Paclitaxel, Trastuzumab, and Pertuzumab in the Treatment of Metastatic HER2-Positive Breast Cancer

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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Westchester
Commack
Basking Ridge
Rockville Centre
Monmouth

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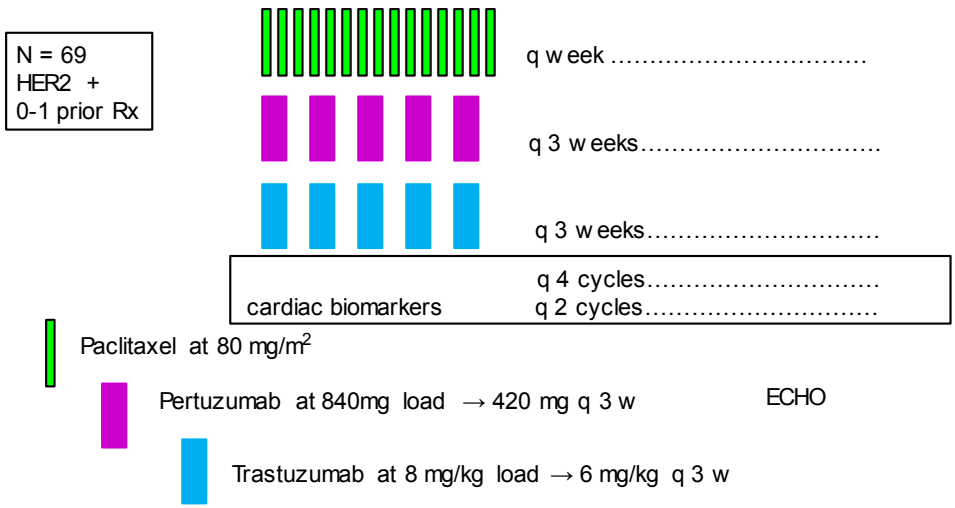
1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a phase II study of pertuzumab in combination with trastuzumab and paclitaxel for the treatment of patients with Stage IV HER2 (+) breast cancer. The regimen will consist of paclitaxel (80 mg/m^2) weekly + trastuzumab every 3 weeks (8 mg/kg loading dose → 6 mg/kg every 3 weeks) + pertuzumab every 3 weeks (840 mg as a loading dose → 420 mg), all given intravenously (IV). Patients may be given trastuzumab weekly in lieu of every 3 weeks (4 mg/kg loading dose → 2 mg/kg every 3 weeks). Patients will be on treatment until progression of disease. Study blood will be collected serially for cardiac biomarker analysis [troponin I (cTnI), brain type natruretic peptide (BNP), neuregulin-1ß (NRG-1ß)]. We will also assess the left ventricular ejection fraction (LVEF) at baseline and every 4th cycle of treatment with an echocardiogram (ECHO) with a strain imaging analysis. When an ECHO cannot be done, a multi-gated acquisition scan (MUGA) may be done.

Schema

Schema

Accrual Goal = 69 patients



- Research bloods (TnI, BNP, NRG-1ß) every other cycle (pre- and post-infusion on day 1). If research blood work is missed on day 1, it can be done on day 8 or 15 pre- and post-infusion.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

- The primary objective of this study will be the proportion of patients who are progression free at 6 months or later. Patients who are considered progression-free at 6 months are deemed successes. Failures are those patients who progressed before the 6 month mark.

- The secondary objectives will be response, safety (including cardiac safety) and tolerability. A cardiac event will be defined as a) "symptomatic" left ventricular systolic dysfunction (LVSD) (deaths and non-deaths), b) non-LVSD cardiac death, or c) probable cardiac death.

3.0 BACKGROUND AND RATIONALE

Anti-HER2 Therapies for Patients with Metastatic HER2-Postive Breast Cancer

HER2/*neu* (c-erb-B2) is a proto-oncogene which encodes the 185kDa HER2 protein, a transmembrane tyrosine kinase receptor that is part of the human epidermal growth factor receptor family[1]. The HER2/*neu* gene is amplified in approximately 20-30% of human breast cancers, leading to HER2 protein overexpression, which is associated with clinically aggressive disease associated with poor prognostic features and shortened disease-free and overall survival.[1] Trastuzumab (Herceptin®) (T) is a humanized monoclonal antibody which binds to the extracellular domain of HER2, and in clinical trials has improved outcomes for patients with both metastatic and early stage HER2 positive breast cancer[2,3,4]. Although the precise mechanism of action of trastuzumab in vivo is not fully understood, its effects may be multiple and include the following: disruption of intracellular signaling leading to cell cycle arrest and apoptosis, [5,6] antibody-dependent cellular cytotoxicity[7], and inhibition of angiogenesis[8].

Trastuzumab has synergistic activity with a number of different chemotherapy agents [2,9-10] and additionally, has activity as a single agent in women with metastatic breast cancer overexpressing HER2 with response rates of 11.6%-15% in pretreated patients and 35% in the first-line setting [11,12,13]. For patients with metastatic breast cancer overexpressing HER2, the addition of trastuzumab to chemotherapy has been shown to improve time to progression (TTP), time to treatment failure (TTF), overall response rate (ORR), duration of response (DOR) and overall survival (OS) when compared to chemotherapy alone as front-line treatment [2,9]. Slamon et al reported the results of the pivotal phase III trial in which chemotherapy alone was compared to chemotherapy plus trastuzumab in 469 women with HER2 overexpressing metastatic breast cancer who had not previously received chemotherapy for metastatic disease[2]. Patients were randomized to an anthracycline or paclitaxel with or without trastuzumab. The results were in favor of chemotherapy with trastuzumab vs chemotherapy alone in terms of TTP (7.4 months vs 4.6 months, $p < .0001$), ORR (50% vs 32%, $p < .001$), DOR (9.1 months vs 6.1 months, $p < .001$), median TTF (6.9 months vs 4.5 months, $p < .001$) and median OS (25.1 months vs 20.3 months, $p = .05$).

Marty et al reported similar results from a randomized trial of trastuzumab plus docetaxel vs docetaxel alone in 186 patients[9]. Patients were randomized to six cycles of docetaxel at 100 mg/m^2 every 3 weeks with or without trastuzumab weekly until disease progression. Trastuzumab plus docetaxel resulted in a significant improvement over docetaxel alone in outcomes in terms of ORR (61% vs 34%, $p = .0002$), median TTF (9.8 vs 5.3 months, $p = .0001$), median TTP (11.7 vs 6.1 months, $p = .0001$), median DOR (11.7 vs 5.7 months, $p = .009$), and median OS (31.2 vs 22.7 months, $p = .0325$).

In other front-line trials trastuzumab resulted in response rates of 62%-84% when combined with vinorelbine [14-17] and of 63%-65% when combined with capecitabine [18-19]. In the pretreated population, trastuzumab combined with various chemotherapy agents with paclitaxel, docetaxel, vinorelbine, gemcitabine, or capecitabine resulted in the response rates of 20%-55% [20-29].

In spite of these results, only a proportion of patients with HER2 positive breast cancer respond to trastuzumab and the vast majority of patients with metastases ultimately experience disease progression despite this treatment. The dependence of HER2 over-expressing breast cancers on this signal transduction pathway for growth has thus motivated the search for more potent and/or complementary anti-HER2 strategies. In this regard, Lapatinib (Tykerb®) has emerged as an active anti-HER2 therapy and is the second agent approved by the US Food and Drug Administration (FDA) for this subtype of breast cancer[30].

Pertuzumab

Pertuzumab is a humanized monoclonal antibody which binds to the extracellular domain of HER2; however unlike trastuzumab which binds at domain IV, pertuzumab binds domain II of the receptor and is thus able to disrupt HER2 dimerization and ligand-activated signaling with other growth factor receptors, including other HER family members [31]. Animal model studies using HER2 positive breast cancer xenografts have shown a synergistic antitumor activity for pertuzumab in combination with trastuzumab [32].

Non-Clinical Studies

In Vitro

In *in vitro* studies pertuzumab blocks heregulin-induced activation of the PI3 kinase cell survival pathway whereas trastuzumab does not. In the breast carcinoma cell line MCF-7, pertuzumab, but not trastuzumab, blocked heregulin-induced activation of the PI3 kinase cell survival pathway, as indicated by a lack of phosphorylation of a key enzyme (Akt) in this pathway. [33] Juntilla showed that pertuzumab blocks ligand-dependent HER2-3 dimerization while trastuzumab blocked ligand-independent HER2-3 dimerization in a HER2+ cell line (SKBR3). [34] The ability of pertuzumab to inhibit ligand activation of HER2 has also been demonstrated in transfected cell lines of fibroblast origin and with purified soluble receptors.[34-35]

Pertuzumab blocks heregulin-dependent in vitro growth of a number of breast cancer cell lines as well as cell lines derived form other solid tumors.[36-38] For example in the heregulin-secreting MDA-MB-175VII breast carcinoma cell line, which expresses low/moderate levels of HER2 protein (1+ by immunohistochemistry), cell proliferation was inhibited in a dose-dependent fashion by both pertuzumab and trastuzumab but the magnitude of the inhibition was far greater with pertuzumab. [37] The calculated pertuzumab concentration at which the half-maximal growth inhibition (IC_{50}) occurred was120 ng/ml or 0.8 nM, and was consistent

with biochemical measurements of pertuzumab inhibition of hereugulin binding or receptor activation. [33, 35, 36] The combination of trastuzumab and pertuzumab was shown to have a synergistic growth inhibiting effect on BT474 breast tumors, which express high levels of HER2 underscoring the complementary mechanism of action of the 2 drugs.[39]

In Vivo

In human tumor xenograft models in mice, pertuzumab is active against various tumors including breast, ovarian, lung, and prostate cancers.[40-43] For example, growth inhibition ranged from 50-70% compared with control in 5 of 18 NSCLC xenografts.[41] Similar studies using 6 different human mammary tumor explants revealed one clear responder with more than 70%.[43] The dosage of 6 mg/kg/week was the optimal schedule to achieve the maximal therapeutic effect.[44]

Pertuzumab also augmented the anti-tumor effect of various cytotoxic drugs, including paclitaxel, irinotecan, cisplatin, gemcitabine, and capecitabine.[45-50]The augmentation by pertuzumab of the various chemotherapeutic agents with differing modes of action is possibly explained by the deprivation of PI3-kinase mediated cellular survival signals after inhibition of the HER2 activation.

Pertuzumab enhances other HER pathway inhibitors, such as erlotinib in human lung cancer xenograft models.[47, 51, 52] In HER2 (+) xenografts derived from HER2-over-expressing NSCLC Calu-3 cells and from KPL-4 breast cancer cells, pertuzumab had synergistic antitumor activity in combination with trastuzumab.[53-54] The synergistic action of pertuzumab and trastuzumab may be explained by their complementary modes of action. While pertuzumab prevents ligand-activated formation of HER2 heterodimers, trastuzumab can block the shedding of HER2 extracellular domain that would result in constitutively activated truncated receptors. Furthermore, as the 2 antibodies are not competing for the same epitope on HER2, their combination may lead to an increased cell killing via antibody dependent cell-mediated cytotoxicity (ADCC).

Non-clinical Pharmacokinetics

Consistent with its IgG1 framework pertuzumab has a long terminal half-life in mice, rats, and primates (approximately 10 days) following an initial rapid distribution phase (<1 day). In nude mice implanted with NSCLC tumors and breast cancer tumors that were given weekly doses (0.4-60 mg/kg), > 80% suppression of tumor growth was seen at steady-state trough levels of 5-25 ug/ml.[55]

Pertuzumab administered weekly IV was well tolerated in primates at doses up to 150 mg/kg. Treatment-related diarrhea was noted at doses of 15 mg/kg and higher. More chronic dosing (>12 weeks of weekly dosing) resulted in diarrhea-related dehydration in monkeys. *In vitro* studies demonstrated that pertuzumab is compatible with human blood and does not cause hemolysis. Tissue binding of the antibody is consistent with HER2 expression. In reproductive toxicity study, pertuzumab administered to pregnant cynomolgus monkeys twice weekly from gestation day (GD) 19-50 caused dose-related increase in embryo-fetal abortion and death. Evidence of delayed renal development was seen at all doses.[55]

Clinical Studies

Summary of Clinical Pharmacology

The clinical information has been obtained from company sponsored studies.[55] In clinical studies, similar pharmacokinetics (PK) was observed across all trials with no change in clearance at doses from 2.0-15.0 mg/kg (140 mg to 1050 mg for a 70 kg patient). A two-compartment model adequately described the concentration-time data with a systemic serum clearance of approximately 0.24 L/day and a terminal half-life of 17 days for a typical patient. Based on these clinical data, a dosing interval of 3 weeks is recommended in clinical studies. In phase II studies, a loading dose of 840 mg (followed by 420 mg every 3 weeks) was capable of attaining a steady state trough and peak concentrations by the second cycle. Population PK modeling of data from Phase I and Phase II studies support the combined use of fixed, non-weight-based dosing in female patients. There was no evidence of an impact of pertuzumab on the co-administered chemotherapeutic agents, docetaxel and capecitabine in Phase Ib studies.[55]

Pharmacokinetics in Single-Agent Studies

In phase II single-agent studies, concentration-time data show that the loading dose of 840 mg (followed by 420 mg q 3 weeks) was capable of attaining steady-state trough and peak concentrations by the second cycle. The PK parameters were estimated using a two-compartment model. The mean systemic clearance of these patients (0.335-0.28 L/day) and the mean volume of distribution of the central compartment (2.70-3.11 L) were similar to that observed in the phase Ia study (TOC2297g).[56] The initial half-life and the serum, terminal half-life were also within the ranges observed across 2-15 mg/kg dose groups in the phase Ib study.

