal saline or D5W.

Storage and Stability: Although the reconstituted cyclophosphamide is stable for six days under refrigeration, it contains no preservatives and therefore should be used within 6 hours. Tablets are stable at room temperature.

Administration: Cyclophosphamide should be diluted in about 150 mL of normal saline or D5W and infused IV. An added dose of IV fluids may help prevent bladder toxicity.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVES

To estimate the pathologic complete response rate (pCR) when pertuzumab is added to weekly trastuzumab/paclitaxel followed by + trastuzumab/FEC neoadjuvant chemotherapy in HER2-positive breast cancer. This study will assess pCR rates separately in ER+ and ER- cancers. Pathologic complete response is defined as no evidence of viable invasive tumor cells at the primary tumor site and axillary lymph nodes in the surgical specimen. Residual Disease (RD) is defined as: Any invasive cancer in the breast or axillary lymph nodes in the surgical specimen.

2.2 SECONDARY OBJECTIVES

(i) To assess the safety and tolerability of the regimen.

a. Assess cardiac safety measured by rates of clinically symptomatic congestive heart failure, asymptomatic decrease in LVEF >10%, and decrease of LVEF below normal level.

b. Assess general tolerability measured by the standard NCI Common Toxicity Criteria.

(ii) To assess clinical response rate according to RECIST criteria (Appendix B).

(iii) To assess Residual Cancer Burden.

(iv) To collect pretreatment and residual cancer tissue after completion of chemotherapy for correlative science studies (biopsies and archival tissue will be collected under a separate protocol, Yale University HIC# 1310012919).

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

This is a single arm, neoadjuvant, Phase II open label trial of pertuzumab and trastuzumab administered concomitant with sequential weekly paclitaxel followed FEC chemotherapies for clinical stage I-III HER 2-positive breast cancer. The primary objective of this study is to estimate the pathologic complete response rate (pCR) of this pertuzumab containing regimen. In this study, pertuzumab is added to a trastuzumab containing chemotherapy regimen that has produced the highest pCR rates in HER2 positive breast cancers so far. Pathologic complete response is defined as absence of any viable invasive cancer in the resected breast and lymph nodes. Because pCR rates differ between estrogen receptor (ER) positive and ER negative breast cancers, we will calculate the sample sizes separately for ER positive and ER negative cancers in this trial. Our goal is to estimate if pCR rates could be improved from 50% to 70% in ER positive breast cancers and from 70% to 90% in ER negative breast cancers by adding pertuzumab to weekly paclitaxel/trastuzumab x12 and FEC/trastuzumab x 4 treatment regimen. This is a single clinical trial but accrual may finish sooner for one ER cohort than for the other.

The historical pCR rate for ER negative patients treated with trastuzumab concomitant with weekly paclitaxel x 12 followed by trastuzumab and 5-fluorouracil/epirubicin/cyclophosphamide x 4 (HT/HFEC) is 70% (n=140). Our goal is to estimate if the pCR rate can be increasing to 90% when pertuzumab is added to the above regimen. With an alpha = 10%, beta = 10%, we will accrue 16 ER negative patients in the first stage and will halt accrual until pathologic CR results become available for all patients. We will stop the study if 11 or fewer patients have pathologic CR. The probability of early termination under the null hypothesis (i.e. the pertuzumab containing combination yields pCR rate equal to the historical data) is 55%. If the study proceeds to full accrual, the total sample size for the ER negative cohort is 25. We declare the treatment of potential interest if more than 20 of 25 patients achieve pathologic CR. The historical pCR rate for ER positive patients treated with HT/HFEC is 50% (n=178). Our goal is to estimate if the pCR rate can be increasing to 70% when pertuzumab is added to the above regimen. With an alpha = 10%, beta = 10%, we will accrue 23 ER positive patients in the first stage and will halt accrual until pathologic CR results become available for all patients; we will stop the study if 11 or fewer patients have pathologic CR. The probability of early termination under the null hypothesis is 50%. If the study proceeds to full accrual, the total sample size for the ER positive cohort is 39. We declare the treatment of potential interest if more than 23 of 39 patients achieve pathologic CR.

Our practice includes 8 full time academic medical oncologists who see breast cancer patients only, and the Cancer Center also acquired 8 community oncology practices; physicians in these practices are members of Yale Cancer Center and provide a broader referral and accrual basis for this trial. We plan to accrue 2-3 patients per months end expect to complete the trial in 24 months.

3.2 RATIONALE FOR STUDY DESIGN

Patients with stage I-III HER2 positive breast cancer who experience pathologic complete response (pCR) to neoadjuvant chemotherapy have excellent overall survival. The US Food and Drug Administration (FDA) has recently recognized this strong association between pCR and long-term survival in this disease subset and expressed interest in accepting this endpoint for accelerated drug approval. Currently, trastuzumab administered concomitantly with weekly paclitaxel and subsequent 5-fluorouracil, epirubicin, cyclophosphamide neoadjuvant chemotherapy achieves the highest pCR rate reported in HER2 positive breast cancer (Bayraktar S et al 2012). All previous studies that combined pertuzumab with trastuzumab or with a trastuzumab and chemotherapy combination have shown improved response rates and increased clinical benefit when pertuzumab was added to a regimen. Our hypothesis is that adding pertuzumab to trastuzumab and weekly paclitaxel FEC will increase pCR rates by 20%, which will translate to pCR rates of 90% and 70% for ER negative and ER positive cancers, respectively.

3.3 OUTCOME MEASURES

3.3.1 Primary Outcome Measure

The primary efficacy endpoint of this study is pathologic complete response rate. Pathologic Complete Response (pCR) is defined as: No evidence of viable invasive tumor cells at the primary tumor site and axillary lymph nodes in the surgical specimen. Residual Disease (RD) is defined as: Any invasive cancer in the breast or axillary lymph nodes in the surgical specimen.

3.3.2 Secondary Outcome Measures

Cardiac safety including rates of clinically symptomatic congestive heart failure (defined as the occurrence of objective findings on clinical examination including rales, S3, elevated jugular venous pressure, confirmed by chest X-ray and/or either MUGA or ECHO) and asymptomatic drops in LVEF >10% or to below normal level.

General tolerability assessed by the standard NCI Common Toxicity Criteria as per http://ctep.cancer.gov/forms/CTCAEv4.pdf.

Clinical response according to RECIST v1. criteria (Appendix B)

Residual Cancer Burden distribution calculated as:
http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

Collection of pretreatment and residual cancer tissue after completion of chemotherapy for correlative science studies.

3.4 SAFETY PLAN

Patients will be evaluated for toxicity at each study visit. Every 3 months cardiac monitoring will continue after surgery to complete a total of 12 months after starting therapy. See Section 4.5 for Study Assessment and Appendix A for Study Flowchart.

See Section 4.3.3 for Dose Modification guidelines.

See Section 5 for reporting of adverse events.

3.5 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Good Clinical Practices (GCPs), and local ethical and legal requirements.

4.1 MATERIALS AND METHODS

4.2 SUBJECTS

Patients will be accrued at the Yale Cancer Center (YCC) Smilow Cancer Hospital. The Breast Center at YCC has 8 medical oncologists and 4 surgeons specializing in breast cancer care and the center sees approximately 400 new cases per year. The hospital is also affiliated with 8 community care centers staffed by physician employees of Smilow Hospital. The study may be extended into a multicenter trial with Sarah Cannon Cancer Research Center that has a strategic alliance with Yale Cancer Center to conduct joint clinical trials to increase accrual rate.

4.2.1 Subject Selection

4.2.2 Inclusion Criteria

Patients must meet each of the following eligibility criteria:

- Patients with histologically confirmed stage I-III, HER2-positive invasive breast cancer for which adjuvant/neoadjuvant chemotherapy is indicated based on physician judgment following NCCN practice guidelines.

  HER2 overexpression or amplification will be based on local test results and is defined as either:

(i) IHC staining of 3+ (uniform, intense membrane staining) in ≥ 10% of invasive tumor cells or,

(ii) Fluorescent in situ hybridization (FISH) result of more than six HER2 gene copies per nucleus or,

(iii) FISH ratio (HER2 gene signals to chromosome 17 signals) of ≥2.0.

These criteria differ from the ASCO - CAP definitions of HER2 positivity, and were requested by the study sponsor in order to make trial results comparable with all previous trastuzumab and pertuzumab clinical trials.

- Patients with synchronous bilateral breast cancers are eligible if at least one of the tumors is HER2-positive.
- Left Ventricular Ejection Fraction (LVEF) greater or equal to 50% at baseline as determined by either ECHO or MUGA, or within the institution's normal limits.
- Women of childbearing potential must have a negative pregnancy test (serum or urine beta HCG) prior to initiation of chemotherapy. Both female and male breast cancer patients who are sexually active have to agree to practice contraception while participating in the trial and for 3 month after completion of therapy.
- Adequate bone marrow function as indicated by the following:

  ANC ≥1500/μL

  Platelets ≥100,000/μL

  Hemoglobin ≥10 g/dL

- Adequate renal function, as indicated by creatinine ≤1.5× upper limit of normal (ULN)
- Adequate liver function, as indicated by bilirubin ≤1.5× ULN and AST or ALT ≤2× ULN.
- Signed informed consent.

4.2.3 Exclusion Criteria

Patients will be excluded from the study based on any of the following criteria:

- Patients who underwent partial excisional biopsy, lumpectomy, segmental mastectomy, modified radical mastectomy or sentinel node biopsy and, therefore cannot be assessed for pathologic response accurately.
- Patients who are high risk for developing the following anthracycline, paclitaxel, trastuzumab or pertuzumab related toxicities including:

History of congestive heart failure, myocardial infarction or cardiomyopathy, uncontrolled hypertension despite adequate medications

Pre-existing peripheral neuropathy ≥ grade 3

Prior anthracycline therapy

Known hypersensitivity to any of the study medications

Patients older than age 65 due to increased risk of cardiotoxicity

- Active infection requiring systemic antibiotic therapy.
- Pregnant or lactating women

4.3 METHOD OF TREATMENT ASSIGNMENT

This is a single arm Phase II trial and all patients will receive the same therapy.

4.3. STUDY TREATMENT

Pertuzumab and Trastuzumab will be provided free of charge by Genentech. The Sponsor Investigator of the study will ensure maintenance of complete and accurate records of the receipt, dispensation, and disposal or return of all study drugs in accordance with 21 Code of Federal Regulations (C.F.R.), Part 312.57 and 312.62 and Genentech requirements. All cytotoxic chemotherapies and supportive medications given in the context of this study are considered standard of care and will be charged to third party insurer.

During the first 1-12 weeks patients will receive pertuzumab, trastuzumab, and paclitaxel concomitantly; during weeks 13-24 patients will receive pertuzumab and trastuzumab concomitantly with 5-fluorouracil, epirubicin, and cyclophosphamide (FEC).

Pertuzumab and trastuzumab administration should precede chemotherapy administration. Patients should be observed for fever and chills or other infusion-associated symptoms.

The standard weekly paclitaxel (T) and 5-fluorouracil, epirubicin, cyclophosphamide (FEC) chemotherapy regimens can be administered as per institutional guidelines. If no institutional treatment orders exist for these regimens then the treatment should be administered as specified in the current protocol.

