A Phase 2 Clinical Trial Assessing the Correlation of Early Changes in Standardized Uptake Value (SUV) on Positron Emission Tomography (PET) with Pathological Complete Response (pCR) to Pertuzumab and Trastuzumab in Patients with Primary Operable HER2-Positive Breast Cancer

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1. Summary

A large proportion of women with early breast cancer will be offered adjuvant chemotherapy in an effort to minimize the risk of disease recurrence. However, because of a lack of predictive biomarkers of sensitivity or resistance, most women receiving adjuvant chemotherapy will either not require or will not benefit from this approach. In addition, chemotherapy is associated with unwanted short and long term side effects, including serious and potentially life threatening complications. The management of women with human epidermal growth factor receptor 2 (HER2)-positive breast cancer has been revolutionized by the development of anti-HER2 therapies such as trastuzumab.[1] The addition of trastuzumab, however, to adjuvant regimens can further potentiate chemotherapy-induced cardiotoxicity. The personalization of systemic treatment for early breast cancer has the potential to both reduce breast cancer recurrences and minimize or eradicate toxicity. We propose a series of clinical trials, which aim to utilize early imaging and plasma/tumor biomarker changes to determine which women with estrogen receptor (ER)negative, HER2-positive breast cancer can be treated with biologic therapy alone and be spared the toxicity of systemic chemotherapy, without compromising efficacy.

Recent studies in the metastatic and neoadjuvant settings indicate that dual anti-HER2 therapy, such as the combination of trastuzumab and pertuzumab (a novel HER2 dimerization inhibitor), is more efficacious than single agent anti-HER2 therapy. The phase 3 CLEOPATRA trial in women with previously untreated HER2-positive metastatic breast cancer (MBC) patients, randomized patients to trastuzumab/docetaxel (control arm) versus trastuzumab/pertuzumab and docetaxel (experimental arm). The median progression-free survival was significantly greater in the experimental arm (18.5 months) compared to the control arm (12.4 months).[2]

NeoSphere was a Phase 2 pre-operative randomized trial comparing trastuzumab/docetaxel (control arm), trastuzumab/pertuzumab/docetaxel, trastuzumab/pertuzumab (dual antiHER2 blockade) and pertuzumab/ docetaxel.[3] The pathological complete response (pCR) rates were 29%, 46%, 17% and 24% respectively. For patients with ER-negative disease, an impressive pCR rate was observed for dual antiHER2 therapy alone (27%). That a large proportion of women treated with biological therapy alone can obtain a pCR, an acceptable surrogate of excellent outcome in neoadjuvant clinical trials, has caused great excitement in the breast oncology community. Clearly, dual antiHER2 blockade has been shown to be superior to trastuzumab or pertuzumab alone and will become a component of standard of care in the near future such that studies investigating biomarkers of response in this setting are imperative. The development of biomarkers able to identify a subgroup of patients with HER2-positive disease that may potentially be treated with biologic therapy alone and be spared systemic chemotherapy is imperative. Several candidate biomarkers can be considered including functional imaging and plasma/tissue biomarkers.

Fluorodeoxyglucose (FDG) positron-emission tomography (PET) may allow for prediction of pCR as early as 2 weeks post commencement of systemic therapy. The NeoALLTO PET substudy is the first study to prospectively evaluate changes in SUV (standardized uptake value) on PET as a predictor of response to neoadjuvant anti-HER2 therapy (n=86).[4] Patients were randomized to receive 6 weeks of antiHER2 therapy followed by the addition of weekly paclitaxel for 12 additional weeks, until definitive surgery.[5] This substudy indicated that metabolic changes in the

primary tumor of patients with invasive operable breast cancer treated with anti-HER2 agents can be detected as early as after 2 weeks of treatment. pCR rates were found to be twice as high in patients who were FDG-PET responders compared to non-responders.

We propose to evaluate for the first time the correlation between early changes in SUV and pCR in men and women with ER-negative, HER2-positive breast cancer receiving trastuzumab and pertuzumab (PT) pre-operatively. This has not previously been evaluated in patients receiving antiHER2 therapy alone and as such is novel and potentially practice changing. The results from this phase 2 biomarker study will be used to plan a randomized study using a predefined cut point for SUV decline such that we can further attempt to identify a group of individuals with HER2positive early breast cancer who do not require cytotoxic chemotherapy in addition to anti-HER2 agents. This non-invasive biomarker approach will be of great interest to breast cancer oncologists and patients by facilitating a personalized approach to managing patients with HER2-positive disease that will undoubtedly spare toxicity and reduce the costs associated with anti-cancer strategies, without compromising efficacy.

Importantly, we will also investigate candidate tissue and plasma biomarkers that may predict sensitivity (pCR) or resistance (no pCR) to anti-HER2 therapy, with a focus on the PI3K pathway.

2. Schema

PET: Baseline and 15 days (+3 days)

Tumor biopsy: Baseline, 15 days (+3 days), prior to additional standard preoperative treatment (if applicable), and at time of definitive surgery; archival sections will also be collected from the diagnostic and surgical specimens in all subjects

Blood sampling: Baseline, 15 days (+3 days), prior to and post- additional standard preoperative treatment (if applicable), and 1-4 weeks following definitive surgery

PT: Pertuzumab (840mg loading dose, 420mg maintenance doses) and Trastuzumab (8mg/kg loading dose, 6mg/kg maintenance doses); 1 cycle = 3 weeks

*Participants with complete clinical response, or without complete clinical response who choose not to receive additional preoperative treatment, should proceed directly to surgery; participants with disease

progression or with residual disease on biopsy after completion of study treatment may proceed to additional standard preoperative treatment.