Pharmacokinetics in Combination Studies

Analyses of the PK of capecitabine (study BO17003) and docetaxel (study BO17021) indicate that pertuzumab does not alter the PK of these two cytotoxic agents.[55] In both of these studies, the PK parameters of pertuzumab were similar to those obtained in single-*agent studies.*

Support for the Dose Regimen

A dosing regimen of pertuzumab given every 3 weeks to patients in Phase II studies (TOC2689g, BO16934) using a fixed dose of 840 mg loading dose (equivalent to 12 mg/kg for a 70 kg patient) for cycle 1 and a fixed dose of 420 mg maintenance dose (equivalent to 6 mg/kg) for subsequent cycles resulted in a steady state trough concentrations of approximately 60 ug/L by the second treatment cycle. In non-clinical dose-response xenograft studies using nude mice implanted with NSCLC and breast cancer tumors (low and high HER2 expression levels), >80% suppression of tumor growth was achieved when steady-state trough concentrations of pertuzumab were 5-25 ug/mL. Thus, the steady-state serum trough concentrations obtained in patients are in excess of concentrations shown to be efficacious in animal tumor models, and thus expected to result in a biologic effect.

A preliminary population PK analysis of the Phase Ia (TOC2297g) and Phase IIa (TOC2689g), BO16934) studies, comprising 153 patients (weight range: 45.0-150.

6 kg) and 1458 concentration-time points, showed that the population variability of steady-state trough concentration and exposure were similar with fixed-, body surface area- (BSA), weight-based dosing. Thus, a dose based on BSA or weight was not superior to a fixed dose supporting the continued use of a fixed dose of pertuzumab in female patients with MBC and ovarian cancer.[55]

Clinical Efficacy

Summary

Clinical activity has been observed in patients with HER2 low expressing tumors who have received pertuzumab either as a single agent or in combination with cytotoxic chemotherapy. Complete responses (CR) have not been observed in any of these trials. Partial responses (PR) or stable disease (SD) lasting for ≥ 6 months have been observed in 11% of patients with ovarian cancer and in 8% of patients with HER2 low expressing breast cancer. No responses were observed for hormone-refractory prostate cancer or NSCLC. Pertuzumab with gemcitabine given to patients with platinum-resistant ovarian cancer showed prolongation of progression-free survival (PFS) over gemcitabine alone. However, in platinum-sensitive ovarian cancer patients, the addition of pertuzumab to carboplatin-based doublet of chemotherapy did not improve PFS. Five CRs and 11 PRs (24% objective response rate) were observed in patients with previously treated HER2 positive MBC following combined treatment with pertuzumab and trastuzumab. The rate and duration of SD observed across all studies suggest that pertuzumab may not have a direct cytotoxic effect, but may function as a cytostatic agent.[55-56]

Efficacy in Breast Cancer

Four studies have been designed to evaluate pertuzumab in breast cancer. Data from the primary analysis of efficacy have been available from 2 studies. The first study, BO16934, studied patients with HER2 low expressing disease. The second study, BO17929, is a trial in patients with HER2 overexpressing MBC. Two additional studies are ongoing. Study WO20697 is evaluating neoadjuvant treatment of HER2 positive early breast cancer and WO20698 is a phase III study of HER2 positive MBC.[55]

Study BO16934 is a phase II study that evaluated the efficacy of pertuzumab given either at 420 mg or 1050 mg to patients with previously treated and low HER2 expressing MBC. During the study, patients received a median of 2 cycles (range 1-24). Two patients in the low dose group had a PR with durations of 18 weeks in 1 patient and 31 weeks in the other. No patients in the higher dose group had an objective response. In total, 32 patients had SD as best response, of which 2 patients in the low dose arm and 2 in the high-dose arm had SD for > 6 months. The overall clinical benefit response rate was 7.7%.[55]

Study BO17929 is a two-stage phase II, single arm study of efficacy and safety of pertuzumab and trastuzumab in patients with HER2 (+) MBC who had relapsed during trastuzumab following up to 3 lines of chemotherapy with or without trastuzumab. Patients in cohorts 1 and 2 (N=66) received trastuzumab plus pertuzumab for up to 8 cycles and those who did not progress by this time were eligible to continue receiving study drug until

progression, intolerable toxicity, or death. The primary endpoints of the study were overall response rate and clinical benefit. Baselga et al presented the result of this study and showed that the response rate was 24% and an additional 17 (25.8%) patients had SD for ≥ 6months, comprising a clinical benefit rate of 50%.[57]The most common toxicities were grade 1 and 2 diarrhea, fatigue, nausea, rash and headache. The mean LVEF in this study did not fluctuate significantly over 12 cycles of therapy. There appeared to be no concerning cardiac signal with the combination of the two antibodies. There were only 3/66 (4.5%) of patients who had asymptomatic LVEF declines of ≥ 10% to < 50%. The promising results of the combination of pertuzumab and trastuzumab led to a protocol amendment to include a 3rd cohort of patients receiving pertuzumab monotherapy, to assess the activity of pertuzumab as a single agent in this clinical setting. If the patient's disease failed to respond to pertuzumab monotherapy or responded and relapsed, then patients could have trastuzumab added to pertuzumab at the physician's discretion. Cortes et al reported the result of pertuzumab as monotherapy.[58] In this study, 29 patients were enrolled, and only 2/29 (7%) patients responded. Fourteen patients then had trastuzumab added to pertuzumab due to inadequate monotherapy response, and 2 of 14 (14%) who had previously progressed on trastuzumab and pertuzumab had a response.

Portera and colleagues conducted a separate study and evaluated the safety and efficacy of pertuzumab with trastuzumab in 11 patients with HER2-positive breast cancer who progressed on prior trastuzumab based treatments.[59] In this small study 2/11 (18%) patients had an objective response and an additional 3/11 (27%) of patients had SD. Thus, the clinical benefit rate occurred in 5/11 (45%) patients. Cardiac evaluation and tumor response were assessed every 3 and 6 weeks, respectively. Overall, 6/11 (54.5%) patients experienced a reduction in left ventricular ejection fraction (LVEF) most of which was asymptomatic [3/11 (27%) had G 1 LVEF decline to 50-55% and 2/11 (18%) had G2 LVEF decline to 40-49%]; 1/11 (9%) patients had clinical congestive heart failure (CHF). These promising phase 2 results have now led to a phase III first-line registration trial where patients with HER2 positive MBC are randomized to treatment with trastuzumab plus docetaxel +/- pertuzumab, namely CLEOPATRA.

Safety

As of November of 2008, approximately 840 patients with advanced cancers or early stage HER2 (+) breast cancer have been treated with pertuzumab. Gastrointestinal toxicities (diarrhea, nausea, vomiting, abdominal pain) and fatigue are the most frequently reported adverse events (AEs) with single therapy.[55]

Diarrhea and rash

Although pertuzumab targets HER2, because of its role in heterodimerization with other members of the HER family (ie: HER1/EGFR), it may cause toxicities associated with the use of EGFR inhibitors. Diarrhea and rash are common events increased with pertuzumab given in combination with chemotherapy compared with chemotherapy alone. Diarrhea has been reported in 60-70% of patients treated with pertuzumab and was mostly of grades 1-2. The mechanism of diarrhea and rash are unknown, but the nature is similar to that of other agents causing HER1 inhibition. In the event of diarrhea, early intervention with anti-

diarrhea medication should be considered and patients treated with fluid and electrolyte replacement, as clinically indicated.[55]

Infusion-related Symptoms

Monoclonal antibodies may cause infusion-associated symptoms such as fever, chills, hypotension, shortness of breath, skin rash, headache, nausea, and/or vomiting. Serious or severe infusion-related symptoms have been rarely observed with 7 patients experiencing serious reactions to date [hypersensitivity, fatal ARDS, pulmonary edema, anaphylaxis, dyspnea with hypertension, infusion-related reaction (unsteady on feet, headache, blurred vision)]. In pertuzumab single-agent studies, less than 5% of patients experienced adverse reactions during pertuzumab infusions. Serious infusion-related reactions with pertuzumab have been infrequently reported (< 1%). Intravenous pertuzumab administration should be performed in the setting with emergency equipment and staff who re trained to monitor medical situations and respond to medical emergencies. Patients should be monitored during each pertuzumab infusion for any adverse effects, as infusion reactions may occur with the first or subsequent doses. The infusion should be stopped for patients who develop dyspnea, clinically significant hypotension, or other clinically significant events. Patients who experience an NCI-CTC grade 4 allergic reaction or acute respiratory distress syndrome should not receive additional peruzumab.[55]

Cardiac Toxicities

Since pertuzumab targets HER2, as with trastuzumab, there is a potential risk of cardiac dysfunction, particularly in patients who have received prior anthracycline treatment. Cardiac toxicities, predominantly asymptomatic left ventricular ejection fraction (LVEF) declines, and 4 cases of cardiac failure have been reported in approximately 840 patients with advanced malignant disease or early stage HER2 (+) breast cancer treated with pertuzumab. Two of these cases occurred in patients with metastatic breast cancer who had received prior anthracyclines and two in patients with ovarian cancer. No clear association between the frequency, nature, and severity of pertuzumab-related toxicities and dose level has been observed.[55]

Patients with significant cardiac disease or baseline LVEF below the institution's lower limit of normal (LLN) should not commence treatment with pertuzumab. Risk factors for pertuzumab-associated cardiac dysfunction are not known at this time. This risk should be carefully weighed against the potential benefit in patients who have received prior anthracyclines. Monitoring of the LVEF is advised while patients are receiving pertuzumab. If symptomatic LVEF decline develops (NCI-CTC grade 3 or 4), the patient must discontinue pertuzumab. Left ventricular dysfunction, symptomatic or not, should be treated and followed according to standard medical practice.

Paclitaxel

The taxanes, paclitaxel and docetaxel, are well established in metastatic breast cancer treatment and can increase response rate and duration of response.[60-61] Taxanes lack cross-resistance with anthracyclines and were therefore quickly deemed worthwhile for evaluation in the adjuvant setting. Results from several randomized trials demonstrate a benefit from the addition of a taxane to an anthracycline-based regimen.[62] There is strong

data to support the use of paclitaxel given on a weekly schedule. There is a large body of evidence that weekly paclitaxel is superior to the same taxane given every 3-weeks in the metastatic setting [20-22] as well as the early setting.[63-64]. Weekly paclitaxel at 80 mg/m^2 has been studied in a prospective randomized phase III study in which it was compared against a previous standard schedule of every 3 week paclitaxel at 175 mg/m^2.[20] In this study, patients with metastatic breast cancer were randomized to every 3 week or weekly paclitaxel. Those with HER2-positive breast cancer had trastuzumab added to chemotherapy and those with HER2-normal disease were randomized to trastuzumab or not. Initially, those on the weekly chemotherapy arm received paclitaxel at 100 mg/m^2 for 6 doses followed by a dose at 80 mg/m^2 continuously. However, due to a 30% incidence of grade 3 sensory neuropathy, the study was amended to start with a reduced dose of 80 mg/m^2 which was then continued until progression of disease or limiting toxicity. Overall, weekly paclitaxel was superior to every 3 week paclitaxel in terms of response, time to progression, and median overall survival. Another study by Seidman et al evaluated the schedule of weekly paclitaxel at 90 mg/m^2 with trastuzumab in patients with HER2-positive and HER2-normal disease. [21] However, the median delivered dose of paclitaxel was 82 mg/m^2. Some of the reasons for the dose reduction included toxicities related to the higher dose of paclitaxel, primarily due to neurotoxicity, when given continuously. Perez ret al have also shown that weekly paclitaxel at 80 mg/m^2 is active against breast cancer. [22]. Sparano and colleagues have shown that weekly paclitaxel after doxorubicin and cyclophosphamide is superior to paclitaxel every 3 weeks in both DFS and OS while docetaxel every 3 weeks was only superior in terms of DFS, but not OS. There were more hematologic and non-hematologic toxicities with every 3-weekly docetaxel. [64] These studies have established weekly paclitaxel at 80 mg/m^2 given continuously as a standard schedule.[20-22, 63-64] Therefore, for this study the continuous weekly paclitaxel schedule at 80 mg/m^2 was chosen. Thus, it is worthwhile to assess the weekly paclitaxel schedule in combination with trastuzumab and pertuzumab.

Cardiac biomarkers

Cardiac toxicity is a potentially grave toxicity of certain cytotoxic chemotherapy agents, particularly the anthracycline drugs. In the adjuvant management of breast cancer when cure is the goal of treatment, the potential long-term cardiac sequelae of treatment are particularly relevant. While it may not be possible to prevent every case of treatment – induced cardiac dysfunction, early detection of evolving cardiac damage and the institution of medical management may help to decrease the morbidity associated with this toxicity. Studies of patients with asymptomatic LV dysfunction have shown that the risk of developing symptomatic heart failure or cardiac death over time can be decreased with the use of angiotensin converting enzyme (ACE) inhibitors.[65-66] With the existence of potential therapeutic interventions, much interest has focused on the development and validation of cardiac biomarkers that might identify subclinical cardiac damage and patients who may benefit from cardioprotective measures. Preliminary data suggest that the use of ACE inhibitors may prevent cardiotoxicity in patients receiving high-dose chemotherapy identified as increased risk on the basis of cardiac biomarkers.[66] To date, the majority of experience with cardiac biomarkers has been in the setting of anthracycline chemotherapy.