AGENT	DOSE	ROUTE	RETREATMENT INTERVAL
Trastuzumab	First dose: 4 mg/kg. Maintenance dose: 2 mg/kg	IV	Once every week x 12 weeks from week 1 to week 12 (**12 doses total**)
Trastuzumab	6 mg/kg	IV	Once every 3 weeks from week 13 to week 24 (**4 doses total**)
Pertuzumab	First dose: 840 mg. Maintenance dose: 420 mg	IV	Once every 3 weeks x 24 weeks **(8 doses total)**
Paclitaxel	80 mg/m2	IV	Once every week from week 1 to week 12 (**12 weekly cycles** total)
5-fluorouracil	500 mg/m2	IV	Once every 3 weeks from week 13 to week 24 (**4 cycles total**)
Epirubicin	75 mg/m2	IV	Once every 3 weeks from week 13 to week 24 (**4 cycles** total)
Cyclophosphamide	500 mg/m2	IV	Once every 3 weeks from week 13 to week 24 (**4 cycles** total)

Treatment weeks 1-12:

- Trastuzumab 4 mg/kg loading dose on Day 1, week 1, over 60 minutes infusion in 250 ml Normal Saline (NS), followed by 2 mg/kg weekly over 30 minutes infusion before each course of paclitaxel chemotherapy from Day 1, weeks 2-12.

- Pertuzumab 840 mg loading dose in 250 ml NS on Day 1, week 1 over 60 min infusion followed by 420 mg once every 21 days over 30 minutes infusion before paclitaxel chemotherapy.

- Paclitaxel 80 mg/m2 administered in 250 mL (NS) IV over 60 minutes.

Premedication will include famotidine 20 mg orally and diphenhydramine 25 mg orally 30 minutes prior to the first two doses of paclitaxel which can be omitted for future doses if no infusion reaction has occurred. Dexamethasone 10 mg IV will be given 30 min prior to the first three doses of paclitaxel. The Dexamethasone dose should be reduced to 4 mg IV after 3rd dose if no infusion related reactions are noted.

Treatment weeks 13-24:

- Trastuzumab 6 mg/kg in 250 ml NS over 30 minutes infusion, once every 21 days before each FEC chemotherapy (loading dose for q 3 week schedule not needed).

- Pertuzumab 420 mg in 250 ml NS over 30 minutes infusion once every 21

days before each FEC chemotherapy.

- 5-Fluorouracil 500 mg/m^2 as a bolus in 50 ml NS once every 21 days for 4 cycles total.

- Epirubicin 75 mg/m^2 over 30 min infusion in 100 ml NS once every 21 days for 4 cycles total.

- Cyclophosphamide 500 mg/m^2 IV over 30 minute infusion in 250 ml NS once every 21 days for 4 cycles total.

Premedications can be given as per local institutional guidelines.

4.3.1 Trastuzumab and Pertuzumab Formulations

Trastuzumab Formulation

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each vial of trastuzumab contains 440 mg of tratuzumab, 9.9 mg of L-histidine 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.

Pertuzumab Formulation

Pertuzumab drug product is provided as a single use formulation containing 30 mg/mL pertuzumab in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20. Each 20 mL vial contains 420 mg of Pertuzumab (14.0 mL/vial). Upon receipt, pertuzumab vials are to be refrigerated at 2°C–8°C (36°F–46°F) until use. Pertuzumab vials should not be used beyond the expiration date provided by the manufacturer. Because the formulation does not contain a preservative, the vial seal may only be punctured once. Any remaining solution should be discarded. Vial contents should be protected from light, and should not be frozen. The solution of pertuzumab for infusion, diluted in PVC or non-PVC polyolefin bags containing 0.9% Sodium Chloride Injection, USP, may be stored for up to 24 hours prior to use. Diluted pertuzumab has been shown to be stable for up to 24 hours at a temperature range of 2°C–25°C. However, since diluted pertuzumab contains no preservative, the diluted solution should be stored refrigerated (2°C–8°C).

4.3.2 Dosage, Preparation, Administration and Storage

All study drugs used in this trial are administered at the FDA recommended doses and routes. Smilow Cancer Hospital pharmacy will follow standard clinical practice in storing, preparing and administering the drugs.

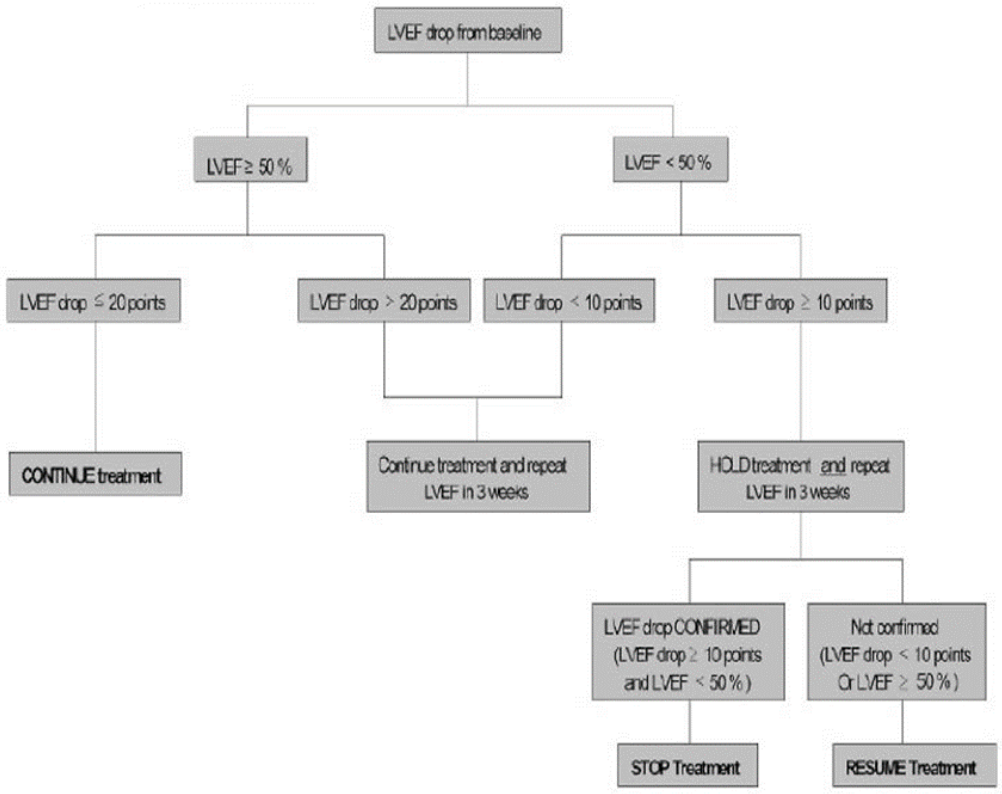
4.3.3. Dosage Modification

Trastuzumab and Pertuzumab

There are no dose modifications for pertuzumab or trastuzumab.

(i) Pertuzumab and trastuzumab should be discontinued in any patient who develops clinical signs and symptoms of CHF. Weekly paclitaxel chemotherapy may be continued alone. CHF should be treated and monitored according to standard medical practice. This included ACE-inhibitors or angiotensin receptor blockers, beta-blockers and diuretics when needed (Appendix C).

(ii) In case of asymptomatic decrease in LVEF, the following algorithm will guide the continuation or discontinuation of pertuzumab and trastuzumab:



If the criteria to hold pertuzumab and trastuzumab are met paclitaxel (but not FEC) can be continued.

For delayed or missed doses, if the time between 2 sequential infusions is less than 6 weeks, the 420 mg IV dose of pertuzumab should be administered along with

Trastuzumab maintenance dose. Do not wait until the next planned dose.

If the time between 2 sequential infusions is 6 weeks or more, the initial dose of 840 mg pertuzumab should be re-administered as a 60 minute IV infusion followed every 3 weeks thereafter by a dose of 420 mg IV administered over 30- 60 minutes. Similarly, Trastuzumab loading dose should be repeated followed by maintenance dose thereafter.

Paclitaxel

(i) For grade 3 peripheral neuropathy (i.e. sensory alteration or paresthesia interfering with normal daily activities) paclitaxel, will be held for a maximum of 14 days. If symptoms improve within this time period to grade 1 or less resume paclitaxel at standard 60 mg/m^2 dose. If neuropathy does not resolve to grade 1 discontinue paclitaxel and start the 5-fluorouracil/epirubicin/cyclophosphamide phase of the treatment.

(ii) If Absolute Neutrophil Count (ANC) is below 1000/microliter or Platelet count is below 100,000/microliter on the day of chemotherapy administration, paclitaxel should be held and complete blood count repeated 24 hours later. Resume administration of paclitaxel at full dose once ANC $\geq$1000 and Plt $\geq$ 100,000. Use G-CSF with subsequent treatments to prevent dose delays as recommended by ASCO practice guidelines.

While paclitaxel is on hold for toxicity Trastuzumab and Pertuzumab should be continued as scheduled.

5-fluorouracil, epirubicin, cyclophosphamide (FEC)

(i) If Absolute Neutrophil Count (ANC) is below 1000/microliter or Platelet count is below 100,000/microliter on the day of chemotherapy administration, FEC chemotherapy should be held and complete blood count repeated 24 hours later. Resume administration of FEC at full dose once ANC $\geq$1000 and Plt $\geq$ 100,000. Use G-CSF with subsequent treatments to prevent dose delays as recommended by ASCO practice guidelines.

(ii) If clinically symptomatic CHF develops discontinue FEC (and also trastuzumab and pertuzumab). CHF should be treated and monitored according to standard medical practice. This included ACE-inhibitors or angiotensin receptor blockers, beta-blockers and diuretics when needed (Appendix C). Patients should be referred to surgery.

(iii) In case of asymptomatic decrease in LVEF, FEC (and also trastuzumab and pertuzumab) should be held and cardiology consultation should be obtained to initiate appropriate therapy. Assessment of LVEF should be repeated within 3 weeks. If LVEF returns to normal, resume therapy. If reversible asymptomatic decrease in LVEF recurs reduce dose of Epirubicin to 50 mg/m^2.

(iv) Grade 3 non-hematologic toxicity, other than alopecia, fatigue, and nausea/vomiting, hold treatment until symptoms resolve to grade 1 or less and resume therapy with 20% dose reduction of all 3 chemotherapy drugs.

4.3.4 Trastuzumab and Pertuzumab Overdosage

There is no experience with overdosage in human clinical trials.

4.4 CONCOMITANT AND EXCLUDED THERAPY

Non-study related anti-tumor therapy is not allowed. There are no restrictions on non-oncology medications.

4.5 STUDY ASSESSMENTS

All pretreatment assessments are standard of care.

4.5.1 Assessments at baseline before treatment

- Determination of tumor HER2, ER and PR status
- Complete medical history including symptom/toxicity assessment.
- Physical examination including breast and regional lymph nodes, weight and height, and vital signs. Record tumor measurements by physical exam.
- ECOG performance status
- Bilateral mammograms and clip placement to mark tumor bed . Other imaging, such as ultrasonography or MRI, is at the discretion of the treating physicians. The most recent breast imaging that documents the size of the cancer must be within the past 6 weeks.
- Ultrasongraphic evaluation of the regional lymph nodes

  If suspicious lymph nodes are detected clinically or by imaging, a diagnostic image guided fine needle aspiration of the suspicious node is required.
- CBC and differential, electrolytes, BUN, creatinine, total bilirubin, AST, ALT, alkaline phosphatase.

- Cardiac function assessment by either MUGA or echocardiography.
- Women of childbearing potential must have a serum or urine beta hCG pregnancy test prior to initiation of first chemotherapy and counseling about need of contraception during treatment and for 6 months after completion of therapy must be performed. Similar counseling about contraception while on therapy is also required for male breast cancer patients.
- Systemic staging with CT scan of the chest/abdomen/pelvis and bone scan or PET scan should follow NCCN staging guidelines and are currently recommended routinely only for patients with clinical Stage III disease.

4.5.2 Assessments during therapy

- CBC and differential within 24 hours before each chemotherapy administration.
- Physical examination including vital signs, weight, breast and regional lymph nodes every 3 weeks.
- Medical history every 3 weeks.
- ECOG performance status every 3 weeks.
- Toxicity evaluation every 3 weeks.
- Cardiac function assessment using the same method as baseline after completion of the 12 courses of paclitaxel, trastuzumab and pertuzumab before starting FEC chemotherapy, and after completion of the 4 cycles of FEC chemotherapy prior to surgery and every 3 months after surgery for a total of 12 months.