3. Hypotheses

We hypothesize that early changes in SUV on FDG-PET will correlate with pCR in men and women with ER-negative, HER2-positive breast cancer receiving pertuzumab and trastuzumab (PT). Our results will be used to develop a randomized study, in which we will use a predefined SUV decline to randomize patients to antiHER2 therapy alone versus antiHER2 therapy plus chemotherapy. We also hypothesize that aberrations of the PI3K pathway, and an immune function gene signature will correlate with pCR in this population. Our long term goal is to identify a group of patients with HER2-positive disease who can be spared chemotherapy. The study will also provide a much needed tissue dataset for future investigation.

4. Objectives

4.1 Primary

4.1.1 To correlate baseline and change (day 15) in SUV on FDG PET with pathological complete response (pCR) in patients treated with preoperative pertuzumab/trastuzumab

4.2 Secondary

4.2.1 To correlate *PIK3CA* mutation status and other genomic alterations (mutations/somatic rearrangements) qualitatively and quantitatively in plasma tumor DNA (ptDNA) with pCR

4.2.2 To correlate PI3K pathway activation (e.g. PTEN low and/or *PIK3CA* mutation, HER 1-4 expression and/or phosphorylation) in tumor samples and pCR

4.2.3 To correlate a gene immune function signature in tumor samples and pCR

4.2.4 To correlate baseline and change (day 15) in Ki67 with pCR

4.2.5 To determine pCR rates to preoperative pertuzumab/trastuzumab + taxane in the setting of histologically confirmed residual cancer after 12 weeks of preoperative pertuzumab/trastuzumab

4.3 Exploratory

4.3.1 To identify baseline biomarkers that may predict sensitivity (pCR) or resistance (no pCR) to anti-HER2 therapy, such as ER, Ki67, serum methylation markers, gene expression profiles, and others to be decided.

4.3.2 To explore changes in biomarkers (e.g., PIK3CA, ER, Ki67, serum methylation markers, gene expression profiles) through serial samples collected from baseline, end of therapy, at the time of definitive surgery and post operatively in an effort to understand resistance to anti-HER2 therapy and obtain preliminary data.

4.3.3 To correlate baseline and change (day 15) in SUV on FDG PET with pCR among patients with histologic confirmed residual disease after 12 weeks preoperative pertuzumab/trastuzumab and subsequent addition of standard therapy per investigator discretion.

5. Background and Rationale

Breast cancer is the most common malignancy in women in Western societies. More than 200,000 women were diagnosed with breast cancer in the US in 2010.[6] Advances in the early detection and optimal adjuvant treatment of breast cancer have led to a significant reduction in disease relapse and death. Nevertheless, more than 40,000 women per year continue to die from this disease.

Traditionally, clinicopathologic factors have been used to guide choice of therapy for women newly diagnosed with breast cancer, i.e., endocrine therapy for tumors that express hormone receptors and trastuzumab for tumors that overexpress HER2. However, it is still difficult to precisely identify who will or will not benefit from (neo) adjuvant chemotherapy. Data from the Oxford Overview,[7] the identification of biologically distinct breast cancer intrinsic subtypes with various prognoses,[8] and multivariate prediction models such as Adjuvant! Online[9] have helped further refine this decision-making process. The recent development of multigene predictive tools has helped with improved prognostication for patients with hormone receptor-positive, HER2negative breast cancer and, in some cases, with prediction of benefit from specific therapies. The molecular tools developed thus far offer little aid in clinical decision-making for women with ERnegative or HER2-positive disease, and improved prognostic and predictive tools for these patients are needed. Consequently, biomarkers able to identify a subgroup of patients that may potentially be spared systemic chemotherapy or who need intensification of standard therapy are of great interest and forms the rationale for our study.

5.1 HER2-Positive Breast Cancer

Increased expression of HER2 or amplification of the *HER2/neu* gene had been associated with a worse prognosis when compared to other breast cancer phenotypes.[10] The NSABP B-31/NCCTG-N9831 and HERA adjuvant clinical trial results indicated that the addition of 1 year of trastuzumab to adjuvant chemotherapy regimens (predominantly anthracycline and taxane-containing) in a HER2-positive population of women resulted in an approximately 50% reduction in breast cancer recurrence and 35% reduction in mortality.[11,12] A recent updated joint analysis at 4 years of the NSABP B-31/NCCTG-N9831 trial has confirmed that the addition of adjuvant trastuzumab to chemotherapy maintains both a significant disease-free and overall survival benefit when compared to chemotherapy alone.[1] Non-anthracycline based regimens have also been evaluated and indicate comparable efficacy to anthracycline-based approaches with a more favorable toxicity profile.[13] It is possible, therefore, that not all women require an anthracycline as part of their (neo) adjuvant treatment strategy. Phase 2 and 3 trials evaluating the use of trastuzumab and chemotherapy in the neoadjuvant setting are described below and have reported high pCR rates, a potential surrogate endpoint for survival that is commonly used in neoadjuvant studies.