Cardiac Troponins

The incidence of elevation of cardiac troponins I or T (cTnI or cTnT) with each chemotherapy cycle may be as high as 30-34% in patients treated with potentially cardiotoxic (primarily anthracycline) chemotherapy across studies.[67] Many of these studies were in pediatric patients and/or those with hematologic malignancies, and in adults with high dose chemotherapy. In addition, methods of troponin detection and cut-off concentrations varied across studies, although most studies used the lowest troponin concentration at which signal-to-noise ratio can be reliably interpreted. In practice, this concentration has been defined across most studies as the concentration measured with an analytic imprecision expressed as the coefficient of variation of 10% or less. The etiology of the troponin rise appears to be non-ischemic as it is not associated with symptoms or classical EKG changes, and it predates LV dysfunction rather than accompanying it.[68] Troponin determination in breast cancer patients has been found to predict the occurrence of clinically significant LV dysfunction as well as the degree and severity of future LV dysfunction[683].

Cardiac Troponin I (cTnI)

Cardinale et al studied 211 patients receiving high dose therapy for breast cancer (mean age 46) using cTnI measurements at six time points before and after each chemotherapy cycle (up to 72 hours after) and correlated these to ECHO findings at 1,2,3,4,7 and 12 months[69]. A positive cTnI was defined as ≥0.5ng/mL. The cTnI level was normal at baseline and before each cycle. The patients were divided into a troponin positive group (at least one positive cTnI value) and a troponin negative group. In this study, 52% of the positive patients had only one positive result. The number of positive results were highest just after chemotherapy and lowest at 72 hours; the number of positive tests was higher after each additional cycle. A close relationship was found between the maximal cTnI value obtained and maximum LVEF decrement seen in follow up. In addition, there was a significant correlation between the number of positive cTnI values and the LVEF maximal decrement. The presence of normal cTnI levels after therapy was associated with no significant decline in LVEF in follow-up.

Another study from the same group in patients receiving high dose therapy measured “early troponin I” (E-cTnI) by taking the highest value from a group of values taken immediately after therapy and at 12, 24, 36 and 72 hours.[68] This was repeated for each cycle of therapy but the single highest troponin value was considered as the E-TnI for analysis. A “late troponin I” (L-cTnI) was taken 1 month after the last administration of HDT. Patients were divided into 3 groups: negative E-cTnI and L-cTnI (-/-), positive E-cTnI and negative L-cTnI (+/-), and positive E-cTnI and L-cTnI (+/+). The persistence of positive troponin values >1 month after chemotherapy (+/+) was related to an 85% risk of major cardiac events within a year of follow-up. Conversely, there was only a 1% incidence of cardiac events over 1 year in the patients with consistently negative results after therapy. Again, there was a significant correlation between the early cTnI value (i.e. the maximum cTnI value seen after any chemotherapy cycle) and LVEF maximal reduction.

A further study used cTnI measurements from 99 healthy volunteers to determine a threshold level of ≥0.08µg/L using ROC area under the curve analysis.[70] Troponin I was assessed in patients receiving high dose therapy at five time points during each cycle. In this study, 32% of patients were found to have positive cTnI (one or more positive results); 61% of these had 2 or more positive results. The ECHOs were performed at 1, 2,3,4,7 and 12 months. Patients with positive cTnI values had a mean decrease in LVEF at 12 months of 18% compared to 2.5% in the cTnI-negative group. A study in 79 patients with leukemia measured cTnI at various intervals during induction therapy, and found elevated cTnI levels at day 7-14 correlated with reversible decreases in LVEF[7].[71] Reports of smaller patient cohorts ranging from 15 to 31 patients showed more variable levels of cTnI detection after anthracycline therapy.[72-74]

B-type natriuretic peptide (BNP)

Natriuretic peptides are rapidly produced by the heart in response to hemodynamic stress. Both BNP and NT-proBNP are widely tested in heart failure as markers of heart failure diagnosis, prognosis, and overall risk assessment.[67] Several studies examined BNP levels at prolonged intervals from chemotherapy. A Dutch study evaluated BNP and NT-BNP levels at two time points after anthracycline chemotherapy at a median of 2.7 years later and a median of 6.5 years afterwards.[75] Elevated BNP levels were found in 14 out of 54 patients 6.5 years after chemotherapy. The BNP levels were significantly higher in patients receiving 450 mg/m^2 epirubicin than those who received 360 mg/m^2. Cardiac function was not assessed. However, a similar study evaluating BNP levels in 63 patients at least 1 year from anthracycline therapy found cardiac dysfunction in 41% and significantly higher mean BNPs in this group.[76] Similarly, Germanakis et al found a high rate of cardiotoxicity (as measured by decreased LV mass) in 19 children a mean of 3.9 years from anthracycline therapy, with significantly higher NT-pro-BNP levels in the patients with reduced LVM.[77] Hayakawa et al also found significantly higher levels of NT-BNP and BNP in pediatric patients previously treated with anthracyclines who had evidence of cardiotoxicity as compared to healthy controls and patients with preserved cardiac function.[78] A Saudi Arabian study in 31 pediatric patients found significant elevation of NT-pro-BNP when checked at least one month after chemotherapy in patients with echocardiographic cardiac toxicity compared with healthy controls and compared to those patients with conserved cardiac function.[74]

Cardiac biomarkers with trastuzumab

Limited data exist regarding the use of cardiac biomarkers as predictors of cardiotoxicity in patients treated with trastuzumab. Plasma cTnI and NT-proBNP levels were assayed in 15 patients taking part in a study of an imaging modality (indium-111 labeled trastuzumab scintigraphy) as a predictor of cardiotoxicity.[79] Patients received trastuzumab as part of a non-anthracycline containing regimen. While the imaging test was not shown to be of value, pre-treatment plasma NT-proBNP levels were found to be higher in patients who developed cardiotoxicity. A pilot study examined established and novel measures of metabolic and vascular risk factors for cardiovascular disease (including BNP) in a small subset of patients participating in the BCIRG 006 adjuvant trastuzumab study.[80] These were compared to

healthy controls. Patients entered the study at a median time from chemotherapy and trastuzumab of 20 months. In this study, 38.4% of patients had an LVEF value 10% or more below their baseline assessment; BNP was significantly elevated in 40% of patients and was a predictor of LVEF on univariate analysis. In N9831, an adjuvant study of AC followed by TH vs chemotherapy alone, a correlative study of potential cardiac biomarkers including BNP, C-reactive peptide (CRP), and troponin T and I, with LVEF identified that elevated BNP and troponin I at baseline and the doubling of BNP may have possible predictive values for cardiac toxicity, but the evaluation was done in a small group.[81]

Neuregulin

Neuregulin-1 (NRG-1) is a cardioprotective, paracrine growth factor released by microvascular endothelial cells. This pathway, which is necessary for the maintenance of cardiac function and survival during states of increased stress, is believed to be an important mediator of heart failure and chemotherapy-induced cardiac dysfunction.[82] In vitro studies demonstrate that NRG-1 administration protects doxorubicin-treated cardiomyocytes from myofibrillar disarray and death, and NRG-1 or ErbB2 deficient mice have dramatically worse survival with anthracycline exposure.[83-84] Trastuzumab, and pertuzumab, specifically target the ErbB2 receptor, a major mediator of NRG-1 activity, and its association with clinical cardiac dysfunction may be related to effects on this signaling pathway.[85]

In order to define the mechanistic and translational significance of NRG-1 signaling in humans, Ky and colleagues recently quantified serum NRG-1ß levels in a large cohort (N=899) of patients with chronic heart failure in the Penn Heart Failure Study.[86] Elevated NRG-1ß levels were significantly associated with more advanced heart failure (NYHA Class IV median 6.2 ng/ml versus those with Class I CHF 4.4 ng/ml, p=0.002). Furthermore, NRG-1ß was independently associated with an elevated risk of all-cause death or cardiac transplantation over a median follow-up of 2.4 months (adjusted HR 1.58, 95% C.I of 1.04-2.3, p=0.003), comparing the 4th vs the 1st quartile of NRG-1ß. Associations differed according to heart failure cause and severity, with stronger relationships observed in those with ischemic heart failure (interaction p=0.008) and advanced NYHA Class III/IV symptoms (interaction, p=0.01). In addition, patients with chemotherapy-induced cardiac dysfunction had significant alterations in their circulating NRG-1β levels compared to all other etiologies (p=0.02).

~~Our goal is to extend these findings and provide mechanistic, translational insight into the potential dysregulation of NRG-1/ErbB signaling with trastuzumab and pertuzumab. In doing so, we will bridge the gap between our basic science and clinical observations and determine the effects and potential clinical relevance of NRG-1ß on chemotherapy-induced cardiac dysfunction. We will quantify the relationship between NRG-1β and incident cardiac dysfunction, and determine the clinical, predictive utility of this biomarker in this important population.~~ As of A(15), we will no longer be quantifying the relationship between NRG-1β and incident cardiac dysfunction since the incidence of cardiac dysfunction based on the LVEF data has been minimal.

Echocardiograms and Strain Imaging Analysis

Monitoring of chemotherapy-induced cardiotoxic effects has relied primarily on the measurement of ejection fraction by echocardiography or radionuclide ventriculography. However, these parameters are too insensitive to detect subtle changes in myocardial dysfunction. When a clear reduction in LV ejection fraction can be measured, functional deterioration often proceeds rapidly.

Strain and strain rate imaging are new ultrasound applications in the field of echocardiography which have emerged in the last few years. Strain measures the deformation of the myocardium during cardiac contraction and relaxation and strain rate measures the speed of the myocardial deformation. This technique provides a more sensitive and quantitative assessment of cardiac contractile function than conventional indices such as ejection fraction and are less affected by loading conditions and translational motion of the heart. Strain imaging has shown to be useful in unmasking subtle cardiac pathology and cardiomyopathy, objective assessment of regional and global function and therapy evaluation in patient follow-up.[875-89] There are limited data available on the usefulness of this technique in the evaluation of anthracycline cardiotoxicity. Using strain imaging, myocardial systolic and diastolic dysfunction have been reported in asymptomatic pediatric patients with normal ejection fraction 5 years after low dose treatment with anthracycline when compared with age matched normal controls.[90] Acute reduction in strain and strain rate have also been demonstrated during low dose anthracycline treatment with no change in the conventional indices of systolic function.[91] A recent study[92] has shown subclinical LV systolic dysfunction in elderly women with early breast cancer treated with a total of 180 mg/m^2 of liposomal doxorubicin (Doxil®) by strain imaging, despite normal ejection fraction and fractional shortening. These results indicate that strain imaging is likely superior to ejection fraction or fraction shortening in the detection of early subclinical myocardial dysfunction. However, these studies are limited by the small sample size and the period of follow-up and are unable to address whether the abnormalities of the deformation parameters noted on strain imaging are clinically significant in predicting development of cardiomyopathy.

Rationale

Based on promising results of the combination of pertuzumab with trastuzumab in a pretreated population, it makes sense to test this combination with paclitaxel given weekly in the treatment of patients with metastatic HER2-positive breast cancer. With the superiority of the weekly over every 3 week schedule of paclitaxel and since there are less toxicities seen with weekly paclitaxel than docetaxel given every 3 weeks, [20-22, 63-64] it is worthwhile to combine weekly paclitaxel with pertuzumab and trastuzumab. There are strong data to support the use of weekly paclitaxel at 80 mg/m^2 continuously.[20-22, , 63-64] Although, CLEOPATRA is evaluating docetaxel every 3 weeks with trastuzumab +/- pertuzumab, it is important to evaluate pertuzumab with a better taxane schedule (weekly paclitaxel) and trastuzumab.

In this study patients with HER2 (+) breast cancer who have had 0-1 prior treatment in the metastatic setting will be enrolled. Trastuzumab in the adjuvant setting is allowed. Slamon

et al showed that the combination of paclitaxel and trastuzumab led to a median TTP of 7.4 months in patients with untreated HER2 (+) metastatic breast cancer.[2] The primary endpoint will be 6 month progression-free survival (PFS). We chose 6 months, as opposed to 7 months, as patients with one prior treatment are included. Patients who are considered progression-free at 6 months are deemed successes. Failures are those patients who progressed before the 6 month mark. Based on results from the Slamon trial, we define 50% as the unpromising rate and 65% as the promising 6 month progression-free rate. Based on these proportions, we would need 69 patients, at alpha level=.05 and 80% power for a single stage design. The secondary endpoints will be the safety (including cardiac safety) and tolerability and the assessments of biomarkers and tissue sample collections.

Patients will be given weekly IV paclitaxel (80 mg/m^2). Trastuzumab will be given IV every 3 weeks (8 mg/kg loading dose → 6 mg/kg every 3 weeks). Trastuzumab may be given IV weekly (4 mg/kg loading dose → 2 mg/kg weekly) in lieu of the every 3 week schedule. Pertuzumab will be given IV at 840 mg as a loading dose → 420 mg every 3 weeks. We will also assess the left ventricular ejection fraction at baseline and after every 4th cycle of treatment with an ECHO, and strain imaging will be evaluated. Study blood will be collected serially for biomarker analysis (ie: TnI, BNP, and NRG-1ß).