  Cardiac evaluation may be repeated at any time if cardiac adverse effects are suspected clinically.
- Mammogram, or the most recent imaging study that was obtained at baseline, of the affected breast after completion of the 12 courses of paclitaxel, trastuzumab and pertuzumab before starting FEC chemotherapy and also after completion of all chemotherapy before surgery. (Other imaging, such as ultrasonography or MRI, is at the discretion of the treating physicians.)

  Breast imaging may be repeated any time during therapy if clinical suspicion of progression of disease.

- BUN, creatinin, total bilirubin, AST, ALT, alkaline phosphatase every 3 weeks before chemotherapy administration.

Surgery will be scheduled 3-6 weeks after completion of last chemotherapy.

The final study follow up visit will be 4 weeks +/- 1 week after completion of surgery and will include complete medical history, toxicity assessment and physical examination.

4.5.3. Treatment after completion of preoperative chemotherapy.

- All patients will undergo breast surgery after completion of preoperative therapy. The decision regarding lumpectomy or mastectomy will be at the discretion of the treating surgeon. All patients will have axillary lymph node sampling with a technique that is deemed appropriate by the surgeon:

  For clinically node negative disease at baseline, defined as negative clinical exam and ultrasonogram or negative lymph node biopsy in case of a suspicious lymph node by imaging, sentinel node sampling is preferred, with subsequent full axillary lymph node dissection at the discretion of the treating physicians.

  For patients with clinically palpable, matted metastatic lymph nodes and/or histologically confirmed node positive disease at baseline, sentinel node sampling is permitted but subsequent full axillary lymph node dissection must be performed even if there is complete clinical response in the nodes after preoperative therapy.

- Patients who progress clinically or by imaging assessment during therapy will be removed from protocol treatment and further treatment including immediate referral to surgery or preoperative radiation or additional chemotherapy will be at the discretion of the treating physician.

- Postoperative adjuvant therapy should follow current standard NCCN practice guidelines. Participation in subsequent adjuvant clinical trials is allowed.

4.6 DISCONTINUATION OF PROTOCOL-SPECIFIED THERAPY

Protocol-specified therapy may be discontinued for any of the following reasons:

- Progressive disease
- Unacceptable toxicity
- Patient election to discontinue therapy (for any reason)

- Physician's judgment

4.7. STUDY DISCONTINUATION

- The study will be terminated after all planned patients are accrued, all primary endpoints are recorded and final data analysis has been completed.
- Genentech and the Principal Investigator has the right to terminate the study at any time. Reasons for terminating the study may include the following:
    - The incidence and severity of adverse events in this or other similar trials indicate unacceptable health hazards for the participants.
    - Unsatisfactory slow enrollment
    - Major violations to the protocol and major inaccuracies in data collection.

4.8. STATISTICAL METHODS

4.8.1 Analysis of the Conduct of the Study

The responsibility for the conduct of the study lies with the Principle Investigators. The Sponsor's Data Safety and Monitoring Committee will conduct annual reviews. The Yale Quality Assurance Compliance and Safety Committee (QUACS) will conduct internal audits at time points they deem appropriate.

4.8.2 Analysis of Treatment Group Comparability

This is a single arm study.

4.8.3 Analysis Plan

a. Primary Endpoint Definition and Reporting

The primary study endpoint is pathologic complete response (pCR) defined as no evidence of viable invasive tumor cells at the primary tumor site and in axillary lymph nodes assessed in the surgical specimen on routine histology examination. Residual Disease (RD) is defined as: Any invasive cancer in the breast or axillary lymph nodes in the surgical specimen. These results will be retrieved from the routine pathology report.

The pCR rates will be reported using descriptive statistics and 95% confidence intervals. This study will assess and report pCR rates separately in ER+ and ER- cancers.

b. Secondary Endpoint Definitions and Reporting

- Cardiac safety endpoints will include (i) clinically symptomatic congestive heart failure (CHF), (ii) asymptomatic drops in left ventricular ejection fraction (LVEF) >10% compared to pre-treatment value (even if it remains above the lower limit of normal), (iii) LVEF drop below normal level. Symptomatic CHF is defined as clinical signs and symptoms of CHF including dyspnea, tachycardia, new unexplained cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and rapid unexplained weight gain, chest X-ray findings suggestive of CHF; these clinical findings must be supported by MUGA or ECHO results indicating systolic or diastolic heart failure. The frequency of cardiac adverse events will be reported as descriptive statistics with 95% confidence intervals.

- Toxicity will be recorded using the standard NCI Common Toxicity Criteria as per http://ctep.cancer.gov/forms/CTCAEv4.pdf.and . Results will be reported in a tabular format by worst grade and occurrence per patient over the entire study period and also by chemotherapy type (paclitaxel versus FEC phases of the treatment)

- Clinical response rate will be assessed by two measures. Mammographic tumor size measurements and physical examination after completion of all chemotherapy compared to baseline. Response will be categorized following RECIST criteria (Appendix B). The results will be reported as descriptive statistics with 95% confidence intervals.

- Residual Cancer Burden (RCB) scores after chemotherapy will be determined by routine pathology examination and scores will be calculated using the http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3 website. Results will be presented as categorical variables (i.e. frequencies of RCB-0, I, II, III response categories) and also as distribution of continuous variable RCB score.

- Baseline and residual cancer tissue will be collected for future biomarker analysis. Considering the small sample size of the study the biomarker results will be exploratory and hypothesis generating. The planned molecular analysis of these tissues will include gene expression profiling, metabolomics profiling, DNA sequencing and establishment of primary tumor cultures to search for potential markers of treatment response.

4.8.4 Missing Data

We do not expect any missing data for the primary endpoint of the study. All patients will undergo breast surgery. Patients who cannot undergo surgery due to deteriorating health related to study medications or progression of cancer will be considered having "Residual Cancer" for the purpose of statistical analysis.

4.8.5 Determination of Sample Size

This study will follow Simons' minimax two-stage trial design based on one-sample binomial test with a goal to minimize the maximum sample size. The primary study endpoint is pathologic complete response (pCR) rate. All patients will be HER2 positive (HER2+) in this study. The pCR rates differ between HER2+/estrogen receptor (ER)-positive and HER2+/ER-negative breast cancers when the same trastuzumab-containing regimen is used. Therefore we calculate the sample size separately for ER-positive and ER-negative cancers. This is a single clinical trial but accrual may finish sooner for one ER cohort than for the other. Setting a single pCR target for a mixed cohort of ER-positive and -negative patients is statistically not sound; the observed overall pCR rate would depend on the proportion of ER-positive versus -negative cases that were accrued to the trial.

The historical pCR rate for ER-negative patients treated with trastuzumab concomitant with weekly paclitaxel x 12 followed by trastuzumab concomitant with 5-fluorouracil/epirubicin/cyclophosphamide x 4 is 70% (n=140). Our goal is to assess if the pCR rate can be increased to 90% when pertuzumab is added to the above regimen. With an alpha = 10%, beta = 10%, p0 (historic pathologic CR rate) = 70% and p1 (expected pathologic CR rate with Pertuzumab) = 90%, **we will accrue 16 ER-negative patients in the first stage** and will halt accrual until pCR results become available for all 16 patients. We will stop the ER-positive arm of the study if fewer than 11 patients had pathologic CR. The probability of early termination under the null hypothesis (i.e. the pertuzumab containing combination yields pCR rate equal to the historical data) is 55%. If the arm proceeds to full accrual, the **total sample size for the ER-negative cohort is 25**. We declare the treatment of potential interest if 20 or more of 25 patients achieve pCR and would recommend conducting a randomize trial to formally compare the Paclitaxel/FEC/Trastuzumab regimen with Pertuzumab plus Paclitaxel/FEC/Trastuzumab in ER-negative cancers. A definitive randomized trial with 90% power and 5% two-sided alpha would require 164 ER-negative patients (82/per arm) to demonstrate an increase from 70 to 90% in pCR rate.

The historical pCR rate for ER-positive patients treated with trastuzumab concomitant with weekly paclitaxel x 12 followed by trastuzumab concomitant with 5-fluorouracil/epirubicin/cyclophosphamide x 4 is 50% (n=178). Our goal is to assess if the pCR rate can be increased to 70% when pertuzumab is added to the above regimen. With an alpha = 10%, beta = 10%, p0 = 50% and p1 = 70%, **we will accrue 23 ER-positive patients in the first stage** and will halt accrual until pCR results become available for all 23 patients; we will stop the study if fewer than 11 patients have pCR. The probability of early termination under the null hypothesis is 50%. If the study proceeds to full accrual, the **total**

sample size for the ER positive cohort is 39. We declare the treatment of potential interest if 23 or more of 39 patients achieve pCR and would recommend conducting a randomize trial to formally compare the Paclitaxel/FEC/Trastuzumab regimen with Pertuzumab plus Paclitaxel/FEC/Trastuzumab in ER positive cancers. A definitive randomized trial with 90% power and 5% two-sided alpha would require 248 ER positive patients (124/per arm) to demonstrate an increase from 50 to 70% in pCR rate.

4.9 DATA COLLECTION AND QUALITY ASSURANCE

The Yale Cancer Center Clinical Trial Office will be responsible to collect, QC and provide data for the Principal Investigator and Sponsor.

5. DEFINITION AND REPORTING OF ADVERSE EVENTS

Adverse Events (AE)

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Events (SAE)

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).

- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

5.1 METHODS AND TIMING FOR ASSESSING AND RECORDING SAFETY VARIABLES

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to the appropriate IRB(s), and Genentech, Inc. in accordance with CFR 312.32 (IND Safety Reports).

Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), seriousness criteria, suspected relationship to the study drugs, and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of the study drugs, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the study drugs; and/or the AE abates or resolves upon discontinuation of the study drugs or dose reduction and, if applicable, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than the study drugs (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to study drugs administration (e.g., cancer diagnosed 2 days after first dose of study drug).

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.) or not identified. 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.

5.2 PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation timepoints should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations. This study will use NCI Common Toxicity Criteria as per obtained from http://ctep.cancer.gov/forms/CTCAEv4.pdf

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 90 days after the last dose of study drug, a report should be completed and expeditiously submitted to Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the study drugs should be reported as an SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior study drug exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party.

If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

h. AEs of Special Interest (AESIs)

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product.

The Trastuzumab and Pertuzumab Events of Special Interest are:

a. Left Ventricular Dysfunction, defined as LVEF drop > 20 points from baseline, or drop to below 50% or below the institution's normal limits. When a cardiac event occurs, the Cardiac Report Form must be submitted within 14 days of learning of the event.

b. Grade 3 or 4 infusion-associated reactions, hypersensitivity reactions, or anaphylaxis.

c. Embryo-fetal toxicity or birth defects.

i. SAE Reporting

Investigators must report all SAEs to Genentech within the timelines described below. The completed report should be faxed within fifteen (15) calendar days of discovery to Genentech Drug Safety at:

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

- Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available.

- Serious AE reports that are related to the trastuzumab and pertuzumab and AEs of Special Interest (regardless of causality) will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date.

- Serious AE reports that are unrelated to the trastuzumab and pertuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.

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

- All Non-serious Adverse Events originating from the Study will be forwarded in a quarterly report to Genentech.

Note: Investigators should also report events to their IRB as required.

The report should include the following information:

- 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 as new information becomes available.

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

Tel: (888) 835-2555
Fax: (650) 225-4682 OR (650) 225-5288

5.3 MedWatch 3500A Reporting Guidelines

MedWatch 3500A forms will be used for reporting safety results to the sponsor Genentech Inc.