5.2 Neoadjuvant Therapy

The neoadjuvant setting has been an attractive area of research for identifying new effective treatment strategies while minimizing treatment-related adverse events, studying drug mechanisms of action, and developing clinically applicable prognostic and predictive biomarkers in an attempt to individualize therapy. Neoadjuvant therapy, also designated primary systemic or preoperative

therapy, was initially employed to downstage inoperable tumors to allow for definitive surgery. Similar survival benefits have subsequently been demonstrated for the administration of chemotherapy before or after surgery. An International Expert Panel, convened three times over the past decade, has recommended that clinicians should consider neoadjuvant chemotherapy in any patient with primary operable disease for whom adjuvant chemotherapy is clearly indicated.[14] The neoadjuvant approach may allow for other benefits such as enhancement of breast conservation, may offer prognostic information, and enables assessment of in vivo response to therapy. Women who achieve a pCR following neoadjuvant therapy are expected to have improved outcome compared to those with extensive residual disease.[15]

Response to treatment in an individual woman may predict her long-term outcome. Despite the varied definitions of pCR in trials completed to date, it has been consistently demonstrated that pCR is associated with improved disease-free and overall survival.[16] Women without residual invasive and noninvasive tumor cells in the breast and in the axillary nodes have substantially improved outcomes compared to women with similar stage and tumor characteristics and extensive residual disease. Furthermore, clinical response to therapy can be assessed as early as following one to two cycles and may help predict who will achieve a pCR. Current consensus recommendations for preoperative chemotherapy include anthracycline- and taxane-based therapy. This is based on results obtained from several prospective trials suggest that preoperative anthracycline and taxane based therapy is associated with high response rates.[17-19]

5.2.1 Neoadjuvant therapy for HER2-positive disease

Women with HER2-positive tumors who are candidates for neoadjuvant chemotherapy should receive a trastuzumab-based regimen.[20] Several small phase 2 studies have reported high pCR rates when trastuzumab was added to neoadjuvant anthracycline and taxane-based regimens. Concurrent paclitaxel and trastuzumab, followed by concurrent FEC (5-FU, epirubicin, cyclophosphamide) and trastuzumab yielded a pCR rate of 60%, without an increase in cardiac toxicity compared to chemotherapy alone.[21] A non–anthracycline-based approach combining carboplatin, weekly paclitaxel, and trastuzumab was associated with a pCR rate of 76%.[22]

In the phase 3 NeOAdjuvant Herceptin (NOAH) trial, the addition of trastuzumab to three cycles of doxorubicin and paclitaxel, followed by four cycles of paclitaxel and three cycles of CMF was associated with an improvement in overall response rate compared to chemotherapy alone (81% vs 73%, $P = .18$) and pCR rate (43% vs 23%, $P = .002$).[23] The NeoALLTO trial investigated the efficacy of lapatinib (a dual HER2/EGFR tyrosine kinase inhibitor) plus paclitaxel, versus trastuzumab plus paclitaxel, versus concomitant lapatinib and trastuzumab plus paclitaxel, when given as neoadjuvant therapy in patients with HER2-positive breast cancer (n=455). Patients were randomized to initially receive 6 weeks of a "biological window" of either lapatinib, or trastuzumab, or lapatinib with trastuzumab. Subsequently the same targeted therapy was continued with the addition of weekly paclitaxel for a further 12 weeks, until definitive surgery. After surgery, patients received 3 cycles of adjuvant FEC followed by the same targeted therapy as in the biological window of the neoadjuvant phase for a further 34 weeks (to complete 52 weeks of anti-HER2 therapy). pCR was significantly higher in the combination arm (lapatinib plus trastuzumab) compared with either trastuzumab or lapatinib alone (51.3% vs. 29.5% vs. 24.7%, respectively; P

< 0.01 for both), indicating that dual blockade of the HER2 pathway is a valid concept. No major cardiac dysfunctions or toxic deaths were observed during the neoadjuvant phase. There was increased, but manageable, toxicity (mainly diarrhea and liver enzyme alterations) in the lapatinib arms. A subset of patients also participated in a PET/CT substudy which is described in a later section.[5] The NeoSPHERE study investigating various combinations of pertuzumab, trastuzumab, and docetaxel in the neoadjuvant setting is described below.

In addition, a number of studies are ongoing or have been completed evaluating dual anti-HER2 therapies alone in the neoadjuvant setting. A phase 2 neoadjuvant study of lapatinib and trastuzumab in patients with operable HER2-positive breast cancer has been presented (TBCRC006). Patients received 12 weeks of anti-HER2 therapy pre-operatively, as well as letrozole +/- goserelin if they had ER-positive disease (approximately 60% of study population). The overall pCR rate was 27%, with a 21% rate for ER-positive patients and 38% for ER-negative. The treatment was found to be well tolerated and clinical responses were observed a few weeks after starting therapy.[24] Another TBCRC study is evaluating whether longer duration (12 versus 24 weeks) of anti-HER2 therapy prior to surgery will result in a higher rate of pCR (TBCRC023).