This is an important study to conduct to assess if pertuzumab in addition to a standard combination (trastuzumab + paclitaxel) has promising activity in the treatment of patients with HER2 (+) MBC. Furthermore, it will be important to identify potential cardiac biomarkers (ie: TnI, BNP) to assess if there is any correlation between elevated markers with any decline in LVEF (asymptomatic or symptomatic). If there is a positive correlation, a future study will be done to assess the role of cardiac medications (ie: an ACE-inhibitor, ß-blocker, alpha-blocker) in those with elevated cardiac biomarkers to determine if there is an improvement in cardiac outcomes in treated patients. Additionally, we will explore if the strain imaging analysis with each ECHO has a role in the detection of early subclinical myocardial dysfunction. Finally, if this treatment combination is proven to be effective and is feasible, then it will be tested in the adjuvant setting against the standard chemotherapy-trastuzumab (ie: AC followed by paclitaxel + trastuzumab) regimen where the goal for a cure is most important.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a phase II study of pertuzumab in combination with trastuzumab and paclitaxel for the treatment of patients with Stage IV HER2 (+) breast cancer. The regimen will consist of paclitaxel (80 mg/m^2) weekly + trastuzumab every 3 weeks (8 mg/kg loading dose → 6 mg/kg every 3 weeks) + pertuzumab every 3 weeks (840 mg as a loading dose → 420 mg), all given intravenously (IV). Patients may be given trastuzumab weekly in lieu of every 3 weeks (4 mg/kg loading dose → 2 mg/kg every 3 weeks). Patients will be on treatment until progression of disease. The primary objective of this study will be the proportion of patients who are progression free at 6 months or later. Patients who are considered progression-free at 6 months are deemed successes. Failures are those patients who progressed before the 6 month mark. The secondary objectives will be response, safety (including cardiac safety) and tolerability. Study blood will be collected serially for cardiac biomarker

analysis [troponin I (cTnI), brain type natriuretic peptide (BNP), neuregulin-1ß (NRG-1ß)]. We will also assess the left ventricular ejection fraction (LVEF) at baseline and after every 4th cycle of treatment with an echocardiogram (ECHO) with a strain imaging analysis. If an ECHO cannot be done, a multi-gated acquisition scan (MUGA) may be done.

4.2 Intervention

This is a phase II study of pertuzumab in combination with trastuzumab and paclitaxel for the treatment of patients with Stage IV HER2 (+) breast cancer. Based on promising results of the combination of pertuzumab with trastuzumab in a pretreated population, it makes sense to test this combination with paclitaxel given weekly in the treatment of patients with metastatic HER2-positive breast cancer. With the superiority of the weekly over every 3 week schedule of paclitaxel and since there are less toxicities seen with weekly paclitaxel than docetaxel given every 3 weeks, [20-22, 63-64] it is worthwhile to combine weekly paclitaxel with pertuzumab and trastuzumab.

The regimen will consist of paclitaxel (80 mg/m^2) weekly + trastuzumab every 3 weeks (8 mg/kg loading dose → 6 mg/kg every 3 weeks) + pertuzumab every 3 weeks (840 mg as a loading dose → 420 mg), all given intravenously (IV). Trastuzumab may also be given weekly (4 mg/kg loading dose → 2 mg/kg) in lieu of the every 3 week schedule. Patients will be on treatment until progression of disease. The primary endpoint of this study will be the proportion of patients who are progression free at 6 months or later. Patients who are considered progression-free at 6 months are deemed successes. Failures are those patients who progressed before the 6 month mark. The secondary endpoints will be response, safety (including cardiac safety) and tolerability. Study blood will be collected serially for cardiac biomarker analysis (TnI, BNP, NRG-1ß) and banked for future studies. We will also assess the LVEF at baseline and after every 4th cycle of treatment with an ECHO with a strain imaging analysis. When an ECHO cannot be done, a MUGA scan may be done. Patients who have surpassed the 6 month period may complete ECHO with strain imaging analysis or MUGA every 6 months +/- 1 month starting from the most recent ECHO scan date.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

Trastuzumab (Herceptin®)

a. Dosage

The recommended initial loading Herceptin dose is 4 mg/kg (for weekly dosing schedules) or 8 mg/kg (for every 3 weeks) administered over approximately 90 minutes. The recommended maintenance Herceptin dose is 2 mg/kg weekly or 6 mg/kg every 3 weeks and can be administered over approximately 30 minutes if the initial loading dose was well tolerated. Herceptin may be administered in an outpatient setting. DO NOT ADMINISTER AS AN IV PUSH OR BOLUS (see ADMINISTRATION).

b. Preparation

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

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

Determine the dose of Herceptin needed. Calculate the correct dose using 21 mg/mL Herceptin solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. DEXTROSE (5%) SOLUTION SHOULD NOT BE USED. Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration. No incompatibilities between Herceptin and polyvinylchloride or polyethylene bags have been observed.

c. Administration

Treatment may be administered in an outpatient setting by administration of a 4 mg/kg Herceptin loading dose for weekly dosing schedules (OR 8 mg/kg Herceptin loading dose for q3wk dosing schedules) by intravenous (IV) infusion given over approximately 90 minutes. DO NOT ADMINISTER AS AN IV PUSH OR BOLUS. If Herceptin is being administered concomitantly with chemotherapy, Herceptin administration may be given before or after chemotherapy administration. Patients should be observed for fever and chills or other infusion-associated symptoms (see ADVERSE REACTIONS). If prior infusions are well tolerated subsequent doses of 2 mg/kg Herceptin weekly (OR 6 mg/kg Herceptin q3wk) may be administered over approximately 30 minutes.

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

d. Storage

Vials of Herceptin are stable at 2°C–8°C (36°F–46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of Herceptin reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2°C–8°C (36°F–46°F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted Herceptin solution should be used immediately and any unused portion must be discarded. DO NOT FREEZE HERCEPTIN THAT HAS BEEN RECONSTITUTED.

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

e. Safety

Infusion-Associated Symptoms. During the first infusion with Herceptin, a symptom complex consisting of chills and/or fever is observed in approximately 40% of patients. Other signs and/or symptoms may include nausea, vomiting, pain, rigors, headache, cough, dizziness, rash, and asthenia. These symptoms are usually mild to moderate in severity, and occur infrequently with subsequent Herceptin infusions. These symptoms can be treated with an analgesic/antipyretic such as meperidine or paracetamol, or an antihistamine such as diphenhydramine.

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

Hematologic Toxicity. In the clinical trials, an increased incidence of anemia was observed in patients receiving Herceptin 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 Herceptin therapy. In the clinical trials, the per-patient incidences of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving Herceptin 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 Herceptin 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 neutropenia has not been determined; the effect of Herceptin on the pharmacokinetics of chemotherapeutic agents has not been fully evaluated. The observed incidence of leukemia among Herceptin-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 Herceptin to the etiology of acute leukemia or myelodysplastic syndrome in these cases is unclear.

Paclitaxel (Taxol®)

a. Dosage

Paclitaxel is an antimicrotubule agent that promotes microtubule assembly and stabilizes tubulin polymers by preventing their depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell functions. The dose of paclitaxel is 80 mg/m^2 IV weekly.

Paclitaxel is commercially available.

b. Preparation
Paclitaxel will be prepared as per MSKCC chemotherapy guidelines (MSKCC and affiliates).

c. Administration

Treatment may be administered in an outpatient setting by administration of an 80 mg/m^2 IV weekly dosing. If paclitaxel is being administered concomitantly with trastuzumab and pertuzumab, chemotherapy administration may be given before or after either antibody. A strict sequence of administration of paclitaxel relative to trastuzumab and pertuzumab is not mandated. Patients should be observed for fever and chills or other infusion-associated symptoms.

d. Storage

Unopened vials of paclitaxel are stable until the date as indicated on the package. Vials of paclitaxel should be stored at 20-25º C (68-77º F) in the original package. Neither freezing nor refrigeration adversely affects the stability of the product. Upon refrigeration, components of paclitaxel may precipitate but will re-dissolve upon reaching room temperature with little or no agitation.

e. Safety

Side-effects include myelosuppression, hypersensitivity (hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, bronchospasm, and tachycardia), sinus bradycardia, complete heart block, sinus tachycardia, premature ventricular beats, ventricular tachycardia, bigeminy, syncope, myocardial infarction, hypotension, hypertension, peripheral neuropathy, taste changes, arthralgia, myalgia, seizures, mood alterations, neuroencephalopathy, motor neuropathy, autonomic neuropathy, alopecia, radiation recall dermatitis, nausea, vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis, ischemic colitis, pancreatitis, elevated liver function, hepatic failure, fatigue, headache, light-headedness, elevated creatinine, elevated triglyceride, and blurred vision.

Pertuzumab

a. Dosage

Pertuzumab is a humanized monoclonal antibody based on the human IgG1 framework sequences and consists of 2 heavy chains and 2 light chains. Pertuzumab will be administered as 840 mg loading dose followed by 420 mg IV every 3 weeks.

b. Preparation

Pertuzumab is produced in Chinese hamster ovary cell cultures and purified by protein A column affinity chromatography, followed by ion exchange column chromatography. Because of the high degree of homology between pertuzumab and trastuzumab, procedures similar to those developed for trastuzumab are used for the manufacturing process, the in-process controls, and the characterization of pertuzumab. No bovine-derived raw materials are used in the manufacture of pertuzumab.

Each lot of the recombinant antibody produced for clinical purposes meets the USP requirements for sterility and safety. Additionally, each lot is extensively characterized and meets the required specifications for identity, purity, and potency.

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

c. Administration

Treatment may be administered in an outpatient setting by administration of 840 mg followed by 420 mg IV every 3 weeks. The first two doses will be given over approximately 60 minutes and the subsequent doses will be given over approximately 30-60 minutes. If

pertuzumab is being administered concomitantly with trastuzumab and paclitaxel, there is no strict sequence of administration of these 3 drugs mandated. Patients should be observed for fever and chills or other infusion- associated symptoms.

d. Storage

Pertuzumab vials are to be refrigerated at 2º C-8º C (36º F-46º F) until use. Pertuzumab vials should not be used beyond the expiration date provided by the manufacturer (Genentech). 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 not be frozen. The solution of pertuzumab fro infusion diluted in PVC or non-PVC polyolefin bags containing 0.9% sodium chloride injection and may be stored at 2º C-8º C (36º F-46º F) for up to 24 hours prior to use. Diluted pertuzumab has been shown to be stable for up to 24 hours at room temperature (2º C-25º C). However, since diluted pertuzumab contains no preservative, the diluted solution should be stored refrigerated at 2º C-8º C.

e. Safety

As of November of 2008, approximately 840 patients with advanced cancers or early stage HER2 (+) breast cancer have been treated with pertuzumab. Gastrointestinal toxicities (diarrhea, nausea, vomiting, abdominal pain) and fatigue are the most frequently reported adverse events (AEs) with single therapy.[53]

Diarrhea and rash

Although pertuzumab targets HER2, because of its role in heterodimerization with other members of the HER family (ie: HER1/EGFR), it may cause toxicities associated with the use of EGFR inhibitors. Diarrhea and rash are common events increased with pertuzumab given in combination with chemotherapy compared with chemotherapy alone. Diarrhea has been reported in 60-70% of patients treated with pertuzumab and was mostly of grades 1-2. The mechanism of diarrhea and rash are unknown, but the nature is similar to that of other agents causing HER1 inhibition. In the event of diarrhea, early intervention with anti-diarrhea medication should be considered and patients treated with fluid and electrolyte replacement, as clinically indicated.[55]

Infusion-related Symptoms

Monoclonal antibodies may cause infusion-associated symptoms such as fever, chills, hypotension, shortness of breath, skin rash, headache, nausea, and/or vomiting. Serious or severe infusion-related symptoms have been rarely observed with 7 patients experiencing serious reactions to date [hypersensitivity, fatal ARDS, pulmonary edema, anaphylaxis, dyspnea with hypertension, infusion-related reaction (unsteady on feet, headache, blurred vision)]. In pertuzumab single-agent studies, less than 5% of patients experienced adverse reactions during pertuzumab infusions. Serious infusion-related reactions with pertuzumab have been infrequently reported (< 1%). Intravenous pertuzumab administration should be performed in the setting with emergency equipment and staff who re trained to monitor medical situations and respond to medical emergencies.

Patients should be monitored during each pertuzumab infusion for any adverse effects, as infusion reactions may occur with the first or subsequent doses. The infusion should be stopped for patients who develop dyspnea, clinically significant hypotension, or other clinically significant events. Patients who experience an NCI-CTC grade 4 allergic reaction or acute respiratory distress syndrome should not receive additional peruzumab.[55]

Cardiac Toxicities

Since pertuzumab targets HER2, as with trastuzumab, there is a potential risk of cardiac dysfunction, particularly in patients who have received prior anthracycline treatment. Cardiac toxicities, predominantly asymptomatic left ventricular ejection fraction (LVEF) declines, and 4 cases of cardiac failure have been reported in approximately 840 patients with advanced malignant disease or early stage HER2 (+) breast cancer treated with pertuzumab. Two of these cases occurred in patients with metastatic breast cancer who had received prior anthracyclines and two in patients with ovarian cancer. No clear association between the frequency, nature, and severity of pertuzumab-related toxicities and dose level has been observed.[55]

Patients with significant cardiac disease or baseline LVEF below the institution's lower limit of normal (LLN) should not commence treatment with pertuzumab. Risk factors for pertuzumab-associated cardiac dysfunction are not known at this time. This risk should be carefully weighed against the potential benefit in patients who have received prior anthracyclines. Monitoring of the LVEF is advised while patients are receiving pertuzumab. If symptomatic LVEF decline develops (NCI-CTC grade 3 or 4), the patient must discontinue pertuzumab. Left ventricular dysfunction, symptomatic or not, should be treated and followed according to standard medical practice.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

- Age $\geq$ 18
- Stage IV HER2 (+) breast cancer
- Histologically documented HER2 (+) breast cancer as defined as IHC 3+ or FISH amplification of $\geq$ 2.0 of primary or metastatic site; results from the local lab are acceptable. (Optional tumor sample collection from primary or metastatic site may be obtained for HER2 testing at MSKCC)
- ECOG performance 0 -1 (Appendix A)
- 0-1 prior treatment in the metastatic setting (ie: hormone, chemotherapy, biologic, targeted agents). Prior anthracycline, paclitaxel, and trastuzumab in the adjuvant setting are allowed. If the patient has one trastuzumab-based treatment in the metastatic setting and is given a break (even intermittently) from the partner drug given with trastuzumab and is continued on trastuzumab alone, this would still be considered as one treatment. For example, if the patient was given paclitaxel + trastuzumab and was later continued on trastuzumab alone or then restarted on paclitaxel + trastuzumab (at the physician's

discretion for any reason), the regimen paclitaxel + trastuzumab followed by trastuzumab alone (or followed by paclitaxel + trastuzumab again) may be considered as one treatment.