5.4 Additional Reporting Requirements for IND Holders

Not applicable

5.5. Reporting to the Yale Human Investigation Committee

All SAEs, whether originating at Yale or a collaborating center, meeting the criteria for expedited reporting will be reported to the Yale University Human Investigation Committee (HIC) using HIC Form 6A within 48 hours of discovery.

The HIC expedited reporting criteria are:

a. Serious AND unexpected AND possibly, probably or definitely related events
b. Anticipated Adverse Events occurring with a greater frequency than expected.

The HIC does not require reporting of any other Adverse Event type. A copy of the HIC reporting policy is available at http://info.med.yale.edu/hic/policy/AdverseEventPolicy.pdf

6. INVESTIGATOR REQUIREMENTS

6.1 STUDY INITIATION

Before the start of this study, the following documents must be on file with Genentech or a Genentech representative:

- Current *curriculum vitae* of the Principal Investigator.
- Written documentation of IRB approval of protocol and informed consent document.
- A copy of the IRB-approved informed consent document.
- A signed Clinical Research Agreement.

6.2 STUDY COMPLETION

The following materials are requested by Genentech when a study is considered complete or terminated:

- Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be mailed to the assigned Clinical Operations contact for the study:
- Email : Pertuzumab-gsur@gene.com
- Fax : 650-360-6908

6.3 INFORMED CONSENT

The informed consent document must be signed by the subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent document must be provided to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language.

Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at any time.

6.4 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.).

6.5 STUDY MONITORING REQUIREMENTS

This investigator initiated study will be monitored by and according to the policies of Yale University Yale Cancer Center.

6.6 DATA COLLECTION

Data will be collected using study-specific electronic case report forms (OnCore) by research personnel assigned to the trial by the Yale Cancer Center Clinical Trials Office.

Case Report Forms

A subset of the National Cancer Institute (NCI) CRFs, in electronic format, will be utilized to record data required by the study. Electronic CRFs (e-CRFs) will reside in the OnCore database. Completion of the electronic CRFs (e-CRFs) will be done in accordance with the instructions in a study specific data capture plan. All e-CRFs will be completed by clinical research staff of the Yale Cancer Center Clinical Trials Office (CTO).

When a cardiac event occurs, the Cardiac Report Form will be submitted within 14 days of learning of the event.

Data Submission Timeline and Forms

Completion of e-CRFs will occur in accordance with NCI guidelines. Baseline (pre-study) e-CRFs (e.g., enrollment, medical history, concomitant medications, disease assessment, etc.) will be completed no later than 14 days after the start of treatment. Treatment e-CRFs (e.g., drug administration, adverse events, chemistries, etc.) will be completed no later than 14 days following each cycle of treatment. Off-treatment information (e.g., follow-up, best response, etc.) will be completed no later than 14 days after the end of protocol treatment.

Research Charts

A research chart (i.e., shadow chart) is maintained at the CTO for each patient enrolled. Copies of significant study source documents will be maintained in the research chart. Examples of source document copies that will be maintained in the research chart include: signed informed consent form, documents that verify eligibility and treatment, and documents that verify Grade 3-4 adverse events and response. This information will be updated on a prospective basis and will be confidentially maintained at the CTO.

Reports

Publications and annual reports for submission to the IRB will be written by the principal investigator using the data captured on the e-CRFs.

6.7 STUDY MEDICATION ACCOUNTABILITY (IF APPLICABLE)

Trastuzumab and Pertuzumab will be provided by Genentech. The recipient will acknowledge receipt of the drug by returning the INDRR-1 form indicating shipment content and condition. Damaged supplies will be replaced.

Accurate records of all study drug dispensed from and returned to the study site should be recorded by using the institution's drug inventory log or the NCI drug accountability log.

All partially used or empty containers should be disposed of at the study site according to institutional standard operating procedure. Return unopened, expired, or unused study drug with the Inventory of Returned Clinical Material form as directed by Genentech.

6.8 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 if appropriate.

6.9 RETENTION OF RECORDS

Records of this study will be retained according the policies of Yale Cancer Center Clinical Trial Office.

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APPENDIX A: Study Flowchart

	Baseline (Days -42 to -1)	Week 1 (Day 1)	Weeks 2-12 (weekly)	Weeks 4-10 (q 3 weeks)	Weeks 12-13	Weeks 13-22 (q 3 weeks)	Weeks 23-26 (Post Chemo)	Final visit (4 wks +/- 1 week after surgery)
Informed Consent	x							
HER2, ER, PR assessment	x							
Tumor assessment by imaging[a]	x				x		x	
Complete medical history	x			x		x		x
Complete physical exam	x			x		x		x
Clinical tumor assessment	x			x		x		x
ECOG performance status	x			x		x		x
Toxicity evaluation	x			x		x		x
MUGA scan/echocardiogram[b]	x				x		x	
Serum or urine pregnancy test[c]	x							
Hematology (CBC, diff)	x		x			x		
Chemistry panel[d]	x			x		x		
Pertuzumab administration		x		x		x		
Trastuzumab administration		x	x			x		
Paclitaxel administration		x	x					
FEC administration						x		
Surgery[e]							x	

[a] Bilateral mammograms and clip placement to mark tumor bed. Other imaging, such as ultrasonography or MRI, is at the discretion of the treating physicians. The most recent breast imaging that documents the size of the cancer must be within the past 6 weeks. Mammogram or the most recent imaging study that was obtained at baseline of the affected breast after completion of the 12 courses of weekly paclitaxel, trastuzumab and pertuzumab before starting FEC chemotherapy and also after completion of all chemotherapy before surgery. (Other imaging, such as ultrasonography or MRI, is at the discretion of the treating physicians.). Regional lymph nodes must be evaluated by ultrasonography; if lymph nodes are suspicious clinically or by imaging, an image guided fine needle aspiration o the suspicious node is required. Baseline staging scans for assessment of distant disease should follow NCCN guidelines and is at the discretion of the treating physicians; scans are recommended for patients with clinical stage III disease.

[b] Week 13 and MUGA scan/echocardiogram should be done before the administration of week 13 treatment. Same testing method must be used for week 13 and week 24-28 cardiac assessment as at Days -42 to -1. Cardiac assessment will continue every 12 weeks after surgery until completion of 12 months total.

[c] Women of childbearing potential. Counseling about need of contraception during treatment and for 3 months after completion of therapy must be performed.

[d] Chemistry panel should include electrolytes, BUN, creatinine, total bilirubin, AST, ALT, alkaline phosphatase.

[e] Surgery will be scheduled 3-6 weeks after receiving the last dose of chemotherapy.

APPENDIX B
Response Evaluation Criteria in Solid Tumors (RECIST Criteria)

The baseline longest diameter of the primary breast cancer assessed by mammogram (or ultrasonogram or MRI at physicians discretion) will be used as the reference by which to characterize objective tumor response in the breast.

- Complete response (CR)

Disappearance of all evidence of tumor for at least two cycles of therapy.

- Partial response

At least a 30% decrease in the sum of the longest diameter of target lesions, taking a reference the baseline sum longest diameter.

- Stable disease (SD)

Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started.

- Progressive disease (PD)

At least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the beginning of treatment or the appearance of one or more new lesions Clinical progressive disease

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

APPENDIX C
HFSA Guidelines
Recommendations for Pharmacological Therapy: Left Ventricular Systolic Dysfunction ß-

Adrenergic Receptor Blockers

Background for Recommendations

The single most significant addition to the pharmacological management of heart failure since the publication of previous guidelines involves the use of ß-receptor antagonists. This represents a noteworthy departure from traditional doctrine in which ß-blocking agents were classified as contraindicated in the setting of left ventricular systolic dysfunction. A solid foundation of both clinical and experimental evidence now firmly supports their use in heart failure with the aim of reducing both morbidity and mortality (16,22,23).

ß-Blocker therapy for heart failure has been advocated by some investigators since the 1970s (24). During the subsequent 2 decades, many small- to medium-sized placebo-controlled trials, which used a variety of agents, showed several common findings: 1) the use of ß-blockers in mild to moderate heart failure was generally safe when initiated at low doses and gradually titrated up under close observation; 2) improvement in left ventricular ejection fraction was observed in all trials that lasted at least 3 months; and 3) there was wide variability in the effects of ß-blockade on exercise tolerance but improvement in outcome and symptomatic benefits was noted in many studies. These generally positive findings stimulated additional, large-scale clinical trials that have provided an impressive body of evidence that supports the use of ß-blockers in patients with heart failure caused by left ventricular systolic dysfunction. The recommendations that follow are derived from nearly 2 decades of research that include basic science data, animal models, and clinical trial experience in over 10,000 patients (25,26).

Although this is a major advance in efficacy, identification of appropriate candidates for ß-blocker therapy is essential to ensure safe and effective treatment. Prescribing physicians should understand the potential risks of ß-blocker therapy, as well as the benefits. The interested practitioner who is unfamiliar with ß-blocker initiation and titration may first seek further education and counsel from sources such as the Heart Failure Society of America or local and regional heart failure specialty centers.

Recommendation 1. ß-blocker therapy should be routinely administered to clinically stable patients with left ventricular systolic dysfunction (left ventricular ejection fraction less than or equal to 40%) and mild to moderate heart failure symptoms (ie, NYHA class II-III, Appendix A) who are on standard therapy, which typically includes ACE inhibitors, diuretics as needed to control fluid retention, and digoxin (Strength of Evidence = A).

The most persuasive outcome in heart failure management remains all-cause mortality. Combined endpoints, including mortality or hospitalization and mortality or hospitalization for heart failure, have also emerged as key outcomes. These latter endpoints reflect a more comprehensive assessment of the influence of therapy on quality of life and disease progression and are assuming more importance as mortality rates decline with treatment advances. The substantial beneficial effect of ß-blocker therapy on these endpoints has been well shown in clinical trials of symptomatic patients (NYHA class II - III) treated with carvedilol, bisoprolol, or metoprolol controlled release/extended release (CR/XL) (27-29). Trials with these agents encompass the combined, worldwide experience with ß-blocker therapy in patients with chronic heart failure who were stable on background therapy, including ACE inhibitors (over 90%) and diuretics (over 90%). Digoxin was common as background therapy, particularly in studies conducted in the United States. Trial results indicate that both selective and nonselective ß-blockers, with and without ancillary properties, have significant efficacy in heart failure. ß-Blocking agents with intrinsic sympathomimetic activity appear to have a negative impact on survival and should not be used in heart failure patients.

Metoprolol. The MDC Study was an early trial that included 383 patients with heart failure caused by nonischemic causes, NYHA class II-III symptoms, and a left ventricular ejection fraction of less than or equal to 40% (30). Patients with coronary artery disease were excluded. Study results showed a 34% reduction in risk in patients treated with metoprolol, although this strong trend toward benefit (*P* = .058) was entirely attributable to a reduction in the frequency of cardiac transplantation listing in the treatment group. In fact, the absolute number of deaths in the metoprolol group was higher than in the placebo group (23 *v* 19, *P* = .69).

The MERIT-HF Trial evaluated the effect of metoprolol CR/XL with all-cause mortality as the primary endpoint. The trial included 3,991 patients with NYHA class II-IV heart failure, although 96% of the study patients were functional class II or III (31). In this study, investigators were allowed to select the starting dose of metoprolol CR/XL. Seventy-nine percent chose 25 mg as the starting dose for class II patients, and 77% chose 12.5 mg for class III-IV patients. The target dose was 200 mg and doses were up-titrated over a period of 8 weeks. Premature discontinuation of blinded therapy occurred in 13.9% of those treated with metoprolol CR/XL and 15.3% of those in the placebo group (*P* = .90). The study results revealed a 34% reduction in mortality in the metoprolol group (relative risk of .66; 95% confidence interval [CI], .53 to .81; p=.0062 after adjustment for interim analyses), with annual mortality rates of 11% in the placebo and 7.2% in the metoprolol CR/XL group (29).