5.3   Pertuzumab

Pertuzumab is a humanized monoclonal antibody and is the first of a novel class of HER2-targeted agents known as HER2 dimerization inhibitors. This agent bind to a distinct epitope on the extracellular domain of the HER2 receptor (the domain II dimerization arm), blocking the interaction between HER2 and other HER family receptors.[25] Potent inhibition of HER-mediated intracellular signaling results in cancer cell growth inhibition and death.[26]

5.3.1 Preclinical Studies

The antitumor activity of pertuzumab and trastuzumab, a HER2-targeted monoclonal antibody, has been evaluated both alone and in combination in HER2-positive breast cancer xenografts. The combination of trastuzumab and pertuzumab was found to have a greatly enhanced antitumor effect, a result not achieved with monotherapy. The enhanced efficacy of the combination was also observed after tumor progression during trastuzumab monotherapy and may relate to the different mechanisms of action of trastuzumab and pertuzumab; inhibition of HER2 dimerization and prevention of p95HER2 formation.[26]

5.3.2 Clinical Studies: Pertuzumab Monotherapy

Pertuzumab was investigated initially as a single agent in metastatic breast cancer, with disappointing results. In a study which evaluated pertuzumab monotherapy in patients with progressive HER2-negative metastatic breast cancer (MBC), the response rate (RR) was 4.9% and the clinical benefit rate (CBR) was 9.8% (n=79). Pertuzumab was relatively well tolerated, with most adverse events being mild to moderate. Decline in left ventricular ejection fraction of $\geq 10\%$ and/or to $\leq 50\%$ was observed in eight patients, with one case of congestive heart failure.[27] In another study in HER2-positive patients with MBC progressing on trastuzumab, the objective RR was 7.4% with a CBR of 11.1% (n=29). The most frequent adverse events were diarrhea (48%),

nausea (34%), and vomiting (24%), and two patients developed an asymptomatic decline in LVEF of > 10% to < 50%. Interestingly, if the tumor failed to respond to pertuzumab monotherapy or responded and then progressed, trastuzumab could be added to pertuzumab. This strategy was undertaken in 14 patients, and 2 of these 14, achieved confirmed response when trastuzumab was added to the pertuzumab. This was the first clinical report providing some evidence of an enhanced effect when the antibodies are combined.[28]

5.3.3 Clinical Studies: Dual anti-HER2 Therapy

The combination of pertuzumab and trastuzumab has yielded promising results in early phase clinical trials. A phase 2 trial in patients with HER2-positive MBC whose disease had progressed during prior trastuzumab-based therapy assessed the efficacy and safety profile of the combination of pertuzumab and trastuzumab (n=66). The objective response rate was 24.2%, and the clinical benefit rate was 50%, with median progression-free survival (PFS) of 5.5 months. Cardiac dysfunction was minimal, and no patients needed to come off study as a result of cardiac-related adverse events.[29]

5.3.4 Clinical Studies: Dual anti-HER2 Therapy and Chemotherapy

This combination of HER2-targeted agents has also been investigated in the neoadjuvant setting. NeoSphere was a Phase 2 randomized trial of preoperative systemic therapy comparing trastuzumab/docetaxel, trastuzumab/pertuzumab/docetaxel, trastuzumab/pertuzumab, and pertuzumab/docetaxel. The pCR rates were 29%, 46%, 17% and 24% respectively. When patients were analyzed based on ER status, a 63% pCR rate was observed in those with ER-negative disease treated with trastuzumab/pertuzumab/docetaxel and 27% in those with ER-negative disease treated with trastuzumab/pertuzumab.[30] Based on data from NeoSphere and other preoperative studies investigating pertuzumab in combination with chemotherapy, the FDA has granted accelerated approval in late 2013 to pertuzumab for use preoperatively in combination with a complete chemotherapy regimen such as that investigated in NeoSphere. That a large proportion of women, however, treated with biological therapy alone in the NeoSphere study, can obtain a pCR has caused great excitement in the breast oncology community. There is a clear need to determine who these patients are upfront as they could potentially be spared the added toxicity of chemotherapy.

A phase 3 trial in previously untreated HER2-positive MBC patients (CLEOPATRA) which randomizes patients to trastuzumab/docetaxel versus trastuzumab/pertuzumab/docetaxel has recently been presented. The median progression-free survival was 12.4 months in the control group, as compared with 18.5 months in the pertuzumab group (hazard ratio for progression or death, 0.62; 95% confidence interval, 0.51 to 0.75; P<0.001). The interim analysis of overall survival showed a strong trend in favor of pertuzumab plus trastuzumab plus docetaxel.[2] In addition, a phase 1 trial is ongoing investigating the combination of trastuzumab, pertuzumab, and paclitaxel in patients with metastatic breast cancer.

5.4 Biomarkers of Response or Resistance

As we enter an era of "personalized" cancer treatment, the preoperative period has been recognized as ideal for evaluating surrogate biomarkers for the prediction of response to therapy and clinical outcome.[31] In a recent meta-analysis which included 3776 patients in 16 studies, pathologic

response was found to be a prognostic indicator for relapse-free survival, disease-free survival and overall survival; suggesting that patients achieving pCR after neoadjuvant chemotherapy have favorable outcomes.[32] Valuable information can be obtained using small numbers of patients in a short time frame, by assessing tumor response to therapy in vivo and measuring biochemical or radiologic changes in malignant tissue prior to, during, and following neoadjuvant therapy. Ideally, these surrogate biomarkers can be used early in the treatment paradigm to predict response to neoadjuvant chemotherapy. For example, a marker that can separate sensitive and resistant tumors to specific agent(s) early in the course of therapy could be used to determine treatment early in the course. This could avoid certain patients receiving toxic and futile therapies, and direct other patients towards an aggressive or investigational approach in an effort to maximize their breast cancer outcome.

Traditionally, standard clinicopathologic factors such as age, receptor status, grade, and proliferation index aid in determining the choice of therapy for those with early breast cancer. In addition to single genes or proteins, gene-expression profiling allows for the rapid assessment of multiple genes rather than single genes using high-throughput DNA sequencing and may be used to predict both response to therapy and clinical outcome. Several trials have evaluated multigene assays as predictors of response to therapy in the preoperative setting, and validation efforts are ongoing.[33,34] The molecular tools developed thus far, however, offer little aid in clinical decisionmaking for women with ER-negative or HER2-positive disease, and improved prognostic and predictive tools for these patients are needed. Of great interest also to the oncology community is the development of biomarkers able to identify a subgroup of patients with HER2-positive disease that may potentially be treated with biologic therapy alone and be spared systemic chemotherapy.