- Measurable or non-measurable disease. Measurable lesions are defined as those that can be measured accurately in at least one diameter, that is ≥ 20 mm using conventional imaging techniques (including incremental CT) or ≥ 10 mm using spiral CT equipment and a lymph node ≥15 mm along the short axis. Non-measurable lesions are all other lesions, including small lesions (longest diameter <10mm, pathological lymph nodes with 10 to less than 15 mm along the short axis, bony metastases, leptomeningeal disease, ascites, pleural/pericardial effusions, inflammatory breast cancer, lymphangitis carcinomatosis, and heavily calcified and cystic/necrotic lesions.
- LVEF ≥ 50%
- Hematologic parameters: white blood cell (WBC) count of ≥ 3000/ul, absolute neutrophil count (ANC) ≥ 1500/ul, platelets ≥ 100,000/ul, hemoglobin ≥ 10.0 g/dl
- Non-hematologic parameters: bilirubin ≤ 1.5 mg/dl, AST/ALT ≤ 2.5 x upper limit of normal (ULN), alkaline phosphatase ≤ 5 x ULN.
- Creatinine ≤ 1.5 mg/dl
- Patients with stable and treated brain lesions of a duration of ≥ 2 months may be enrolled.

6.2 Subject Exclusion Criteria

- History of prior cardiac morbidities within 12 months (unstable angina, myocardial infarction, CHF, uncontrolled ventricular arrhythmias)
- Prior pertuzumab
- History of prior ≥ G 3 hypersensitivity (HSR) or any toxicity to trastuzumab that warranted permanent cessation of this antibody
- History of prior ≥ G 3 HSR or any toxicity to paclitaxel warranted permanent cessation of this chemotherapy
- ≥ G 2 peripheral neuropathy
- Patients with a history of chronic hepatitis B or C should be excluded from the study as paclitaxel is potentially hepatotoxic
- Pregnant patients

7.0 RECRUITMENT PLAN

This study is open to patients with metastatic HER2 (+) breast carcinoma at MSKCC or MSKCC satellites. These patients will be identified and recruited from the breast cancer patients seen at the Breast Cancer Center at MSKCC or MSKCC satellites.

Patients who are potentially eligible will be evaluated at the Breast Cancer Center at MSKCC or MSKCC satellites. This initial encounter will include a discussion of the proposed treatment and the rationale for its use. Eligible patients will be required to review and sign an informed consent.

8.0 PRETREATMENT EVALUATION

- Evidence of disease evaluation within 4 weeks prior to starting treatment:
  - CT of chest/abdomen +/- pelvis or MRI of chest/abdomen +/- pelvis
  - Bone scan and PET are optional
- ECHO (with strain imaging if possible). When an ECHO cannot be done, a MUGA scan may be done within 4 weeks of treatment.
- EKG within 4 weeks of treatment
- History and physical examination (including vitals and ECOG performance status) within 4 weeks prior to starting treating
- Blood work within 2 weeks prior to starting treatment
  - CBC
  - Comprehensive profile
  - Pregnancy test (serum if pre- or perimenopausal)

9.0 TREATMENT/INTERVENTION PLAN

The regimen will consist of paclitaxel (80 mg/m^2) weekly + trastuzumab every 3 weeks (8 mg/kg loading dose → 6 mg/kg every 3 weeks) + pertuzumab every 3 weeks (840 mg as a loading dose → 420 mg), all given intravenously (IV) (+/- 3 days). Trastuzumab may be given weekly (2 mg/kg) at the physician's discretion.

Due to the potential for an allergic reaction to paclitaxel, all patients should be premedicated as per institutional guidelines: approximately 30-60 minutes prior to paclitaxel infusion with a steroid, H1 and H2 receptor antagonists (ie: dexamethasone 10-20 mg IV or 20 mg orally night and morning of paclitaxel, diphenhydramine 50 mg IV, and ranitidine 50 mg IV or another H2 antagonist equivalent). If the patient tolerates the first dose of paclitaxel, the premedications may be modified at the physician's discretion. Both trastuzumab and pertuzumab may cause an infusion related reaction.

Premedications for trastuzumab and pertuzumab should include acetaminophen 650 mg orally and diphenhydramine 25-50 mg IV for the loading dose (diphenhydramine dose that

is used for paclitaxel premedication does not need to be repeated for trastuzumab). Subsequent doses of trastuzumab and pertuzumab do not require premedications.

Patients will be on treatment until progression of disease.

Each cycle will consist of 3 weeks (days 1, 8, and 15) of weekly paclitaxel (+/- 3 days) and trastuzumab and pertuzumab given on day 1 every 3 weeks (+/- 3 days). Trastuzumab may be given weekly at the physician's discretion.

Dose Reductions and Modifications:

This study will use the NCI Common Toxicity Criteria (CTC) AE version 4.0 for toxicity.

Note: Actual weight should be used rather than the ideal body weight.

Note: If a weight change of ≥ 10% occurs, the dose of paclitaxel, trastuzumab, and pertuzumab should be adjusted accordingly.

Paclitaxel

- After each paclitaxel treatment, dose adjustments of the taxane should be based on hematologic and non-hematologic toxicities. Patients experiencing neutropenic fever (ANC <1,000/μL and body temperature ≥ 38.5°C) should use filgrastim with subsequent treatments, in accordance with ASCO guidelines. If patients experience febrile neutropenia again despite the use of filgrastim, then a 25% dose reduction is allowed. If on the day that paclitaxel is due and ≥ Grade 3 non-hematologic toxicities have not recovered to ≤ Grade 2 (except neuropathy), treatment may be delayed up to 3 weeks. Up to 3 consecutive weeks of a delay in the administration of paclitaxel is allowed. If there is a delay of > 3 consecutive weeks from the intended treatment date due to toxicities, the patient will be removed from study. Once the patient is re-started on paclitaxel, a dose reduction is allowed. If ≥ Grade 3 non-hematologic toxicities occur again with one dose reduction, another dose reduction is allowed. A maximum of 2 dose reductions are allowed (from 80 mg/m^2 → 60 mg/m^2 and from 60 mg/m^2 → 45 mg/m^2).

- If on the day that paclitaxel is due, platelet counts are <75,000/μL and/or ANC <1000/μL have not recovered to ≤ Grade 2 (except neuropathy), treatment should be delayed by ≤ 1 week and CBC and toxicity grading should be repeated. Filgrastim should be used with the subsequent chemotherapy dose (in accordance with ASCO guidelines) if the delay in treatment is due to an ANC of <1000/μL. If the platelet counts are still <75,000/μL and/or ANC <1000/μL and/or non-hematologic toxicities have not recovered to ≤ Grade 2 (except for neuropathy), a further delay of ≤ 1 week is required. Up to 3 consecutive weeks of a delay in the administration of paclitaxel is allowed. If there is a delay of > 3 consecutive weeks from the intended treatment date due to toxicities, the patient will be removed from study.

- In terms of neuropathy, if on the day that paclitaxel is due and neuropathy has not recovered to ≤ Grade 1, then treatment may be held up to 3 weeks. If neuropathy has recovered to ≤ G 1 within these 3 weeks, then the patient may be re-challenged with

paclitaxel but at one 25% dose reduction (from 80 mg/^{2} → 60 mg/m^2). If ≥ G2 neuropathy occurs again at a reduced paclitaxel dose and has not recover to ≤ G 1 on the day that paclitaxel is due, paclitaxel may be held again up to 3 weeks. If neuropathy has recovered to ≤ G 1 within these 3 weeks, then the patient may be re-challenged with paclitaxel at a second dose reduction (from 60 mg/m^2 → 45 mg/m^2). Only 2 dose reductions are allowed. If the patient's neuropathy does "not" return to G ≤ 1 within 3 weeks at any point, the patient should not be re-challenged with paclitaxel. Upon re-challenging the patient with paclitaxel and at the physician's discretion, there may be one treatment break from paclitaxel per cycle in order to avoid cumulative neurotoxicity (ie: paclitaxel given on days 1 and 8 and off on day 15).

- In terms of nail toxicities and paronychia, if on the day that paclitaxel is due and the patient has ≥ Grade 2 nail changes, then treatment may be held up to 3 weeks. Once the nail changes are managed, upon treatment resumption, the patient may be re-challenged with paclitaxel at one 25% dose reduction (from 80 mg/m^2 → 60 mg/m^2). Another dose reduction may be allowed if necessary (from 60 mg/m^2 → 45 mg/m^2). Only 2 dose reductions are allowed. Upon re-challenging the patient with paclitaxel and at the physician's discretion, there may be one treatment break from paclitaxel per cycle in order to avoid cumulative nail toxicities (ie: paclitaxel given on days 1 and 8 and off on day 15).

- If paclitaxel cannot be given on day 1 (for any reason), trastuzumab and pertuzumab may be given as planned, and subsequent weekly doses of paclitaxel will be given when appropriate.

- Hypersensitivity reactions: Please see Appendix B for treatment. Treatment should be discontinued for Grade 4 hypersensitivity reactions. There are no dose reductions for hypersensitivity reactions. Consider discontinuation of taxane therapy if patient experiences 2 episodes of Grade 3 hypersensitivity reaction.

- **Note:** At 6 months if the patient is deemed to have responded to the combination of paclitaxel, trastuzumab, and pertuzumab, at the physician's discretion, the paclitaxel may be stopped and the patient may continue on trastuzumab and pertuzumab alone. If the patient has met the 6 month PFS endpoint and paclitaxel is stopped per physician's discretion, the patient may have paclitaxel re-initiated at a later time based on clinical indications per the treating physician. The patient will be followed and safety data will be collected. This would mimic real-life practice. This will not affect the primary or secondary end-points. Once paditaxel is re-initiated, patient will not need to have ECHO (or MUGA) every 4th cycle as the cardiac toxicity of these 2 antibodies have been minimal. ECHO (or MUGA) may be ordered infrequently at physician's discretion. If patient had paclitaxel re-initiated due to progression of disease, the interval between CT of chest/abdomen +/- pelvis is at physician's discretion. It does not have to be every 4th cycle as the patient would have already met the primary 6-month PFS endpoint. This will not affect study's primary endpoint.

- **Note:** Paclitaxel is metabolized by the CYP3A4 and CYP2C8 enzymes. There is a potential for drug interaction with paclitaxel and CYP3A4 inhibitors and inducers as well as

caution should be taken with CYP2C8 substrates. Appendix D is a list of CYP3A4 inhibitors and inducers and CYP2C8 substrates.

Trastuzumab

Trastuzumab loading dose (8 mg/kg) IV will be administered on same day as day 1 of cycle 1 of paclitaxel and is continued every 3 weeks at 6 mg/kg. Trastuzumab may be given weekly (4 mg/kg loading dose → 2 mg/kg weekly). There is no dose modification for trastuzumab.

Pertuzumab

Pertuzumab loading dose (840 mg) IV will be administered on same day as day 1 of cycle 1 of paclitaxel and is continued every 3 weeks at 420 mg. There is no dose modification for pertuzumab.

Note: Vital signs should be monitored before, during, and after each pertuzumab infusion.

Note: If there is a > 2 week delay from the last administration of trastuzumab and pertuzumab, then a re-loading of both antibodies is recommended. Up to 3 weeks of delay in the administration of trastuzumab and pertuzumab may be allowed for any reason.

Note: Patients will have both trastuzumab and pertuzumab held for significant asymptomatic LVEF declines as outlined in the table below.

RELATIONSHIP OF LVEF TO THE LOWER LIMIT OF NORMAL (LLN)	**ABSOLUTE DECREASE OF < 10 PERCENTAGE POINTS**	**ABSOLUTE DECREASE OF 10 TO 15 PERCENTAGE POINTS**	**ABSOLUTE DECREASE OF ≥ 16 PERCENTAGE POINTS**
Within radiology facility's normal limits	Continue T + P	Continue T + P	Hold T + P and repeat LVEF within 3 weeks
1 to 5 percentage points below the LLN	Continue T + P	Hold T + P and repeat LVEF within 3 weeks	Hold T + P and repeat LVEF within 3 weeks
≥ 6 percentage points below the LLN	Continue T + P and repeat LVEF within 3 weeks	Hold T + P and repeat LVEF within 3 weeks	Hold T + P and repeat LVEF within 3 weeks

T=Trastuzumab

P=Pertuzumab

A repeated LVEF may be assessed by an ECHO or MUGA.