Bisoprolol. The CIBIS Study evaluated the effects of bisoprolol in 641 patients with left ventricular systolic dysfunction caused by ischemic or nonischemic causes and NYHA class III-IV heart failure (32). The primary endpoint was all-cause mortality, and hospitalization for worsening heart failure was one of the

secondary outcomes of interest. The initial bisoprolol dose was 1.25 mg/day, which was increased to a maximum dose of 5 mg/day. The trial found no significant reduction in all-cause mortality in patients treated with bisoprolol (20% reduction bisoprolol *v* placebo, *P* = .22) (32). The risk of hospitalization was significantly reduced by 34% (28% placebo group *v* 19% bisoprolol group, *P* < .01).

The favorable trends seen in CIBIS led to the larger CIBIS II Study, which ultimately was prematurely terminated as a result of a significant reduction in mortality in the bisoprolol arm (28). These results were obtained in 2,647 patients who were followed for an average of 1.3 years. Over 80% of the patients were judged to be NYHA class III at enrollment. Background therapy included ACE inhibitors in 96% and diuretic in 99% of the study patients, whereas 52% were taking digoxin. In contrast to the original CIBIS study, CIBIS II had a similar starting dose of 1.25 mg but had a greater target dose of 10 mg daily of bisoprolol. More stringent criteria for defining ischemic cardiomyopathy were used. Treatment with bisoprolol reduced the annual mortality rate by 34% (13.2% placebo *v* 8.8% bisoprolol; hazard ratio .66; 95% CI, .54 to .81; *P*< .0001). Hospitalizations for worsening heart failure were also decreased by 32% (18% placebo *v* 12% bisoprolol, hazard ratio .64; 95% CI, .53 to .79; *P*< .0001).
Although a post hoc
analysis of the CIBIS Study had suggested benefit might be consigned to patients without coronary disease, the survival benefit, with significant reductions apparent in both ischemic or nonischemic patients, was not influenced by disease origins.

Carvedilol. Carvedilol, a nonselective ß-blocker and -blocker, has been extensively investigated for treatment of heart failure caused by left ventricular systolic dysfunction. In the United States carvedilol trials, 4 separate study populations were examined and the data from 1,094 patients were combined to evaluate the effect of carvedilol therapy on the clinical progression of heart failure (27). Clinical progression was defined as worsening heart failure leading to death, hospitalization, or, in one study, a sustained increase in background medications. Patients with a left ventricular ejection fraction of 35% or less and NYHA class II-IV were eligible if they tolerated 6.25 mg of carvedilol twice per day for a 2-week, open-label, run-in period. Although this run-in phase biased the ultimately randomized patient population, less than 8% of eligible patients failed the open-label challenge. Target dosages for the studies were 50 to 100 mg/day of carvedilol that were administered in divided doses twice daily. Patients completing the run-in period were randomized based on results from their 6-minute walk test into mild, moderate, or severe trials. These studies were prematurely terminated (median follow-up 6.5 months) by the Trial Data and Safety Monitoring Board because of reduced mortality across the 4 combined trials of patients treated with carvedilol.

Data from these combined trials indicated a substantial benefit from carvedilol treatment. The risk of mortality was 65% lower (7.8% placebo *v* 3.2% carvedilol; 95% CI, 39% to 80%; *P*< .001) and the combined risk of hospitalization or death was reduced by 38% (20% on placebo *v* 14% on carvedilol; 95% CI, 18% to 53%; *P*< .001). A significant mortality reduction was also noted when deaths that

occurred in the run-in period were included in the analysis. The statistical validity of the survival analysis across the trials has been questioned because mortality was not the primary endpoint, and only 1 of the 4 trials achieved a significant result when analyzed based on the primary endpoint. Nevertheless, the magnitude of the survival benefit and the reduction in hospitalization were impressive. The survival benefit was not influenced by the cause of disease, age, gender, or baseline ejection fraction. Overall, 7.8% of the placebo group and 5.7% of the carvedilol group discontinued study medication. Data from the individual trials, PRECISE and MOCHA, which evaluated patients with moderate to severe heart failure, found that carvedilol reduced the risk of the combined endpoint of mortality or heart failure hospitalization by 39% to 49% (33,34). The MOCHA Study provided strong evidence for increased benefit from higher dosages (25 mg twice per day) versus lower dosages (6.25 mg twice per day) of carvedilol, so uptitration of carvedilol dosages to 25 mg twice per day is generally recommended. However, favorable effects were noted at 6.25 mg twice per day, so intolerance of high doses should not be a reason for discontinuation of therapy.

The Australia-New Zealand Carvedilol Trial enrolled 415 patients with ischemic cardiomyopathy and a left ventricular ejection fraction of less than 45% (35). Although patients with NYHA functional classes I-III were eligible, the majority enrolled were NYHA functional class I (30%) or II (54%). ACE inhibitors were used in 86% of the participants, whereas 76% were on diuretic therapy, and 38% were on digoxin. This trial also had a run-in phase during which 6% of the patients discontinued ß-blocker therapy. During an average follow-up of 19 months, carvedilol decreased the combined risk of all-cause mortality or any hospitalization by 26% (relative risk .74; 95% CI, .57 to .95; P= .02). Overall mortality was 12.5% in the placebo group and 9.6% in the carvedilol group which was not statistically significant (relative risk .76; 95% CI, .42 to 1.36; P > .10).

Unreported or Ongoing Trials. Studies that are underway will provide additional data concerning specific aspects of the efficacy of ß-blocker therapy in heart failure. The effect of bucindolol on mortality and morbidity in patients with moderate to severe heart failure has been evaluated in the BEST Study. This study enrolled a substantial number of women so the potential influence of gender on the efficacy of ß-blocker therapy can be investigated. The trial has been stopped, and no results are available for analysis.

The COPERNICUS Trial is designed to assess the effect of carvedilol treatment on disease progression and survival in patients with advanced heart failure with symptoms at rest or on minimal exertion. The COMET protocol is a 3,000 patient study that directly compares the survival benefit of carvedilol versus metoprolol. This trial will provide important data concerning the relative efficacy of a selective ß-blocker versus a nonselective ß-blocker with ancillary properties.

Recommendation 2. ß-blocker therapy should be considered for patients with left ventricular systolic dysfunction (left ventricular ejection fraction less than or equal to 40%) who are asymptomatic (ie, NYHA class I) and standard therapy, including ACE inhibitors

(Strength of Evidence = C).

Data from the SOLVD Prevention Trial prospectively illustrated the efficacy of ACE inhibitors in delaying the onset of heart failure symptoms and the need for treatment or hospitalization for heart failure in asymptomatic patients with a left ventricular ejection fraction less than or equal to 35% (36). Similar controlled, clinical trial data that support the use of a ß-blocker in this clinical circumstance are not available. However, significant support for the use of ßblocker therapy in patients with asymptomatic left ventricular dysfunction can be derived from clinical trials in coronary artery disease and hypertension. Previous data indicate that ß-blocker therapy should be used in patients after myocardial infarction (MI) and in patients with myocardial revascularization who have good symptomatic and functional recovery but residual ventricular systolic dysfunction. Trials in hypertension indicate that ß-blocker therapy decreases the risk of developing heart failure. Given the potential of ß-blockers to retard disease progression and improve ventricular function, the risk to benefit ratio seems sufficiently low to support ß-blocker use in asymptomatic patients with left ventricular dysfunction, especially when the dysfunction is marked, and coronary artery disease is present.

Recommendation 3. To maximize patient safety, a period of clinical stability on standard therapy should occur before ß-blocker therapy is instituted. Initiation of ß-blocker therapy in patients with heart failure requires a careful baseline evaluation of clinical status (Strength of Evidence = B).

Initiation of ß-blocker therapy has the potential to worsen heart failure signs and symptoms. This risk increases with the underlying severity of the heart failure that is present. To minimize the likelihood of worsening failure, a period of treatment with standard therapy and evidence of clinical stability without acute decompensation or fluid overload is recommended before initiation of ß-blocker therapy. The majority of the large-scale, ß-blocker heart failure trials required that chronic heart failure be present 3 months or more before initiation of ß-blocker therapy. Patients enrolled in these trials were typically treated with ACE inhibitors (if tolerated), diuretic, and digoxin for at least 2 months and were observed to be clinically stable for 2 to 3 weeks before beginning ß-blocker therapy. Thus, many heart failure clinicians favor a minimum of 2 to 4 weeks of clinical stability on standard therapy before ß-blocker therapy is instituted. Likewise, most clinicians discourage the initiation of ß-blocker therapy in the hospital setting after treatment for new or decompensated heart failure (with or without associated inotrope administration). Some experienced clinicians initiate ß-blocker therapy in the hospital in selected patients who have responded well to inpatient treatment and who can be followed closely after discharge.

Recommendation 4. There is insufficient evidence to recommend the use ofß-blocker therapy for inpatients or outpatients with symptoms of heart failure at rest (ie, NYHA class IV) (Strength of Evidence = C).

ß-Blocker therapy cannot be routinely recommended for NYHA class IV patients because there are currently no clinical trial data to indicate favorable long-term efficacy and safety of ß-blocker therapy in this patient population. A substantial body of observational data indicates that successful institution of ß-blocker therapy in patients with this degree of heart failure is problematic. If used, these agents may precipitate deterioration, and patients so treated should be monitored by a physician who has expertise in heart failure.

The number of patients with class IV heart failure at the time of ß-blocker initiation in controlled clinical trials is small. Available trials, which report data on patients with severe heart failure mostly labeled as NYHA class III, show the potential problems of ß-blocker therapy in this part of the heart failure spectrum. This experience is reflected in a 14-week study that evaluated the effects of ß-blocker therapy in 56 patients (51 NYHA class III and 5 NYHA class IV at randomization) with severe left ventricular dysfunction (average left ventricular ejection fraction of 16% ± 1% and left ventricular filling pressure of 24 mm Hg ± 1 mm Hg) (37). These patients had significant impairment of exercise capacity (mean VO2 max of 13.6 mL/kg/min ± 0.6 mL/kg/min) despite ACE-inhibitor, digoxin and diuretic therapy. Patients were believed to be clinically stable (requiring no medication adjustments) for a 2-week period before an open-label challenge was conducted. Seven patients (12%) failed to complete the open-label, run-in period, during which 5 died and 2 had nonfatal adverse reactions. Clinical parameters did not distinguish these patients from those who were able to continue in the trial. Eighteen of the 49 patients (37%) completing the run-in period experienced worsened dyspnea or fluid retention during this phase. Also, 22% experienced dizziness and required medication adjustment, which delayed up-titration during the run-in. Subsequently, an additional 12% of the patients randomized to carvedilol withdrew from the blinded arm of the study. One of the United States carvedilol trials studied patients with severe left ventricular dysfunction who had markedly reduced exercise capacity as assessed by the 6-minute walk test (38). In this trial, 131 patients with a mean left ventricular ejection fraction of 22% and severe impairment in quality of life underwent a 2-week, open-label challenge phase of 6.25 mg of carvedilol twice per day. Ten of these 131 patients (8%) were unable to complete this run-in phase, most because of worsening heart failure, dyspnea, or dizziness. Subsequently, 11% of the patients randomized to carvedilol withdrew, as did a similar number of patients (11%) in the placebo group. In the recently completed large-scale BEST Trial, the mortality trend in NYHA class III-IV patients favored the ß-blocker bucindolol, but the difference from placebo was not significant. Further analysis of these preliminary findings is necessary, but the data suggest that the striking benefit of ß-blockers in mild-to-moderate heart failure may not be extrapolated to those with severe symptoms.