5.4.1 Functional Imaging to Predict Response to Neoadjuvant Therapy

Imaging techniques may also provide early information regarding tumor response. PET may allow for early prediction for pathologic response as early as 2 weeks post commencement of systemic therapy.[35] Although several studies have evaluated the role of FDG PET in predicting response to therapy, a clear role in this regard has not been established. It must also be noted that the majority of studies performed to date included few if any patients with HER2-positive disease and so the role of PET in this population is not as clearly defined.

There are a few advantages to using FDG-PET for our study. We have some preliminary data from the NeoALLTO PET substudy described below that it may be predictive of response to therapy in a HER2-positive population, it is widely available such that all sites can easily do, the baseline study is covered by insurance, and finally response criteria for FDG-PET have been established (PERCIST).[36] Other imaging modalities may be of interest in imaging biomarker studies, such as fluoro-L-thymidine (FLT)-PET. Although FLT-PET is promising and interesting, it is not FDA approved for use at this time unlike FDG-PET, it is difficult to make available for multi-site studies. In addition, other imaging modalities such as MRI have not been proposed for cost reasons and due to challenges in multicenter standardization.

5.4.1.1 Predicting Response to Chemotherapy

Wahl and colleagues, our collaborator at Johns Hopkins, have conducted serial FDG PET studies in 11 patients with primary breast cancer >3 cm in size. They observed a rapid and significant decrease in glucose metabolism as early as 8 days after the administration of one cycle of combination chemotherapy in women who responded to the regimen.[37] Patients with a partial response had a reduced FDG uptake later in the course of the treatment, while non-responders had no significant change in uptake. In another study, FDG PET studies were obtained before and after 1, 4, and 8 cycles of doxorubicin-based combination chemotherapy administered every 3 weeks. Mean baseline values of FDG uptake were higher in lesions that subsequently showed a partial or complete pathological response compared to those which did not.[38] Moreover, the breast lesions that responded to the chemotherapy (clinical or pathological complete response demonstrated a statistically significant reduction in FDG uptake following a single cycle of the chemotherapy. These findings were confirmed in another study in which 22 patients with primary breast cancer underwent FDG PET imaging before and after 1 and 2 cycles of combination epirubicin and cyclophosphamide or epirubicin and paclitaxel. Women who had minimal residual disease had a decline in tracer uptake while those who had gross residual disease showed only little change in tracer uptake.[39] These studies demonstrate that FDG PET response following one cycle of chemotherapy is predictive of complete pathological response to the therapy (sensitivity 90-100% and specificity 74-91%).

Investigators at Johns Hopkins have also conducted a prospective study in women with stage 2 and 3 breast cancer who received neoadjuvant chemotherapy with FDG PET being performed at baseline and 7 days after the first cycle of chemotherapy. Six of nineteen eligible women underwent radiological imaging of FDG-PET/CT and MRI for at least two cycles of treatment. Greater reduction in SUV was observed in responders (38%) compared to the non-responder (22%; $P = 0.03$). MRI volumes decreased after cycle 1 by 42% (responders) and 35% (non-responder; $P = 0.11$). Proliferation index Ki-67 performed on biopsy specimens declined in responders in the first cycle (median = 47%, range = 29-20%), but increased (4%) in the non-responder.[40] Finally, a multi-institutional neoadjuvant clinical trial being led by our group at Johns Hopkins has reached full patient accrual (n=60) and indicates feasibility of completing studies which incorporate serial biopsies and PET imaging with central review in multiple sites (.[35]

The results of the studies outlined above suggest that early changes in FDG uptake after the first cycle of therapy are predictive of subsequent clinical and pathological response to combination chemotherapy (4-8 cycles) for breast cancer. To our knowledge, few studies have evaluated change in FDG uptake shortly after the administration of combination chemotherapy (7 days). Because FDG PET detects changes in tumor biology, it is a very attractive modality to evaluate in our clinical model.

Many other studies have evaluated changes in PET as a biomarker of response to neoadjuvant therapy at later time points and have identified varying cut offs felt to best predict response to therapy. A study of 52 patients undergoing neoadjuvant chemotherapy for stage 2 and 3 breast cancer aimed to evaluate early axillary lymph node response. Fifty per cent of baseline SUV was considered the best cutoff value to distinguish responders from non-responders. The sensitivity, specificity, negative predictive value and accuracy of FDG PET after one course of chemotherapy

were, respectively, 96, 75, 95, and 84%; suggesting that the pathological status of regional axillary lymph nodes could be accurately predicted after one course of neoadjuvant chemotherapy using PET.[41] In another neoadjuvant study (n=52) in which PET was performed after the 1st and 2nd cycle of therapy, patients were classified as non responders (NR) when the decrease of SUVmax in the primary tumor was less than 15% at the time of the second PET per EORTC criteria.[42] The 3-year disease-free survival (DFS) rate was significantly longer for metabolic responders than for nonresponders (respectively 94% vs. 69%). This prospective study showed that a decrease in the SUV less than 15% after the first chemotherapy course was a very potent predictor of failure to respond to neoadjuvant chemotherapy.[43]