Rules for interpreting and applying "repeat" LVEF results:

- T+ P must be permanently discontinued when two consecutive "hold" categories occur.
- T + P must be permanently discontinued when three intermittent "hold" categories occur. (At the investigator's discretion, T + P may also be permanently discontinued prior to the occurrence of three intermittent "hold" categories.)
- If LVEF is maintained at a "continue and repeat LVEF" or improves from a "hold" to a "continue and repeat LVEF" category, an additional ECHO (or MUGA scan) prior to the next scheduled LVEF assessment will be at the investigator's discretion.
- Patient who experiences a significant "asymptomatic" LVEF decline while on trastuzumab + pertuzumab which result in permanent discontinuation of T + P will be seen in the cardiology clinic.
- If a patient experiences G 3-4 symptomatic congestive heart failure at any time, trastuzumab and pertuzumab should be stopped and she/he will be removed from study and be seen by a cardiologist.

Note: If a patient has any temporary or permanent cessation of trastuzumab and pertuzumab due to asymptomatic or symptomatic LVEF declines, weekly paclitaxel may be continued as planned.

Diarrhea and Rash Management

Pertuzumab may cause diarrhea and rash. Below are guidelines in place for management of these toxicities as follows:

Pertuzumab Diarrhea and Rash Management

Diarrhea		
Grade	**Management**	**Dose Delay**
1	Loperamide at 1st onset	None
2	Loperamide at 1st onset	None; If unacceptable, hold pertuzumab ≤ 21 days, resume pertuzumab at same dose *
≥ 3	Loperamide at 1st onset	Hold pertuzumab + paclitaxel ≤ 21 days until ≤ G 1, resume pertuzumab at same dose and paclitaxel at 25% dose reduction **

Rash		
Grade	**Management**	**Dose Delay**
1	None	None
2	minocycline, topical tetracycline or clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisone	None; If unacceptable, hold pertuzumab ≤ 21 days, resume same dose [#]
≥ 3	Same as above	Hold pertuzumab ≤ 21 days until ≤ G 1, resume at same dose [##]

Note: Refer to **Appendix C** for diarrhea and rash management.

* If the patient experiences a G 2 diarrhea that is "unacceptable", hold pertuzumab for ≤ 21 days and resume at the same dose. If G 2 diarrhea recurs that is "unacceptable" despite a held dose previously for G 2 toxicity, then can hold pertuzumab again for ≤ 21 days and should reduce paclitaxel by 25% (80 mg/m^2 → 60 mg/m^2). Trastuzumab should be continued as planned. There is no dose reduction for trastuzumab and pertuzumab.

** If the patient experiences G ≥ 3 diarrhea, hold pertuzumab and paclitaxel for ≤ 21 days until ≤ G 1 and then resume pertuzumab at the same dose and should reduce paclitaxel by 25% (80 mg/m^2 → 60 mg/m^2). If > 3 weeks are required for the toxicity to reach to ≤ G 1, then pertuzumab should be held permanently and the patient should be taken off the study (The patient may remain on paclitaxel and trastuzumab at the physician's discretion). If G 3 diarrhea recurs upon being re-challenged with pertuzumab and paclitaxel (at a reduced dose), then the patient will be removed from study. There is no dose reduction for trastuzumab and pertuzumab.

[#] If the patient experiences a G 2 rash that is "unacceptable", hold pertuzumab for ≤ 21 days and resume at the same dose. If G 2 rash recurs that is "unacceptable" despite a held dose previously for G 2 toxicity, then can hold pertuzumab again for < 21 days. Trastuzumab and paclitaxel should be continued as planned. There is no dose reduction for trastuzumab and pertuzumab.

[##] If the patient experiences G ≥ 3 rash, hold pertuzumab ≤ 21 days until ≤ G 1 and then resume pertuzumab at the same dose. If > 3 weeks are required for the toxicity to reach to ≤ G 1, then pertuzumab should be held permanently and the patient should be taken off the study (The patient may remain on paclitaxel and trastuzumab at the physician's discretion). If G 3 rash recurs upon being re-challenged with pertuzumab, then the patient will be removed from study. There is no dose reduction for trastuzumab and pertuzumab.

Note: If the patient is found to have brain metastasis that requires treatment but is responding to this study regimen systemically, she may be allowed to take a break from receiving paclitaxel for up to 6 weeks in order to have treatment for brain metastasis (ie: whole brain radiation, stereotactic radiation, surgical resection, etc). It is encouraged that trastuzumab and pertuzumab should be

continued every 3 weeks if possible. However, a >3 week delay is allowed from trastuzumab and pertuzumab while the patient is receiving care for brain metastases.As it is not known that paclitaxel, trastuzumab, or pertuzumab can cross the blood-brain barrier well, a central nervous system progression will not be considered a treatment failure. This will be the one time that a patient may have up to a 6 week break from paclitaxel while undergoing treatment for brain metastasis.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

- Patients will be seen at every cycle of treatment preferably on day 1 of each cycle, while receiving paclitaxel. If there is a schedule conflict, patients may be seen on day 8 or 15. Patients who are no longer receiving paclitaxel (i.e. at the 6 month mark) may be seen every other cycle (+/- 3 weeks) of antibody treatment per the physician's discretion. Each evaluation will consist of the following:
    - History and physical examination with vital signs, ECOG performance status.
    - Recording of the adverse events
        - Investigators will assess the occurrence of AEs and SAEs at all patient evaluation time points during the study. All AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be recorded in the patient's medical record and on the appropriate AE or SAE CRDB page. Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity, regulatory seriousness criteria, if applicable, suspected relationship to the investigational product, and actions taken. Since this trial's secondary objective is to measure safety and tolerability of the intervention by assessing toxicity, suspected relationships or attributions of non-hematological adverse events will only be collected if assessed as grades ≥ 2, except for the following events: rash and diarrhea. AE grading (severity) scale found in the NCI CTCAE, Version 4.0 will be used for AE reporting.
    - Labs
        - CBC prior to each paclitaxel (paclitaxel, trastuzumab, and pertuzumab can be given +/- 3 days). A CBC can be completed up to 3 days prior to the infusion.
        - Comprehensive profile once per cycle of treatment for patients receiving paclitaxel. The patient does not have to wait for the result to come back to start treatment on the same day. The result will not necessarily be used to guide treatment on the same day but may be used to guide subsequent treatments. The specific labs that are used

to guide paclitaxel treatment are the AST, ALT, and bilirubin. On the day of each paclitaxel treatment, the AST, ALT, and bilirubin obtained within 3 weeks may be used.

- Patients who have surpassed the 6 month period and are on antibody therapy alone (off chemotherapy) may have CBC and comprehensive labs drawn at any frequency per physician's discretion. This is allowed as these labs are not used to dose trastuzumab or pertuzumab.

- Patients will have cardiac biomarkers drawn at every other cycle (ie: cycle 1, 3, 5, 7, etc), preferably on day 1 (pre- and post- infusion of paclitaxel, trastuzumab, and pertuzumab) of every other cycle. If biomarkers were not done on day 1, these may be drawn on day 8 or 15 (pre- and post- infusion of paclitaxel). These biomarkers will be done at every other cycle up to 6 time-points.

- Patients may have their treatment schedule modified as follows:

    - Patients may have a delay of treatment for up to 3 weeks (for any reason).

    - If there is a delay of > 2 weeks since the last infusion of trastuzumab and pertuzumab, then a re-loading of trastuzumab and pertuzumab is advised.

- Patients will be assessed radiographically after every 4th cycle +/- 2 weeks (i.e. day 1 of cycle 5 ± 2 weeks, day 1 of cycle 9 ± 2 weeks, etc). Patients who have surpassed the 6 month period may complete these radiologic assessments every 6 months +/- 1 month starting form the most recent radiology scan date. Patients who have surpassed the 6 month period and have progression will be censored but may have paclitaxel re-initiated; at this point the frequency of radiographic assessment is determined by physician's discretion.

- Radiographic assessment will include:

    - CT of chest and abdomen +/- pelvis or MRI of chest/abdomen +/- pelvis (+/- 1 month)

    - Bone scan and PET are optional (+/- 1 month)

- Patients will have an ECHO with strain imaging analysis after every 4th cycle +/- 2 weeks (i.e. day 1 of cycle 5 ± 2 weeks, day 1 of cycle 9 ± 2 weeks, etc). Patients who have surpassed the 6 month period may complete ECHO with strain imaging analysis every 6 months +/- 1 month starting from the most recent ECHO scan date. Patients who have surpassed the 6 month period and have progression will be censored but may have paclitaxel re-initiated; at this point the frequency of ECHO (or MUGA) assessment is determined by physician's discretion. When an ECHO by the GE Vivid 7 or E9 system (GE Healthcare, Milwaukee, WI) is not available for strain imaging analysis, then an LVEF by a standard ECHO machine per facility may be done. If an ECHO cannot be done, a MUGA scan may be done (+/- 1 month). The same modality should be used throughout the study.

<u>Biomarker Evaluations:</u>

Troponin

From each patient at every other cycle for up to 6 time-points, five mL of peripheral blood will be collected pre- and post-infusion in a green top (heparin) tube for the measurement of TnI. For the troponin assay, plasma will be separated from peripheral blood and samples will be frozen for analysis and will not be known to the investigator until the patient has completed the study. The TnI samples will be evaluated by Dr. Martin Fleisher's laboratory at MSKCC. At all MSKCC sites, blood samples should be clearly labeled before transfer to Main campus. This labeling should include the study identifier (IRB-assigned protocol number), MSKCC-assigned patient identifier (once available), test ("troponin") and the time point of sample measurement.

TnI concentrations will be determined by a fluorometric enzyme immunoassay analyzer (Tosoh Bioscience, Inc., San Francisco, CA) with a low end sensitivity of 0.06 ng/ml. TnI levels will be classified as <0.06 ng/mL ("undetectable"), 0.06-0.31 ng/mL ("minimal elevations"), or >0.31 ng/mL ("above normal range"). In addition to categorizing TnI values in this way, the absolute change (represented by the difference between the baseline value and the maximum value observed during follow-up) will be calculated for each patient.

Brain Natriuretic Peptide

From each patient at every other cycle for up to 6 time-points, five mL of peripheral blood will be collected pre- and post-infusion into a lavender top (EDTA) tube for measurement of BNP. The BNP assay will be performed within 24 hours of blood collection. The BNP samples will be evaluated by Dr. Martin Fleisher's laboratory at MSKCC. BNP specimens can be refrigerated following collection. Samples from MSKCC Regional Network sites will be delivered to the MSKCC Main Campus at least once daily per usual Regional practice for preparation and frozen storage. At all MSKCC sites, blood samples should be clearly labeled before transfer to Main campus. This labeling should include the study identifier (IRB-assigned protocol number), MSKCC-assigned patient identifier (once available), test ("BNP") and the time point of sample measurement.

BNP is assayed in the Clinical Chemistry STAT lab. The BNP assay is performed on a Biosite Triage analyzer (Biosite, San Diego, California) using fluorescence immunoassay on EDTA anticoagulated whole blood. Average within day imprecision is 8.5% at 71 pg/ml and 11% at 630 pg/ml. BNP results less than or equal to 100 pg/ml are representative of normal values in patients without CHF. BNP will be classified as ≤100 pg/mL ("within normal range") or >100 pg/mL ("above normal range"). In addition to categorizing BNP values in this way, the absolute change (represented by the difference between the baseline value and the maximum value observed during follow-up) will be calculated for each patient.

Neuregulin 1ß

From each patient at every other cycle for up to 6 time-points, five mL of peripheral blood will be collected pre- and post-infusion in a lavender top (EDTA) tube for the measurement NRG-1ß. For the NRG-1ß assay, plasma will be separated from peripheral blood and samples will be frozen for analysis and will not be known to the investigator until the patient has completed

the study. A reproducible assay for assessing serum NRG-1ß levels using an indirect sandwich ELISA technique has been developed and validated in the Sawyer Lab at Vanderbilt University. The monoclonal capture antibody used in this assay is against a biologically active and cardiac-specific peptide sequence, which has been studied extensively by Sawyer et al. This peptide has been shown to play an important role in the activation of the ErbB receptor and downstream signaling pathways in ventricular myocytes [93]. The coefficient of variation (CV) of this assay is 5.6-13%.

The first generation form of this assay was used in a pilot study performed by Dr. Sawyer's group which sought to define the effects of exercise on circulating NRG-1ß levels in humans [94]. This study demonstrated that there was no significant difference in serum NRG-1ß levels pre- and post- exercise in healthy human subjects, but these levels were associated with cardiopulmonary exercise capacity.

A second generation ELISA assay with lower detection limits has since been developed with NRG-1ß being detected in >98% samples tested to date. This assay was used to quantitate NRG-1ß from serum samples in 899 subjects with chronic heart failure. [82] NRG-1ß levels were detected in all non-heart failure samples and all but 12 heart failure patients. Extensive validation of the second generation form of this assay has been performed. The effects of multiple freeze/thaw cycles and the differences between plasma and serum NRG-1ß levels have also been assessed. NRG-1ß is detectable in both serum and plasma with a strong correlation (R^2=0.91), with plasma levels being 50% higher than serum. Repeated measures of NRG-1ß levels after exposure to multiple freeze/thaw cycles are highly correlated (R^2=0.92), but decrease 25.7% per freeze/thaw cycle. Because of these findings, we will use frozen, previously unthawed plasma for our study.