Recommendation 5. ß-Blocker therapy should be initiated at low doses and up-titrated slowly, generally no sooner than at 2-week intervals. Clinical reevaluation should occur at each titration point and with worsening of patient symptoms. Patients who develop worsening heart failure or other side effects after drug initiation or during titration require adjustment of concomitant medications. These patients may also require a

reduction in ß-blocker dose and, in some cases, temporary or permanent withdrawal of this therapy (Strength of Evidence = B).

ß-Blocker therapy should be initiated at doses substantially less than target doses. Clinical trials required patient reassessment at up-titration of each dose. This careful evaluation by trained nurses and/or heart failure specialists likely contributed to the relatively low withdrawal rates and safety profiles observed in the clinical trials.

Treatment for symptomatic deterioration may be required during ß-blocker titration, but with appropriate adjustments in therapy, most patients can be maintained and generally achieve target doses. There is a risk of worsening heart failure, and vasodilatory side effects may occur with certain agents. Worsening heart failure is typically reflected by increasing fatigue, lower exercise tolerance, and weight gain. Increased diuretic doses may be required for signs and symptoms of worsened fluid retention. Treatment options also include temporary down-titration of the ß-blocker to the last tolerated dose. Abrupt withdrawal should be avoided. A minimum period of stability of 2 weeks should occur before further up-titration is attempted. Hypotensive side effects may often resolve with reduction in diuretic dose. Temporary reductions in ACE inhibitor dose may be helpful for symptomatic hypotension not obviated by staggering the schedule of vasoactive medications. Administration of carvedilol with food may alleviate vasodilatory side effects as well.

If ß-blocker treatment is interrupted for a period exceeding 72 hours and the patient is still judged a candidate for this therapy, drug treatment should be reinitiated at 50% of the previous dose. Subsequent up-titration should be conducted as previously described.

Recommendation 6. In general, patients who experience a deterioration in clinical status or symptomatic exacerbation of heart failure during chronic maintenance treatment should be continued on ß-blocker therapy (Strength of Evidence = C).

Clinical decompensation that occurs during stable maintenance therapy is less likely caused by chronic ß-blocker therapy than other factors (diet or medication noncompliance, ischemia, arrhythmia, comorbid disease, infection, or disease progression). In these situations, maintaining the current ß-blocker dose while relieving or compensating for the precipitating factor(s) is most often the best course. Data from patients randomized to continue or discontinue ß-blocker therapy in this setting are not currently available. However, studies of the withdrawal of ß-blocker therapy in patients with persistent left ventricular systolic dysfunction but improved and stable clinical heart failure have revealed a substantial risk of worsening heart failure and early death after discontinuation of ß-blocker therapy (39,40).

Recommendation 7. Patient education regarding early recognition of symptom exacerbation and side effects is considered important. If clinical uncertainty exists, consultation with clinicians who have expertise in heart

failure and/or specialized programs with experience in ß-blocker use in patients with heart failure is recommended (Strength of Evidence = B).

In certain patients, frequent return visits for dose-titration may be difficult to accommodate in a busy clinical practice. Trained personnel, including nurse practitioners, physicians' assistants, and pharmacists with physician supervision, may more efficiently perform patient education and reevaluation during up-titration. Heart failure specialty programs are more likely to have the resources to provide this follow-up and education (41). Consultation or referral may be particularly beneficial when the clinical heart failure status of the patient is uncertain or problems arise during initiation of therapy or dose-titration that may cause unwarranted discontinuation of therapy. Ideal patients for ß-blocker therapy should be compliant and have a good understanding of their disease and their overall treatment plan. Patients should be aware that symptomatic deterioration is possible early in therapy and that symptomatic improvement may be delayed for weeks to months.

Unresolved Therapeutic Issues

Combining ß-Blocking Agents With Amiodarone Therapy. Concomitant use of amiodarone was generally precluded in the trials evaluating carvedilol and most other ß-blockers. However, the use of this agent for rate control of atrial arrhythmia or for maintenance of sinus rhythm is common in heart failure patients. Drug interactions between ß-blockers and amiodarone are possible, including symptomatic bradycardia, and may limit the maximum tolerated dose of the ß-blocker. When the combination is used, the smallest effective dose of amiodarone should be employed. Given the lack of a clear survival benefit, amiodarone is not a substitute for ß-blocker therapy in heart failure patients who are candidates for this therapy.

Implantation of Cardiac Pacemakers. Given the strength of evidence that supports ß-blocker therapy in patients with symptomatic heart failure, some physicians would consider pacemaker implantation when symptomatic bradycardia or heart block occur during the initiation of this therapy, although no data are available to support such use. Consideration should be given, after weighing risks and benefits, to the withdrawal of other drugs that may have bradycardia effects.

Duration of Therapy. Whether patients experiencing marked improvement in left ventricular systolic dysfunction and heart failure symptoms during therapy can be successfully withdrawn from ß-blocker therapy remains to be established. Concern continues that such patients would experience worsening after ß- blocker withdrawal, either in systolic function or symptoms, over a time period that is undefined. Until clinical trial data indicate otherwise, the duration of ßblocker therapy must be considered indefinite.

Digoxin

Background for Recommendations

Although little controversy exists as to the benefit of digoxin in patients with symptomatic left ventricular systolic dysfunction and concomitant atrial fibrillation, the debate continues over its current role in similar patients with normal sinus rhythm. Recent information regarding digoxin's mechanism of action and new analyses of clinical data from the DIG Trial and the combined PROVED and RADIANCE Trial databases provide additional evidence of favorable efficacy that was unavailable to previous guideline committees (42-47). In fact, this information has recently formed the basis of Food and Drug Administration (FDA) approval of digoxin for the treatment of mild to moderate heart failure (48). Digoxin, a drug that is inexpensive and can be given once daily, represents the only orally effective drug with positive inotropic effects approved for the management of heart failure. The committee's consensus is that digoxin, when used in combination with other standard therapy, will continue to play an important role in the symptomatic management of the majority of patients with heart failure.

The efficacy of digoxin for the treatment of heart failure caused by systolic dysfunction has traditionally been attributed to its relatively weak positive inotropic action that comes from inhibition of sodium-potassium adenosine triphosphatase (ATPase) that results in an increase in cardiac myocyte intracellular calcium. However, in addition to positive inotropy, digitalis has important, neurohormonal-modulating effects in patients with chronic heart failure, including a sympathoinhibitory effect that cannot be ascribed to its inotropic action (49,50). Digoxin also ameliorates autonomic dysfunction as evidenced by studies of heart rate variability, which indicates increased parasympathetic and baroreceptor sensitivity during therapy (51).

Recommendation 1. Digoxin should be considered for patients who have symptoms of heart failure (NYHA class II-III, Strength of Evidence = A and NYHA class IV, Strength of Evidence = C) caused by left ventricular systolic dysfunction while receiving standard therapy.

Digoxin increases left ventricular ejection fraction and alleviates symptomatic heart failure as evidenced by drug-related improvement in exercise capacity and reductions in heart-failureassociated hospitalization and emergency room visits. Digoxin should be used in conjunction with other forms of standard heart failure therapy including ACE inhibitors, diuretics and ß-blockers.

The DIG Trial, a randomized, double-blind, placebo-controlled trial in over 7,000 patients with heart failure, showed a neutral effect on the primary study endpoint and mortality from any cause during an average follow-up of approximately 3 years (42). In the main trial, 6,800 patients with left ventricular ejection fraction less than or equal to 45% were randomized to digoxin or placebo, in addition to diuretics and ACE inhibitors. A total of 1,181 deaths occurred on digoxin (34.8%) and 1,194 on placebo (35.1%) for a risk ratio of .99 (95% CI, .91 to 1.07; P = .80). These results differ from other oral agents with inotropic properties that have been associated with an adverse effect on mortality. In addition, the need for hospitalization and cointervention (defined as

increasing the dose of diuretics and ACE inhibitors or adding new therapies for worsening heart failure) was significantly lower in the digoxin group, even in those patients who were not previously taking digoxin. Fewer patients on digoxin compared with placebo were hospitalized for worsening heart failure (26.8%*v* 34.7%; risk ratio .72; 95% CI, .66 to .79; *P* < .001). These long-term data are consistent with recent results obtained from an analysis of the combined PROVED and RADIANCE databases (45). In this analysis, patients who continued digoxin as part of triple therapy with diuretics and an ACE inhibitor were much less likely to develop worsening heart failure (4.7%) than those treated with a diuretic alone (39%, *P* < .001), diuretic plus digoxin (19%, *P* = .009) or diuretic plus an ACE inhibitor (25%, *P* = .001).

Although there are no clinical trial data (level A evidence) for the efficacy of digoxin in patients with NYHA Class IV heart failure, there is evidence that digoxin works across the spectrum of left ventricular systolic dysfunction. A prespecified subgroup analysis of patients enrolled in the DIG Trial with evidence of severe heart failure (as manifested by left ventricular ejection fraction less than 25%, or cardiothoracic ratio [CTR] greater than .55) showed the benefit of digoxin (48). The following reductions in the combined endpoint of all-cause mortality or hospitalization were seen on digoxin compared with placebo: 16% reduction (95% CI, 7% to 24%) in patients with a left ventricular ejection fraction of less than 25%, and a 15% reduction (95% CI, 6% to 23%) in patients with a CTR of greater than .55 (43). Reductions in the risk of the combined endpoint of heart-failure related mortality or hospitalization were even more striking: 39% (95% CI, 29% to 47%) for patients with left ventricular ejection fraction less than 25%, and 35% (95% CI, 25% to 43%) for patients with a CTR greater than .55 (48).

Evidence for the efficacy of digoxin in patients with mild symptoms of heart failure has been provided by a recent retrospective, cohort analysis of the combined PROVED and RADIANCE data (52). The outcome of patients in these trials who were randomized to digoxin withdrawal or continuation was categorized by using a prospectively obtained heart failure score based on clinical signs and symptoms. Patients in the mild heart failure group (heart failure score of 2 or less) who were randomized to have digoxin withdrawn were at increased risk of treatment failure and had deterioration of exercise capacity and left ventricular ejection fraction compared with patients who continued digoxin (all *P* < .01). Patients in the moderate heart failure group who had digoxin withdrawn were significantly more likely to experience treatment failure than either patients in the mild heart failure group or patients who continued digoxin (both *P* < .05). These data suggest that patients with left ventricular systolic dysfunction benefit from digoxin despite only mild clinical evidence of heart failure.

In summary, a large body of evidence supports the efficacy of digoxin in patients with symptomatic heart failure caused by left ventricular systolic dysfunction. Digoxin has been shown to decrease hospitalizations, as well as emergency room visits; decrease the need for co-intervention; and improve exercise capacity (42-44,53,54). Taken as a whole, these clinical trial data provide support for digoxin's beneficial effect on morbidity and neutral effect on mortality (42).

Recommendation 2. In the majority of patients, the dosage of digoxin should be .125 mg to .25 mg daily (Strength of Evidence = C).

Recent data suggest that the target dose of digoxin therapy should be lower than traditionally assumed. Although higher doses may be necessary for maximal hemodynamic effects (55), beneficial neurohormonal and functional effects appear to be achieved at relatively low serum digoxin concentrations (SDC) typically associated with daily doses of .125 mg to .25 mg of digoxin (55-57). The utility of lower SDC is supported by recent clinical trial data; the mean SDC achieved in the RADIANCE Trial was 1.2 ng/mL and in the DIG Trial was 0.8 ng/mL (42,44). Recent retrospective, cohort analysis of the combined PROVED and RADIANCE databases indicates that patients with a low SDC (less than .9 ng/mL) were no more likely to experience worsening symptoms of heart failure on maintenance digoxin than those with a moderate (.9 to 1.2 ng/mL) or high (greater than 1.2 ng/mL) SDC (41). All SDC groups were significantly less likely to deteriorate during follow-up compared with patients withdrawn from digoxin.