A recent meta-analysis of 19 studies and 920 patients with pCR aimed to predict histopathological response in primary breast lesions by PET. The pooled sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio were 84%, 66%, 50%, 91%, and 11.90, respectively. Subgroup analysis showed that performing a post-therapy PET early (after the 1st or 2nd cycle of chemotherapy) was significantly better than later (accuracy 76% vs. 65%, $P = 0.001$). In addition, the best correlation with pathology was yielded by employing a reduction rate (RR) cutoff value of standardized uptake value between 55 and 65%. Further prospective studies were recommended to adequately position PET in treatment management for breast cancer patients.[44]

Finally, the impact of ER status on the role of PET as a biomarker in this setting remains to be defined. A considerable distinction in metabolic activity has been observed between the different subtypes of breast cancer. The mean SUVmax in lymph node metastases of ER-positive, triplenegative and HER2-positive tumors was 6.6, 11.6 and 6.6, respectively (n=38).[45] In a study that evaluated 41 patients receiving neoadjuvant anthracycline/taxane based chemotherapy, PET scans were obtained at baseline and post 2 cycles of therapy. Thirty-three patients (80%) presented SUV reduction > 50% including all 10 patients with pCR (included minimal residual disease) and 23 patients with no pCR. On the contrary, none of the 8 patients with SUV reduction ≤ 50% achieved a pCR and these 8 patients had ER-positive tumors. Hence, out of a total of 24 patients with ERpositive tumors, one third was correctly identified early as not obtaining a pCR.[46] These results indicate that PET may play a role in early identification of patients with ER-positive tumors who are unlikely to obtain a pCR.

5.4.1.2 Predicting Response to Anti-HER2 Therapy

The NeoALLTO PET substudy is the first study to prospectively evaluate changes in SUV on PET as a predictor of response to neoadjuvant anti-HER2 therapy (n=86, 77 evaluable). As a reminder, patients in the NeoALLTO study were randomized to initially receive 6 weeks of a "biological window" of either lapatinib, or trastuzumab, or lapatinib with trastuzumab. Subsequently, the same targeted therapy was continued with the addition of weekly paclitaxel for a further 12 weeks, until definitive surgery. PET scans were performed at baseline, week 2, and week 6 after starting therapy. A metabolic response was defined as a >15% reduction in SUV at 2 weeks, or >25% reduction at 6 weeks per EORTC criteria.[42] At week 2, metabolic responders had a pCR rate of 42% and non-responders 21%. At week 6, metabolic responders had a pCR rate of 44% and nonresponders 19%. A more stringent cut off of 30% reduction in SUV from baseline to 2 weeks

is observed in approximately 50% of the population and is associated with a pCR rate of 40% approx. It must be emphasized that the patients in this small PET substudy are from all arms of NeoALLTO and received only one biologic therapy for the first 6 weeks of the "biological window" while the PET scans were being performed, but subsequently also received paclitaxel. Therefore, the pathological response reflects response to the chemotherapy and biologic therapy while the response on PET reflects response to the biologic therapy alone.

The association between early changes in SUV on PET in an advanced HER2-positive breast cancer population receiving trastuzumab and lapatinib has also recently been presented (TBCRC003 PET substudy).[47] Metabolic responses were detected as early as 1 week with FDGPET and the PET scan at week 1 correlated with the PET scan at week 8 after initiating therapy. In exploratory analyses, there appeared to be a relationship between early metabolic response and more traditional measures of clinical outcome, such as clinical benefit rate and PFS. For example, 45% (9/20) of patients who achieved a metabolic partial response at week 1 had clinical benefit by RECIST and 23% (5/23) of patients who achieved metabolic stable disease at week 1 had clinical benefit by RECIST.

We propose to evaluate for the first time the correlation between early changes in SUV and pCR in men and women with ER-negative, HER2-positive breast cancer receiving trastuzumab and pertuzumab. Based on the low pCR rate observed in those with ER-positive disease, we propose to investigate this question in an ER-negative population alone. For the purposes of this clinical trial we have decided to use a cut off of <10% for ER/PR staining. The publication in 2011 by Iwamoto et al supports this approach as it indicates that few tumors with ER staining of 1-9% show molecular features similar to those of ER-positive, potentially endocrine-sensitive tumors, whereas most show ER-negative, basal-like molecular characteristics.[48] In addition, as we are focusing on a select population of patients with HER2-positive disease (approximately 8-10% of early breast cancers), and hope to avoid difficulties with accrual, we felt it would be in the best interest of the trial to include patients with ER staining 1-10%. These patients will be recommended adjuvant hormonal therapy after completion of adjuvant chemotherapy per standard of care.

The results from this phase 2 biomarker study will be used to plan a randomized study using a predefined cut point for SUV decline such that we can further attempt to identify a group of individuals with HER2-positive early breast cancer who do not require cytotoxic chemotherapy in addition to anti-HER2 agents. The preliminary data from NeoALLTO described below and our ongoing experience in HER2-negative disease suggest that this non-invasive biomarker approach will be of great interest to breast cancer oncologists and patients by facilitating a personalized approach to managing patients with HER2-positive disease which will undoubtedly spare toxicity and reduce the costs associated with anti-cancer strategies, without compromising efficacy.