A third generation form of this assay has also been developed and validated at the University of Pennsylvania Translational Core Lab led by Theodore Mifflin, PhD and Daniel Rader, MD under Dr. Ky's direction and Dr. Sawyer's collaboration. This assay has lower detection limits and an improved coefficient of variation. At all MSKCC sites, blood samples should be clearly labeled before transfer to University of Pennsylvania Translational Core Laboratory. This labeling should include the study identifier (IRB-assigned protocol number), MSKCC-assigned patient identifier (once available), test ("NRG-1ß ") and the time point of sample measurement.

Note: As the clinical significance of an elevated TnI, BNP, or NRG-1ß in an asymptomatic patient is unknown, the TnI and NRG-1ß samples drawn at various time intervals as described previously for each patient will be stored frozen and analyzed but the results will be blinded to the treating physician until the end of the study, "not in real time". Patients and their treating physicians will not have the results in real time and thus, treatment decisions will not be affected. The BNP assay will be performed within 24 hours of sample collection, but the results will not be released to the treating oncologist until the end of the study, as the significance of an elevated BNP in an asymptomatic patient is not known. The results will instead be stored for interpretation at the end of the study.

As of A(15), we will no longer be quantifying the relationship between NRG-1ß and incident cardiac dysfunction since the incidences of cardiac dysfunction based on the LVEF data have been minimal.

Echocardiograms (and Strain Imaging Analysis for research purposes):

Echocardiograms will be performed every 4th cycle of treatment as defined in the study. Patients who have surpassed the 6 month PFS period may complete ECHO with strain imaging analysis every 6 months +/- 1 month starting from the most recent ECHO scan date. Strain imaging will be analyzed off-line using 2D image loops from the routine echocardiographic examination. The ECHO machine for the strain imaging analysis should be the Vivid 7 or E9 machine (GE healthcare, Milwaukee, WI). In addition to being stored in the main ECHO PACS system, these studies will also be stored in an external GE workstation which contains the software needed to perform the strain imaging analysis. To calculate strain and strain rate, the LV myocardium is traced in a click-to-point approach. Subsequently, the software automatically defines an epicardial and midmyocardial line and processes all frames of the loop. The myocardium in each of the 3 standard apical views is divided into 6 segments. The software will automatically calculate strain and strain rate for each of the 18 segments plus a global value for the entire myocardium. These measurements will be made by a designated investigator (Dr. Jennifer Liu) blinded to patient identification, demographics and clinical characteristics at the end of the study. When possible any ECHOs done outside of MSKCC are requested to be done on the GE Vivid 7 or E9 machine (GE healthcare, Milwaukee, WI), and the disc to be sent to Dr. Jennifer Liu for the strain imaging analysis.

Schedule of Activity

	Pre-Treatment	**Prior to each chemo-therapy treatment [4]**	**Every Cycle [6-7]**	**Every other cycle**	**After every 4th cycle**	**After Treatment Completion [5]**
Medical History	X (w/i 4 weeks)		X			X
Physical examination	X (w/i 4 weeks)		X			X
ECOG PS	X (w/i 4 weeks)		X			
CBC	X (w/i 2 weeks)	X	X			
Comprehensive Profile	X (w/i 2 weeks)		X			
EKG	X (w/i 4 weeks)					
LVEF [1]	X (w/i 4 weeks)				X	
Correlative blood-work[2]				X		
Pregnancy test	X (w/i 2 weeks)					
Evidence of disease evaluation (EOD) [3]	X (w/i 4 weeks)				X	
Signed informed	X (w/i 4 weeks)					

consent						
Adverse event/toxicity assessment			X			

1. LVEF is assessed via an ECHO with strain imaging. When an ECHO by the GE Vivid 7 or E9 system (GE Healthcare, Milwaukee, WI) is not available for strain imaging analysis, then an LVEF by a standard ECHO machine per facility may be done. If an ECHO cannot be done, a MUGA scan may be done. The LVEF will be assessed after every 4th cycle +/- 2 weeks (i.e. day 1 of cycle 5 ± 2 weeks, day 1 of cycle 9 ± 2 weeks, etc.). Patients who have surpassed the 6 month period may complete ECHO or MUGA every 6 months +/- 1 month starting from the most recent scan date.

2. Correlative bloodwork will consist of TnI, BNP, and NRG-1ß. These will be done pre- and post-infusion on day 1 of every other cycle, up to six time points (i.e. Cycle 1, 3, 5, 7, 9, and 11). If day 1 bloodwork was missed, it can be done on day 8 or 15.

3. EOD evaluation will consist of a CT of chest and abdomen +/- pelvis or MRI of chest/abdomen +/- pelvis. Bone scan and PET are optional. EOD evaluation will be assessed after every 4th cycle (+/- 2 weeks). Patients who have surpassed the 6 month period may complete these radiologic assessments every 6 months +/- 1 month starting from the most recent scan date.

4. Prior to the first cycle of treatment, a height and weight must be obtained.

5. After treatment completion, the patient will be seen for routine follow-ups (ie: medical history, physical examination) at the discretion of the treating physician.

6. A comprehensive profile and CBC may be omitted for patients who have reached the 6 month mark and are no longer receiving paclitaxel,

7. Patients who have reached the 6 month mark and are no longer receiving paclitaxel may be seen at every other cycle of treatment per the physician's discretion.

11.0 TOXICITIES/SIDE EFFECTS

Trastuzumab (Herceptin®)

Infusion-Associated Symptoms. During the first infusion with Herceptin, a symptom complex consisting of chills and/or fever is observed in approximately 40% of patients. Other signs and/or symptoms may include nausea, vomiting, pain, rigors, headache, cough, dizziness, rash, and asthenia. These symptoms are usually mild to moderate in severity, and occur infrequently with subsequent Herceptin infusions. These symptoms can be treated with an analgesic/antipyretic such as meperidine or paracetamol, or an antihistamine such as diphenhydramine.

Serious Infusion-Associated Events. Serious adverse reactions to Herceptin infusion including dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation and respiratory distress have been reported infrequently. In rare cases (4 per 10,000), these events were associated with a clinical course culminating in a fatal outcome.

Serious reactions have been treated with supportive therapy such as oxygen, beta-agonists, corticosteroids and withdrawal of Herceptin as indicated.

Hematologic Toxicity. In the clinical trials, an increased incidence of anemia was observed in patients receiving Herceptin 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 Herceptin therapy. In the clinical trials, the per-patient incidences of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving Herceptin 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 Herceptin 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 neutropenia has not been determined; the effect of Herceptin on the pharmacokinetics of chemotherapeutic agents has not been fully evaluated. The observed incidence of leukemia among Herceptin-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 Herceptin to the etiology of acute leukemia or myelodysplastic syndrome in these cases is unclear.

Paclitaxel (Taxol®)

Side-effects include alopecia, myelosuppression, fatigue, neuropathy, arthralgia, myalgia, onycholysis, taste changes, amenorrhea, teratogenesis, hypersensitivity (hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, bronchospasm, and tachycardia), fever, sinus bradycardia, complete heart block, sinus tachycardia, premature ventricular beats, ventricular tachycardia, bigeminy, syncope, myocardial infarction, hypotension, hypertension, dizziness, visual changes, headaches, radiation recall, nausea and vomiting, mouth sores, abdominal pain, diarrhea, typhlitis, ischemic colitis, abnormal liver function, pancreatitis, abnormal triglyceride, and seizures.

Pertuzumab

Diarrhea and rash

Although pertuzumab targets HER2, because of its role in heterodimerization with other members of the HER family (ie: HER1/EGFR), it may cause toxicities associated with the use of EGFR inhibitors. Diarrhea and rash are common events increased with pertuzumab given in combination with chemotherapy compared with chemotherapy alone. Diarrhea has been reported in 60-70% of patients treated with pertuzumab and was mostly of grades 1-2. The mechanism of diarrhea and rash are unknown, but the nature is similar to that of other agents causing HER1 inhibition. In the event of diarrhea, early intervention with anti-diarrhea medication should be considered and patients treated with fluid and electrolyte replacement, as clinically indicated.[55]

Infusion-related Symptoms

Monoclonal antibodies may cause infusion-associated symptoms such as fever, chills, hypotension, shortness of breath, skin rash, headache, nausea, and/or vomiting. Serious or severe infusion-related symptoms have been rarely observed with 7 patients experiencing serious reactions to date [hypersensitivity, fatal ARDS, pulmonary edema,

anaphylaxis, dyspnea with hypotension, infusion-related reaction (unsteady on feet, headache)]. In pertuzumab single-agent studies, less than 5% of patients experienced adverse reactions during pertuzumab infusions. Serious infusion-related reactions with pertuzumab have been infrequently reported (< 1%). Intravenous pertuzumab administration should be performed in the setting with emergency equipment and staff who re trained to monitor medical situations and respond to medical emergencies. Patients should be monitored during each pertuzumab infusion for any adverse effects, as infusion reactions may occur with the first or subsequent doses. The infusion should be stopped for patients who develop dyspnea, clinically significant hypotension, or other clinically significant events. Patients who experience an NCI-CTC grade 4 allergic reaction or acute respiratory distress syndrome should not receive additional peruzumab.[55]

Cardiac Toxicities

Since pertuzumab targets HER2, as with trastuzumab, there is a potential risk of cardiac dysfunction, particularly in patients who have received prior anthracycline treatment. Cardiac toxicities, predominantly asymptomatic LVEF declines, and 4 cases of cardiac failure have been reported in approximately 840 patients with advanced malignant disease or early stage HER2 (+) breast cancer treated with pertuzumab. Two of these cases occurred in patients with metastatic breast cancer who had received prior anthracyclines and two in patients with ovarian cancer. No clear association between the frequency, nature, and severity of pertuzumab-related toxicities and dose level has been observed.[55]

Patients with significant cardiac disease or baseline LVEF below the institution's lower limit of normal (LLN) should not commence treatment with pertuzumab. Risk factors for pertuzumab-associated cardiac dysfunction are not known at this time. This risk should be carefully weighed against the potential benefit in patients who have received prior anthracyclines. Monitoring of the LVEF is advised while patients are receiving pertuzumab. If symptomatic LVEF decline develops (NCI-CTC grade 3 or 4), the patient must discontinue pertuzumab. Left ventricular dysfunction, symptomatic or not, should be treated and followed according to standard medical practice.

Other Side-effects

Other side-effects of pertuzumab include low blood count and fatigue.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Patients with measurable or non- measurable lesions are included in this study. Measurable lesions are defined as those that can be measured accurately in at least one diameter, that is $\geq$ 20 mm using conventional imaging techniques (including incremental CT) or $\geq$ 10 mm using spiral CT equipment. Non- measurable lesions include bony metastases, leptomeningeal disease, ascites, pleural/pericardial effusions, inflammatory breast cancer, lymphangitis carcinomatosis, and heavily calcified and cystic/necrotic lesions.

Patients will have an evidence of disease evaluation (EOD) evaluation after every 4th cycle of study treatment which will consist of CT of chest and abdomen +/- pelvis. Bone scan and PET scan are optional. Evaluable patients include all patients who started therapy and for whom we are able to assess PFS status at 6 months. Patients who withdraw before 6

months for non-toxicity reasons (ie: personal reasons, non-cancer related illness, and non-compliance) are considered inevaluable. We do not anticipate a significant early withdrawal for non-toxicity reasons. Patients who discontinue therapy due to toxicity before the 6 month endpoint are considered evaluable and their progression status at 6 months will be assessed during followup. Patients who have surpassed the 6 month period may complete these radiologic assessments every 6 months +/- 1 month starting from the most recent scan date.

The primary endpoint is PFS and secondary endpoint will include the response rate using the RECIST criteria (version 1.1). The definitions are included as below:

- Progression-free survival (PFS) is defined from time from treatment assignment to disease progression or death, whichever comes first.
- Partial response (PR) is at least a 30% reduction in the sum of longest diameter of target lesions
- Complete response (CR) is the disappearance of all target lesions.
- Stable disease (SD) is neither a PR nor a POD.
- Progression of disease (POD) requires a 20% increase in the sum of the longest diameters or the appearance of new lesions.

13.0 CRITERIA FOR REMOVAL FROM STUDY

- 1) > 2 dose reductions of paclitaxel
- 2) > 3 consecutive weeks of a delay in treatment due to toxicities
- 3) Progressive disease
- 4) Unacceptable toxicity
- 5) Intercurrent, non-cancer related illness that prevents continuation of protocol therapy or follow-up
- 6) Major protocol violation that would render the patient inevaluable for efficacy
- 7) Repeated non-compliance by the patient with protocol requirements
- 8) Changes in the patient's condition or study drug related toxicity such that, in the opinion of the investigator, continued participation in the protocol would compromise patient well-being
- 9) Withdrawal of patient's consent for personal reasons
- 10) Death

Patients removed due to criteria listed in 5, 6, 7, and 9 are considered inevaluable for the primary 6 month PFS endpoint.