Therefore, patients with left ventricular systolic dysfunction and normal sinus rhythm should be started on a maintenance dosage of digoxin (no loading dose) of .125 or .25 mg once daily based on ideal body weight, age, and renal function For patients with normal renal function, a dosage of digoxin of .25 mg/day will be typical. Many patients with heart failure have reduced renal function and should begin on .125 mg daily. In addition, patients with a baseline conduction abnormality, or who are small in stature or elderly, should be started at .125 mg/day, which can be up-titrated if necessary. Once dosing has continued for a sufficient period for serum concentration to reach steady state (typically in 2 to 3 weeks), some clinicians consider the measurement of a SDC, especially in elderly patients or those with impaired renal function in which the digoxin dose is often not predictive of SDC. SDC measurements may be considered when 1) a significant change in renal function occurs; 2) a potentially interacting drug (amiodarone, quinidine, or verapamil) is added or discontinued; or 3) confirmation of suspected digoxin toxicity is necessary in a patient with signs or symptoms and/or electrocardiographic changes consistent with this diagnosis. Samples for trough SDC should be drawn more than 6 hours after dosing. Otherwise, the result is difficult to interpret because the drug may not be fully distributed into tissues.

Recommendation 3. In patients with heart failure and atrial fibrillation with a rapid ventricular response, the administration of high doses of digoxin (greater than .25 mg) for the purpose of rate control is not recommended. When necessary, additional rate control should be achieved by the addition of ß-blocker therapy or amiodarone (Strength of Evidence = C).

Digoxin continues to be the drug of choice for patients with heart failure and atrial fibrillation. However, the traditional practice of arbitrarily increasing the dose (and SDC) of digoxin until ventricular response is controlled should be abandoned because the risk of digoxin toxicity increases as well. Digoxin alone is

often inadequate to control ventricular response in patients with atrial fibrillation, and the SDC should not be used to guide dosing to achieve rate control. Therefore, digoxin should be dosed in the same manner as in a patient with heart failure and normal sinus rhythm.

Digoxin slows ventricular response to atrial fibrillation through enhancement of vagal tone. However, with exertion or other increases in sympathetic activity, vagal tone may decrease and ventricular rate accelerate. Addition of a ß-blocker or amiodarone 1) complements the pharmacological action of digoxin and provides more optimal rate control; 2) allows the beneficial clinical effects of digoxin to be maintained; and 3) limits the risk of toxicity that may occur if digoxin is dosed to achieve a high SDC (58). For patients who have a contraindication to ß-blockers, amiodarone is a reasonable alternative. If amiodarone is added, the dose of digoxin should be reduced, and the SDC should be monitored so that the serum concentration can be maintained in the desired range. Some clinicians advocate the short-term, intravenous administration of diltiazem for the acute treatment of patients with very rapid ventricular response, especially those with hemodynamic compromise. This drug is not indicated for long-term management because its negative inotropic effects may worsen heart failure.

Unresolved Therapeutic Issues

Combination With ß-blockers. ß-Blocker therapy has become pivotal in the management of heart failure. However, the majority of patients enrolled in controlled clinical trials that study the efficacy of digoxin were not taking ß-blockers. Therefore, it is uncertain whether or not digoxin should be routinely included as part of a ß-blocker regimen for symptomatic heart failure caused by left ventricular systolic dysfunction. There are attractive features of combining digoxin with ß-blocker therapy in the treatment of heart failure. The majority of heart failure patients have coronary artery disease and may be at risk for transient episodes of myocardial ischemia that could cause catecholamine release and sudden cardiac death. Combining digoxin with a ß-blocker may preserve the beneficial effects of digoxin on the symptoms of heart failure while minimizing the potential detrimental effects of this therapy on catecholamine release in the setting of ischemia (47).

Combination with Diuretics. Non-potassium-sparing diuretics can produce electrolyte abnormalities such as hypokalemia and hypomagnesemia, which increases the risk of digoxin toxicity. The combination of digoxin with a potassium- sparing diuretic would be a potentially safer alternative. Further study will be necessary to carefully elucidate the efficacy and safety of combining digoxin with these agents.

Anticoagulation and Antiplatelet Drugs
Background for Recommendations

Patients with heart failure are recognized to be at increased risk for thromboembolic events that can be arterial or venous in origin. In addition to atrial fibrillation and poor ventricular function (which promote stasis and increase the risk of thrombus formation), patients with heart failure have other

manifestations of hypercoagulability. Evidence of heightened platelet activation; increased plasma and blood viscosity; and increased plasma levels of fibrinopeptide A, ßthromboglobulin, D-dimer, and von Willebrand factor (59-61) have been found in many patients. Despite a predisposition, estimates regarding the incidence of thromboemboli in patients with heart failure vary substantially between 1.4 and 42 per 100 patient years (62-65). Although variability in the reported incidence likely results from differences in the populations studied and the methods used to identify these events, the consensus is that pulmonary and systemic emboli are not common in heart failure patients. Traditionally, the issue of anticoagulation in patients with heart failure centered on warfarin. Growing recognition of the importance of ischemic heart disease as a cause of heart failure suggests that the role of antiplatelet therapy must be considered in patients with this syndrome as well.

Previous guidelines have recommended warfarin anticoagulation in patients with heart failure complicated by atrial fibrillation and in heart failure patients with prior thromboembolic events (18,19). Warfarin anticoagulation specifically was not recommended in patients with heart failure in the absence of these indications. There have been no randomized, controlled trials of warfarin in patients with heart failure. Therefore, recommendations regarding its use, in the absence of atrial fibrillation or clinically overt systemic or pulmonary thromboemboli, must be made on the basis of cohort data and expert opinion. The likely incidence of thromboembolic events and the possibility of averting them with warfarin are important considerations for any guideline recommendation. In addition, the potential beneficial effects of warfarin on coronary thrombotic events, independent of embolic phenomenon, must be taken into account. The substantial clinical trial data that reflect the beneficial effects of antiplatelet therapy in patients with ischemic heart disease suggest that new guideline recommendations for heart failure should address the role of this form of therapy in patients with left ventricular dysfunction.

Anticoagulation

Recommendation 1. All patients with heart failure and atrial fibrillation should be treated with warfarin (goal, international normalized ratio (INR) 2.0 to 3.0) unless contraindicated (Strength of Evidence = A).

The committee agrees with previous guideline recommendations that concern warfarin therapy in patients with heart failure complicated by atrial fibrillation. The benefit of warfarin anticoagulation in this setting is well established through several randomized trials (66). Patients with heart failure commonly have atrial fibrillation. Warfarin anticoagulation should be implemented in all of these patients unless clear contraindications exist.

Recommendation 2. Warfarin anticoagulation merits consideration for patients with left ventricular ejection fraction of 35% or less. Careful assessment of the risks and benefits of anticoagulation should be undertaken in individual patients (Strength of Evidence = B).

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Cohort analyses examining the relationship between warfarin use and noncoronary thromboembolism in patients with heart failure have not consistently yielded positive findings (62,63,65,67-69). It is possible that the lack of consistent benefit was related to the low incidence of identifiable embolic events in these populations. However, these studies do not make a convincing argument for the use of warfarin to prevent embolic events in the absence of atrial fibrillation or a previous thromboembolic episode.

In contrast, a recent cohort analysis of the SOLVD population focused on the relation between warfarin use and the risk of all-cause mortality rather than risk for embolic events (70). After adjustment for baseline differences, patients treated with warfarin at baseline had a significantly lower risk of mortality during follow-up (adjusted hazard ratio .76; 95% CI, .65 to .89, *P* = .0006). In addition to a mortality benefit, warfarin use was also associated with a significant reduction in the combined endpoint of death or hospitalization for heart failure (adjusted hazard ratio .82; 95% CI, .72 to .93, *P* = .002). In the SOLVD population, the benefit associated with warfarin use was not significantly influenced by 1) presence or absence of symptoms (treatment trial *v* prevention trial), 2) randomization to enalapril or placebo, 3) gender, 4) presence or absence of atrial fibrillation; 5) age, 6) ejection fraction, 7) NYHA class, or 8) origins of disease.

The benefit associated with warfarin use in the cohort analysis of the SOLVD population was related to a reduction in cardiac mortality. Specifically, there was a significant reduction among warfarin users in deaths that were identified as sudden, in deaths associated with heart failure, and in fatal MI. In contrast (yet in agreement with previous cohort analyses), there was no significant difference in deaths considered cardiovascular but noncardiac, including pulmonary embolism and fatal stroke. Some caution is needed in consideration of this finding because the number of cardiovascular deaths that were noncardiac was far less than the number of cardiac deaths.

Reduction in ischemic events is one potential explanation for the apparent benefit from warfarin in the SOLVD Study. Warfarin users showed a reduced rate of hospitalization for unstable angina or nonfatal MI. Prior investigations of patients after acute MI showed that warfarin anticoagulation, when started within 4 weeks, reduces the incidence of fatal and nonfatal coronary events, as well as pulmonary embolus and stroke (71).

As with other post hoc, cohort analyses, it is possible that the findings from the SOLVD Study may result from differences between the treatment groups that were not identified and for which statistical correction could not adequately adjust. For this reason, evidence from any cohort study must be considered less powerful compared with evidence derived from randomized, controlled trials. Nevertheless, in the absence of randomized data, the SOLVD cohort analysis represents reasonable evidence to support more aggressive use of warfarin anticoagulation than previously recommended in patients with reduced left ventricular ejection fraction and sinus rhythm. The data from this analysis provide no information regarding the ideal warfarin dose in this patient population. Therefore, the dosing recommendation should likely conform to that derived from previous randomized trials performed in patients without mechanical prosthetic valves (INR 2.0 to 3.0).

Antiplatelet Drugs

Recommendation 1. With regard to the concomitant use of ACE inhibitors and acetylsalicylic acid (ASA), each medication should be considered on its own merit for individual patients. Currently, there is insufficient evidence concerning the potential negative therapeutic interaction between ASA and ACE inhibitors to warrant withholding either of these medications in which an indication exists (Strength of Evidence = C).

Strong evidence supports the clinical benefit of aspirin in ischemic heart disease and atherosclerosis (72-75). However, recent post hoc analyses of large randomized trials involving ACE inhibitors in heart failure and post-MI suggest the possibility of an adverse drug interaction between ASA and ACE inhibitors (76-78). A retrospective cohort analysis of the SOLVD Study found that patients on antiplatelet therapy (assumed to be ASA in the great majority of patients) derived no additional survival benefit from the addition of enalapril. Data from CONSENSUS II and GUSTO-1 in post-MI patients, suggest not only no additive benefit, but the possibility of a negative effect on mortality from the combination of ASA and ACE inhibition. In contrast, an unadjusted, retrospective registry study in patients with chronic coronary artery disease did not support an adverse interaction (79). Interestingly, in an adjusted analysis of the subset of patients with heart failure in this study, the beneficial effects of aspirin seemed less evident in patients taking ACE inhibitors. Despite these provocative post hoc findings, no prospective studies have yet been reported that concern the possible adverse interaction between ACE inhibitors and aspirin. To date, there is no clear evidence of harm from the combination of ASA and ACE inhibitors in patients with heart failure (76).

There is also some evidence that the potential interaction between ASA and ACE inhibitors may be dose related. A recent meta-analysis of all hypertension and heart failure patients who have received both ASA and ACE inhibitors suggests that ASA at doses equal to or less than 100 mg showed no interaction with ACE inhibitors (80). Any interaction, if observed, occurred at higher doses of aspirin.