The NeoALLTO study defined a metabolic responder as a patient with ≥ 15% decline in SUV at 2 weeks (EORTC criteria). Our collaborators at Johns Hopkins (Wahl and colleagues) have concern that obtaining this 15% reduction in SUV may at times be due to chance and believe that randomizing patients with a greater reduction in SUV as defined by PERCIST criteria is more appropriate when aiming to preselect a population with a high chance of obtaining a pCR.[36] The EORTC criteria were proposed initially in 1999 when limited data was available in this regard.

The PERCIST criteria is based on more modern data which indicates that larger drops in SUV of more than 30-35% is associated with a good outcome for cancer patients, and thus they propose a 30% cut off for SUV decline for tumor response. We anticipate that we will identify an SUV reduction from baseline which will accurately predict pCR and plan in our next study to randomize patients with this identified decline in SUV from baseline to PT versus PT + chemotherapy.[36] We chose to evaluate day 15 changes to correlate with findings from ongoing studies of endocrine therapies, chemotherapies, and novel agents that are commonly evaluating changes in imaging and tissue markers approximately 2 weeks after treatment initiation. We will also be able to evaluate concomitant changes in FDG uptake and in molecular markers that may be associated with response or resistance to chemotherapy detected in the breast cancer tissue obtained at the time of imaging. The results of our study can be used to design new studies with novel agents such as the randomized design described.

5.4.2 The PI3K Pathway as a Biomarker of Response to Therapy

An important focus of this study will be the correlation of tumor *PIK3CA* mutation status and PI3K pathway activation status with pCR. Similar to the HER2 pathway, the PI3K pathway is involved with several cellular systems, regulating proliferation, growth, and survival. Prior studies have shown that trastuzumab has antitumor effects only in the presence of a normal PI3K pathway and dysregulation of the PI3K pathway through genetic alterations results in trastuzumab resistance.[49] Thus, it can be hypothesized that PI3K pathway dysregulation, causing trastuzumab resistance, may be associated with inability to achieve pCR and transitively, poorer clinical outcomes. Preclinical data also supports a link between PI3K/Akt pathway and pertuzumab with or without trastuzumab.[26,50] This question is very important because the use of trastuzumab is now standard in the treatment of HER2-positive breast cancer, and assessment of PI3K pathway activation in this patient population may provide a biomarker to identify patients less likely to respond to trastuzumab-based therapy.[51]

A recent study was conducted to determine the frequency of *PIK3CA* mutations and human epidermal growth factor receptor-2 (HER2) phosphorylation status (pHER2-Tyr1221/1222) and if PIK3CA, phosphatase and tensin homolog (PTEN), or pHER2 had an impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab. Samples from 240 patients receiving adjuvant chemotherapy and subsequent trastuzumab were evaluated for PTEN and pHER2 expression by immunohistochemistry as well as *PIK3CA* mutations (exons 9 and 20) by pyrosequencing. *PIK3CA* mutation were identified in 26%, 24% were PTEN low, 45% pHER2 high, and 47% patients had increased PI3K pathway activation (PTEN low and/or *PIK3CA* mutation). No significant correlations were observed between the clinicopathological variables and PIK3CA, PTEN, and pHER2 status. In both univariate and multivariate analyses, patients with *PIK3CA* mutations or high PI3K pathway activity had a significant worse OS despite adequate chemotherapy and trastuzumab.[52] Other studies have investigated the expression or activation of HER3 as a predictive or prognostic biomarker in patients with HER2-positive disease or those treated with pertuzumab. Chiu et al. reported that HER3 overexpression was identified in 10.0% of tumors in patients with breast cancer in a randomized study and was a clinically significant marker of reduced survival in such patients.[3] In a randomized phase 2 study, it was found that low HER3 levels corresponded with response to

pertuzumab in patients with platinum-resistant ovarian cancer.[4] In the NEOPSHERE and CLEOPATRA studies, the level of HER3 protein and mRNA did not correlate with treatment outcome. However, it has recently been reported that HER3 expression and activation is modulated in response to HER2, HER3, or PI3K pathway inhibition, which may not be reflected by the analysis of primary tumors but perhaps in biopsies obtained subsequent to commencement of anticancer therapy.[53] We propose to investigate these questions in a prospective fashion using both archival and frozen tumor samples before, during, and after therapy.

In addition, we propose to evaluate BEAMing (Beads, Emulsification, Amplification and Magnetics) as a technique to identify *PIK3CA mutations* in an ER-negative, HER2-positive early breast cancer population at baseline and during therapy, and assess if a correlation exists between mutation status and pCR. Because BEAMing is quantitative, we will also determine the correlation between pre and post treatment ptDNA blood levels with pCR. Traditional screening for mutations such as the *PIK3CA* mutation relies upon sequencing of archival tumor tissue. However, limitations exist with this method including the need to select specimens with a sufficient number of tumor cells, tumor heterogeneity, and variable quality of extracted and stored DNA that can interfere with accurate analyses. There is a growing need to develop accurate and less invasive methods of screening. Our group designed a prospective study to evaluate the feasibility of detecting hotspot *PIK3CA* mutations in both archival tumor and peripheral blood samples from patients with metastatic breast cancer using the BEAMing technique, and compared the results to those obtained by traditional sequencing of FFPE tissue. BEAMing was also performed on any remaining genomic DNA extracted from archival tissue samples used for sequencing.[53,54]

BEAMing of blood samples revealed that 28% (17/60) of patients had an identifiable hotspot *PIK3CA* mutation. Standard sequencing indicated that 27% (14/51) of tumors (12 primary, 2 metastatic) had *PIK3CA* mutation. Of the 51 samples for which both a tissue and plasma sample was available, 39 (76.5%) yielded the same results (either all wild type or same hotspot *PIK3CA* mutation identified) by both sequencing and BEAMing. The concordance between results obtained by BEAMing and sequencing of the same archival tissue sample was 100%. The investigators concluded that BEAMing was a non-invasive method of identifying these mutations. The 23.5% discordance observed between mutational status in archival tissue samples and blood samples drawn later in the disease course supports recent reports that *PIK3CA* mutational status changes during the progression of breast cancers. The study overall suggest that patients should optimally be selected for trials of PI3K inhibitors based on *PIK3CA* mutational status at time of enrollment, rather than on mutational status of archival tissue.