14.0 BIOSTATISTICS

This is a single stage phase II trial of paclitaxel given with pertuzumab and trastuzumab in patients with metastatic HER2-positive breast cancer. Patients with HER2 (+) breast cancer who have had 0-1 prior treatment in the metastatic setting are eligible and will be enrolled. The primary objective of this trial is to determine the efficacy of this regimen. The primary endpoint of this study will be the proportion of

patients who are progression free at 6 months or later. Patients who are considered progression-free at 6 months are deemed successes. Failures are those patients who progressed or died before the 6 month mark. Evaluable patients include all patients who started therapy and for whom we are able to assess PFS status at 6 months. Patients who withdraw before 6 months for non-toxicity reasons (ie: personal reasons, non-cancer related illness, and non-compliance) are considered inevaluable. We do not anticipate a significant early withdrawal for non-toxicity reasons. Patients who discontinue therapy due to toxicity before the 6 month endpoint are considered evaluable and their progression status at 6 months will be assessed during followup. Patients will be followed until progression of disease at which point further follow-up is not mandated by the study. There is no minimal time on study treatment before patients are considered evaluable. The secondary endpoints are response, safety (including cardiac safety) and tolerability. Serial blood measurements of cardiac markers (cTnI, BNP, and NRG-1ß) will be examined. We will also assess the LVEF at baseline and every 4th cycle of treatment with an ECHO with a strain imaging analysis. When an ECHO cannot be done, a multi-gated acquisition scan (MUGA) may be done.

A single stage design will be used to evaluate the efficacy of this regimen. We elected a target progression-free rate below 50% as the unpromising rate and 65% or higher as the promising 6 month progression-free rate. These target rates are based on data from Slamon et al which showed that the combination of paclitaxel and trastuzumab led to a median TTP of 7.4 months in patients with untreated HER2 (+) metastatic breast cancer. We chose 6 months, as opposed to 7 months, as patients with one prior treatment are included. Based on these proportions, we would need 69 patients, at an alpha level of 5% and 80% power for a single stage design. The secondary endpoints will be the safety (including cardiac safety) and tolerability and the assessments of biomarkers. Baselga et al reported that in 66 patients treated with trastuzumab and pertuzumab, only 3/66 (4.5%) of patients experienced an asymptomatic LVEF decline of $\geq$ 10% to < 50% and 0/66 (0%) patient had symptomatic CHF.[57] However, Portera et al reported that in 11 patients treated with these 2 antibodies, 2/11 (18%) had asymptomatic LVEF decline of $\geq$ 10 % to < 50%, and 1/11 (9%) patient experienced symptomatic CHF.[59] The differences in cardiac events (asymptomatic and symptomatic) between the 2 studies could be due to sample sizes and variations in patient inclusion criteria. Nevertheless, in our study we will consider an asymptomatic LVEF decline (of $\geq$ 10% to < 50%) rate of $\leq$ 20% (or 14 of 69 patients) as acceptable, based on the Baselga and Portera studies [57, 59]. We will consider a symptomatic CHF rate of $\leq$ 4% as acceptable to be consistent with what has been reported in previous trials with trastuzumab alone (symptomatic CHF rates ranging from 2-4%) [95]. For this study, to be homologous to the large phase III trial, CLEOPATRA, a cardiac event is defined as a) "symptomatic" left ventricular systolic dysfunction (LVSD) (deaths and non-deaths), b) non-LVSD cardiac death, or c) probable cardiac death. Other cardiac non-death events that do not meet this definition are not counted as a cardiac event (personal communications with Dr. Melissa Brammer and Dr. Christina Pelizon for CLEOPATRA). For safety, we have built in a stopping rule based on symptomatic cardiac events and asymptomatic LVEF declines. The trial will be closed if: 1) 4 patients experience a cardiac event while on study treatment at any point in the study or 2) if 15 patients have an asymptomatic LVEF decline (of $\geq$ 10% to < 50%) at any point while on study. This corresponds to an overall cardiac event (as defined above) rate of 5.8% (4/69) and an overall asymptomatic LVEF decline (of $\geq$ 10% to < 50%) rate of 22% (15/69).

Upon completion of the study, the 6 month PFS rate will be estimated and an exact confidence interval will be constructed. Progression-free survival and median overall

survival will also be estimated by the Kaplan-Meier method. Selected non-hematologic and hematologic toxicities will be described by frequency and grade, by each cycle and all cycles, with the maximum grade over all cycles used as the summary measure per patient. Each of the cardiac markers and LVEF will be examined graphically over the study period. Particular attention to the trajectories of the cardiac markers will be given to patients who have had significant LVEF decline (defined in section 10.0). We anticipate an accrual rate of 2 patients per month over the course of 3 years. Additional patients will be accrued to take place of inevaluable patients as necessary. We expect this early dropout to be a rare event, occurring 5% of the time, and will be examined closely as a source for potential bias.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System .

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The Clinical Research Database (CRDB) will be used for data collection. The data will be reported to the institution (IRB) and the sponsor (Roche) as appropriate.

It is estimated that 2 patients will be accrued per month and it will take 3 years to accrue.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals, protocol compliance, eligibility verification, informed consent procedure, data accuracy, and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://www.cancer.gov/clinicaltrials/conducting/dsm-guidelines/page1. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://smskpsps9/dept/ocr/OCR%20Website%20Documents/Clinical%20Research%20Quality%20Assurance%20(CRQA)/MSKCC%20Data%20and%20Safety%20Monitoring%20Plan.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

16.3 Regulatory Documentation

Participating sites that are consulting and/or conducting specimen or data analysis should submit this protocol to their IRB according to local guidelines. Copies of any site IRB correspondence should be forwarded to MSKCC.

17.0 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible toxicities and side effects. Every effort will be made to keep study records private. Neither the patient's name nor anything else that could identify the patient will be used in any reports or publications that result from this study. Trained staff at Memorial Hospital, the Food and Drug Administration, or the study supporters will be able to review the medical records if necessary. The patient may terminate her participation in the study at any time during the trial.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1 Genentech Serious Adverse Event (SAE) Reporting

All SAE reports must also be forwarded as soon as possible to:

Genentech Drug Safety

Pertuzumab Safety Monitor

Tele: 1-888-835-2555

Fax: (650) 225-4682

Reporting SAEs:

All SAEs that are serious and reasonably or probably related to the use of pertuzumab (this applies to both expected and unexpected events) should be recorded on an MSK CRDB SAE report and faxed as soon as possible to:

Genentech Drug Safety Contact Line

Tele: 1-888-835-2555

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

AND:

Study Coordination Center/Principal Investigator: Chau Dang, M.D.

Contact Information phone: 646-888-4554 fax : 646-888-4555

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

Study Drug Relationship:

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

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

The AE cannot be attributed solely to concurrent/underlying illness,

Adverse Event Reporting Definitions:

A serious treatment emergent adverse event (STEAE) is any sign, symptom or medical condition that emerges during pertuzumab treatment or during a post-treatment follow-up period that (1) was not present at the start of pertuzumab treatment and it is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of pertuzumab treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect
- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

Assessing Causality:

The event should be assessed to decide whether there is a reasonable possibility that pertuzumab caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to pertuzumab administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to pertuzumab administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

For **Investigator Sponsored IND Studies**, there are some additional reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR § 600.80.

Events meeting the following criteria need to be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

***7 Calendar-Day Telephone or Fax Report*:** The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the pertuzumab Package Insert or Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and Genentech within 7 calendar days of first learning of the event.

***15 Calendar-Day Written Report*:** The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any **serious**, **unexpected** AE that is considered reasonably or **possibly related** to the use of pertuzumab. An **unexpected** adverse event is one that is not already described in the Package Insert or Investigator Brochure for pertuzumab.

- Written IND Safety Reports should include an **Analysis of Similar Events** in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.
- Written IND safety reports with Analysis of Similar Events are to be submitted the FDA, Genentech Drug Safety, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500 Form but alternative formats are acceptable (e.g. summary letter).

All written IND Safety Reports submitted to the FDA by the Sponsor-Investigator must also be faxed to:

Genentech Drug Safety

Pertuzumab Safety Monitor

Tele: 1-888-835-2555

Fax: (650) 225-4682

For questions related to safety reporting, contact:

Genentech Drug Safety

Pertuzumab Safety Monitor

Tele: 1-888-835-2555

Fax: (650) 225-4682

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20. APPENDICES

Appendix A ECOG Performance Scale

Appendix B Hypersensitivity Management

Appendix C Diarrhrea and Rash Management

Appendix D CYP3A4 Inducers and Inhibitors and CYP2C8 Substrates

APPENDIX A

ECOG PERFORMANCE STATUS SCALE

Grade	Description
0	Fully active, able to carry out all normal activity without restriction (Karnofsky 100)
1	Restricted in physically strenuous activity but ambulatory and able to carry out light work (Karnofsky 90-80)
2	Ambulatory and capable of all self-care but unable to carry out any work. Up and about more than 50% of waking hours (Karnofsky 70-60)
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours (Karnofsky 50-40)
4	Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair (Karnofsky 30-20)
5	Death

APPENDIX B

MANAGEMENT OF ACUTE HYPERSENSITIVITY

Severity of Symptoms	Treatment Guidelines
Mild symptoms: localized cutaneous reactions such as mild pruritus, flushing, rash	· consider decreasing the rate of infusion until recovery from symptoms, stay at bedside and monitor patient · then, complete taxane infusion at the initial planned rate
Moderate symptoms: any symptom that is not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP > 80 mm Hg	· interrupt taxane infusion · give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV; monitor patient until resolution of symptoms · resume taxane infusion after recovery of symptoms; depending on the physician's assessment of the patient, taxane infusion should be resumed at a slower rate, then increased incrementally to the initial planned rate*, (eg. infuse at an 8 hour rate for 5 minutes, then at a 4-h rate for 5 minutes, and then finally, resume at the 3-h infusion rate)* · depending on the intensity of the reaction observed, additional oral or IV premedication with an antihistamine should also be given for the next cycle of treatment, and the rate of infusion should be decreased initially and then increased back to the initial planned rate, *(eg. infuse at an 8 hour rate for 5 minutes, then at a 4-h rate for 5 minutes, and finally, administer at the 3-h infusion rate)*
Severe symptoms: any reaction such as bronchospasm, generalized urticaria, systolic BP ≤ 80mm Hg, angioedema	· immediately discontinue taxane infusion · give diphenhydramine 50 mg IV with or without dexamethasone 10 mg IV and/or epinephrine as needed; monitor patient until resolution of symptoms · the same treatment guidelines outlined under moderate symptoms (i.e. the third and fourth bullets) should be followed.
Anaphylaxis (NCI grade 4 reaction)	· NO FURTHER STUDY DRUG THERAPY

APPENDIX C

Supportive Care Guidelines for Pertuzumab

Although pertuzumab targets HER2, because of its role in hetero-dimerization with other members of the HER family (ie: EGFR), it may cause toxicities associated with the use of EGFR inhibitors such as diarrhea and rash.

Rash: The following agents may be used: minocycline, topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisone.

Diarrhea: Loperamide is recommended at the first sign of diarrhea. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg q 2–4 hr until diarrhea free for 12 hr.

Diarrhea Management Guidelines:

1. Uncomplicated grade 1-2 diarrhea:
   - Stop all lactose containing products;
   - Drink 8-10 large glasses of clear liquids a day;
   - Eat frequent small meals;
   - Grade 2 diarrhea, consider holding cytotoxic chemotherapy;
   - Administer standard dose of loperamide:
     - Initial dose 4mg followed by 2mg every 4 hours or after every unformed stool.
     - We suggest continuation of loperamide until diarrhea free for 12 hours.

2. For grade 3 or 4 diarrhea or grade 1 or 2 with complicating features (severe cramping, severe nausea/vomiting, decreased performance status, fever, sepsis, grade 3 or 4 neutropenia, frank bleeding, dehydration):
   - Use intravenous fluids as appropriate, consider hospital admission;
   - Use prophylactic antibiotics as needed (example fluoroquinolones), especially if diarrhea is persistent beyond 24 hours or there is fever or grade 3-4 neutropenia;
   - Hold cytotoxic chemotherapy.

   These broad general management principles are recommended to proactively try and avoid more serious complications by active management of the diarrhea syndrome.

   Guidelines such as these should never replace sound clinical judgment.

   Other anti-diarrheal agents are allowed at the physician's discretion.

APPENDIX D

CYP3A4 inducers and inhibitors

Drug Class	Agent	Wash-out[1]
CYP3A4 Inducers		
Antibiotics	All rifamycin class agents (ie: rifampicin, rifabutin, rifapentine)	14 days
Anticonvulsants	phenytoin, carbamazepine, barbituates (ie: phenobarbital)	14 days
Antiretrovirals	efavirenz, nevirapine	14 days
Glucocorticoids	cortisone (>50 mg), hydrocortisone (>40 mg), prednisone (>10 mg), methylprednisolone (>8 mg), dexamethasone (>1.5 mg)[2]	14 days
Other	St. John's Wort, modafinil	14 days
CYP3A4 Inhibitors		
Antibiotics	clarithromycin, erythromycin, troleandomycin	7 days
Antifungals	itraconazole, ketoconazole, fluconazole (>150 mg daily), voriconazole	7 days
Antiretrovirals, Protease Inhibitors	declaviridine, nelfinavir, amprenavir, ritonavir, indinavir, saquinavir, lopinovir	7 days
Calcium channel blockers	verapamil, diltiazem	7 days
Antidepressants	nefazodone, fluvoxamine	7 days
GI agents	cimetidine, aprepitant	7 days
Other	grapefruit, grapefruit juice,	7 days
	amiodarone	6 months
Miscellaneous		
Antacids	Mylanta, maalox, tums, rennies	1 hour before and after dosing
Herbal supplements[3]	Ginkgo biloba, kava, grape seed, valerian, ginseng, echinacea, evening primrose oil	14 days

CYP2C8 Substrates:

-Amodiaquine

-Torsemide

Cerivastatin

-Repaglinide

Rosiglitazone