A potential mechanism for the hypothesized adverse interaction between ASA and ACE inhibitors in patients with heart failure involves prostaglandin synthesis. ACE inhibition is believed to augment bradykinin which, in turn, stimulates the synthesis of various prostaglandins that may contribute vasodilatory and other salutary effects. In the presence of ASA, the bradykinin-induced increase in prostaglandins should be attenuated or blocked, which potentially reduces the benefits of ACE inhibition. Invasive hemodynamic monitoring has shown that the acute hemodynamic effect of enalapril is blunted by concomitant administration of aspirin (81). Another possibility is that ASA and ACE inhibitors act in a similar fashion in heart failure, therefore no added benefit is gained from the combination. ACE inhibitors appear to reduce ischemic events in heart failure patients possibly through antithrombotic effects, which could mimic those of

antiplatelet agents. Recent study results that suggest ASA may have independent beneficial action on ventricular remodeling support the hypothesis of similar mechanisms of action for ACE inhibitors and ASA (82).

Development of the adenosine diphosphate (ADP) antagonists, ticlopidine and clopidogrel, provides alternative therapy for platelet inhibition that does not appear to influence prostaglandin synthesis (83). In direct comparison with aspirin, large-scale clinical trial results have established the efficacy of clopidogrel in the prevention of vascular events in patients with arteriosclerotic disease (84). Clinical data are limited with ADP antagonists in heart failure. However, hemodynamic evaluation found a similar reduction in systemic vascular resistance in heart failure patients treated with the combination of ACE inhibitors and ticlopidine versus ACE inhibitors alone, which suggests no adverse hemodynamic interaction with ACE inhibition with this type of antiplatelet compound (85). Definitive resolution of the therapeutic implications of the ASA/ACE inhibitor interaction and the appropriate alternative therapy, if any, in heart failure awaits the results of additional clinical research studies.

Angiotensin II Receptor Blockers

Background for Recommendations

Angiotensin ll (AT) receptor blockers (ARBs) differ in their mechanism of action compared with ACE inhibitors. Rather than inhibiting the production of AT by blockade of ACE, ARBs block the cell surface receptor for AT. ARBs that are currently available are selective and only effectively inhibit the AT1 subtype of this receptor. Theoretical benefits of ARBs include receptor blockade of AT produced by enzymes other than ACE and maintenance of ambient AT to maintain or increase stimulation of AT2 receptors. AT1 receptor antagonism is important because this receptor appears to mediate the classical adverse effects associated with AT in heart failure. In contrast, the AT2 receptor subtype appears to counterbalance AT1 receptor stimulation by causing vasodilation and inhibiting proliferative and hypertrophic responses (86). Thus, the selective receptor blockade of the current ARBs may be particularly advantageous. Theoretical concerns about ARB therapy include the potential deleterious effects of increased AT levels and AT2 receptor-mediated enhancement of apoptosis. Whether ARBs have beneficial effects similar to ACE inhibitors on the course of coronary artery disease remains to be determined. ARBs may or may not influence bradykinin concentrations, which are anticipated to rise with ACE inhibitor therapy and may contribute to their efficacy.

The hemodynamic actions of ARBs have, thus far, been similar to ACE inhibitors for
reduction of blood pressure in hypertension and lowering of systemic vascular resistance in heart failure (87). ARBs have a similar mild-to-modest effect on exercise capacity and produce a comparable reduction in norepinephrine relative to ACE inhibitors (88).

Recommendation 1. ACE inhibitors rather than ARBs continue to be the agents of choice for blockade of the renin-angiotensin system in heart failure, and they remain the cornerstone of standard therapy for patients

with left ventricular systolic dysfunction with or without symptomatic heart failure (Strength of Evidence = A).

At present, it is not possible to predict where ARBs will ultimately reside among accepted therapies for heart failure. Although the initial small ELITE Trial suggested a greater benefit from a losartan dosage of 50 mg daily than from a captopril dosage of 50 mg 3 times daily on mortality in elderly patients with heart failure (89), the ELITE II Mortality Trial, which included more than 3,000 patients (90), showed no comparative benefit from losartan and a trend for a better outcome and fewer sudden deaths with captopril (91). This result provides no evidence that the low dose (50 mg) of losartan that was tested is better than an ACE inhibitor for treating heart failure, but it does not exclude the efficacy of a higher dose designed to provide continuous inhibition of the AT1 receptor. Tolerability of losartan was better than of captopril, primarily because of an ACE inhibitor cough. But the well-established efficacy of the ACE inhibitors on outcome in the post-MI period, in diabetes, in atherosclerosis, and in heart failure mandates that this drug group remains agents of choice for inhibiting the renin-angiotensin system in heart failure. The RESOLVD Trial suggested no major differences in efficacy of candesartan and enalapril, with a trend favoring enalapril during the study period of 43 weeks (92). The OPTIMAAL and VALIANT Studies will provide information specifically about the role of ARBs versus ACE inhibitors in the post-MI population.

Currently, ACE inhibitors continue to be regarded as the therapy of choice to inhibit the renin-angiotensin system in patients with asymptomatic and symptomatic left ventricular dysfunction. There is no current rationale to recommend initiating ARBs in patients with new onset heart failure or for switching from a tolerated ACE-inhibitor regimen to an ARB in patients with chronic heart failure.

Recommendation 2. All efforts should be made to achieve ACE inhibitor use in patients with heart failure caused by left ventricular dysfunction. Patients who are truly intolerant to ACE inhibitors should be considered for treatment with the combination of hydralazine and isosorbide dinitrate (Hyd-ISDN) (Strength of Evidence = B) or an ARB (Strength of Evidence = C).

Previous large-scale trials do not specifically address the role of ARB and Hyd-ISDN in patients who are intolerant to ACE inhibitors. One arm of the CHARM Study has been specifically designed to test the effectiveness of candesartan in patients with systolic dysfunction who are intolerant to ACE inhibitors. The primary endpoint in this study will be a composite of cardiovascular death and time until first hospitalization for heart failure. For now, ARBs offer a reasonable alternative in the heart failure or post-MI patient who is truly intolerant to ACE inhibition. Intolerance because of cough should always trigger a careful reevaluation for congestion. If congestion is present, cough should abate with increases in diuretic that should allow ACE-inhibitor use to continue (93). It should be emphasized that patients intolerant to ACE inhibitor because of renal dysfunction, hyperkalemia, or hypotension are often intolerant to ARBs as well.

ACE inhibitor intolerance because of persistent symptomatic hypotension in advanced heart failure may represent severe dependence on the hemodynamic support of the renin-angiotensin system, which generally would predict hypotension with ARB use as well.

The combination of Hyd-ISDN has not been studied in the post-MI population, but sufficient experience exists to support its use in the ACE-inhibitor-intolerant patient with symptomatic heart failure. Hydralazine blocks the development of nitrate tolerance, which argues for the use of combination therapy. Although they were not studied alone in a heart failure mortality trial, oral nitrates represent another reasonable alternative for patients intolerant to both ACE inhibitors and hydralazine.

Unresolved Therapeutic Issues

Combination Therapy With ACE Inhibitors and ARBs. Interest has grown in the potential utility of combining ACE inhibitors and ARBs in patients with heart failure. Initial data suggest that the combination yields more vasodilation and decreased blood pressure than either agent alone. The addition of losartan to an ACE inhibitor has been found to improve exercise capacity compared with an ACE inhibitor alone (94). Preliminary data from the RESOLVD Trial suggest that ventricular dilation and neuroendocrine activation may be best reduced with combination therapy, but other endpoints were not clearly affected. Trials are currently underway to determine the safety, as well as benefit, of more complete blockade of the renin-angiotensin system. The Val-HeFT Trial is a large-scale investigation of the effect of valsartan in addition to ACE inhibitors on morbidity and mortality in symptomatic patients with heart failure caused by systolic dysfunction. One arm of the CHARM Study will also examine the effect of the addition of candesartan in patients with symptomatic, systolic dysfunction treated with an ACE inhibitor. Preliminary data from the RESOLVD Trial suggest that combination therapy may be even more efficacious when used in conjunction with ß-blocker treatment. Results from Val-HeFT and CHARM in the subset of patients treated with ß-blocker therapy will provide more information concerning this strategy.

Combination therapy represents a rational option when treating severe hypertension or other vasoconstriction but cannot, at present, be recommended as routine therapy in the absence of a proven superiority to ACE-inhibitor therapy alone.

HFSA Guidelines
Criteria for NYHA functional classification for chronic heart failure patients, functional capacity

CLASS 1	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea.
CLASS 2	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnea.
CLASS 3	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation or dyspnea.
CLASS 4	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

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HFSA Guidelines
Glossary of Clinical Trials

AVID	Antiarrhythmics Versus Implantable Defibrillators
BEST	Beta-blocker Evaluation of Survival Trial
CAMIAT	Canadian Amiodarone Myocardial Infarction Arrhythmia Trial CAPRIE Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events CASH Cardiac Arrest Study Hamburg
CHF-STAT	Congestive Heart Failure-Survival Trial of Antiarrhythmic Therapy
CHARM	Candesartan Cilexetil in Heart Failure Assessment of Reduction in Mortality and Morbidity
CIBIS	Cardiac Insufficiency BIsoprolol Study
CIBIS II	Cardiac Insufficiency Bisoprolol Study II
CIDS	Canadian Implantable Defibrillator Study
COMET	Carvedilol or Metoprolol European Trial
CONSENSUS	Cooperative North Scandinavian Enalapril Survival Study
CONSENSUS II	Cooperative New Scandinavian Enalapril Survival Study II
COPERNICUS	Carvedilol Prospective Randomized Cumulative Survival Trial
DEFINITE	Defibrillators in Nonischemic Cardiomyopathy Treatment Evaluation
DIAMOND	Danish Investigation of Arrhythmia and Mortality on Dofetilide
DIG	Digitalis Investigation Group
ELITE	Evaluation of Losartan In The Elderly
ELITE II	Losartan Heart Failure Survival Study - ELITE II
EMIAT	Infarction Amiodarone Trial
GESICA	Grupo de Estudio de Sobrevida en Insuficiencia Cardiaca en Argentina
GUSTO 1	Global Utilization of Streptokinase and TPA for Occluded coronary arteries
MADIT	Multicenter Automatic Defibrillator Implantation Trial
MADITII	Multicenter Automatic Defibrillator Implantation Trial II Metoprolol in Dilated Cardiomyopathy trial
MERIT-HF	Metoprolol CR/XL Randomized Intervention Trial in Heart Failure
MOCHA	Multicenter Oral Carvedilol in Heart-failure Assessment
MTT	Myocarditis Treatment Trial
OPTIMALL	Optimal Therapy in Myocardial Infarction with the
Angiotensin II	Antagonist Losartan
PRECISE	Prospective Randomized Evaluation of Carvedilol In Symptoms and Exercise
PROVED	Prospective Randomized study Of Ventricular failure and the Efficacy of Digoxin
RADIANCE	Randomized Assessment of Digoxin on Inhibitors of the Angiotensin Converting Enzyme
RALES	Randomized Aldactone Evaluation Study

RESOLVD	Randomized Evaluation of Strategies for Left Ventricular Dysfunction
SAVE	Survival And Ventricular Enlargement
SCD-HeFT	Sudden Cardiac Death in Heart Failure: Trial of prophylactic amiodarone versus implantable defibrillator therapy
SOLVD	Studies Of Left Ventricular Dysfunction
SWORD	Survival With Oral D-sotalol
ValHeFT	Valsartan Heart Failure Trial
VALIANT	Valsartan in Acute Myocardial Infarction

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APPENDIX D

Genentech
A Member of the Roche Group

SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

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

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Principal Investigator	
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Reporter name	
Reporter Telephone #	
Reporter Fax #	

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

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

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

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