New unpublished preliminary data from Dr. Park's laboratory demonstrate that second generation digital PCR technologies, specifically droplet digital PCR (ddPCR, Bio-Rad) can also detect *PIK3CA* mutations in plasma of patients with early stage breast cancer. *PIK3CA* mutations were detected in 14 of 15 patients tested, including eight patients with Stage Ia (T1, N0, M0) disease, yielding a sensitivity of 93.3% and specificity of 100% PIK3CA mutations can therefore be reproducibly detected in pre op plasma samples (~2ml of plasma containing as few as 1000 genome equivalents) from early stage breast cancer patients with minimal tumor burden. In addition, newer

technologies using next generation sequencing will enable us to use all mutations/genomic rearrangements as "biomarkers" that can be qualitatively and quantitatively measured in plasma as a surrogate measure of disease burden and response to therapies. As this technology matures, we envision expanding this portion of the biomarker study to include other mutations (e.g. TP53) and genomic rearrangements and then coupling this with BEAMing and other digital PCR technologies to assess how changes in ptDNA correlate with pCR.

5.4.3 Immune enrichment signatures as a Biomarker of Response to Therapy

There has been a surge in interest in the role of the immune system, and agents targeting the immune system, in breast cancer. Breast carcinomas are often infiltrated by inflammatory cells, particularly macrophages and T lymphocytes. These inflammatory cells may represent a cellmediated immune response against the carcinoma. A recent study indicated that tumor-infiltrating CD8+ T lymphocytes have antitumor activity in breast cancer based on the observed favorable effect on patients' survival, suggesting a potential therapeutic strategy that can be harnessed.[55] The prognostic and predictive roles of tumor-infiltrating lymphocytes (TILs) were also assessed in a phase 3 adjuvant breast cancer trial. In the HER2-positive population, there was a significant interaction between increasing stromal lymphocytic infiltration (10% increments) and benefit with anthracycline-only chemotherapy.[56] The importance of the immune system in HER2-positive breast cancer was also determined in a meta-analysis (n >2,100) in which gene expression modules related to key biological processes in breast cancer were evaluated together with clinical factors and several prognostic signatures.[57] Multivariate analysis demonstrated that the tumor invasion and immune response modules were significantly associated with survival in the HER2-positive tumors. Finally, the adaptive immune system and immune checkpoints were found to be associated with response to chemotherapy and anti-HER2 therapy with pertuzumab and trastuzumab in the NeoSPHERE neoadjuvant study.[ref] High PD-L1 expression was consistently associated with lower pCR rate in all chemotherapy containing arms, with a similar trend in the anti-HER2 therapy alone arm. This further supports our understanding of the key role of the immune system in contributing to HER2-targeted antibody therapy on top of signaling inhibition.[58]

Our collaborators at the Mayo clinic have recently completed transcriptome analysis of 1282 patient samples from the N9831 adjuvant trastuzumab trial.[59] RNA was extracted and DASL microarray analysis was carried out on 433 patients who received chemotherapy alone and 849 who received chemotherapy plus trastuzumab. They identified 87 genes which are linked to immune functions and which have significant Cox HRs, using relapse free survival as a continuous variable end point. A modified surface mapping tool was used to assort HER2 tumors, based on the expression of these immune function genes, into two cohorts: those that are enriched for immune function genes and those that are not enriched. Both immune-enriched and immune notenriched tumors were equally responsive to chemotherapy alone (AC->T, HR=0.98, p=0.94). Among trastuzumab-treated patients, those with immune not-enriched tumors exhibited relapsefree similar to that observed in patients treated with chemotherapy alone (HR=01.00, p=0.99). However, trastuzumab-treated immune enriched tumors exhibited considerably long relapse free survival (HR 0.54, p<0.001), when compared to immune not-enriched tumors. It was concluded that there is a subset of HER2 tumors that manifest a highly activated immune status prior to onset of therapy, and it appears that most of the benefit from adjuvant trastuzumab resides

within this cohort of tumors. These data are consistent with the concept that immune feature, present in the tumor prior to onset of therapy, play an integral, perhaps essential role in the mechanism of action of trastuzumab.

Based upon the data described above, we hypothesize that tumors with an enhanced immune signature, defined as described above, are more likely to undergo pCR after neoadjuvant trastuzumab or pertuzumab. We anticipate that we will be able to use RNA from formalin-fixed, paraffin-embedded biopsy samples to measure the expression of the genes that we identified from the N9831 analysis. A surface mapping model will be used to assign tumors to immune enriched and not-enriched cohorts.

5.4.4 Change in Apoptosis and Proliferation as a Marker of Response to Therapy

A change in the proliferation index Ki67 has been evaluated predominantly in women receiving hormonal treatments for breast cancer. The IMPACT trialists evaluated short term changes in Ki67 during neoadjuvant treatment of primary ER-positive breast cancer with anastrozole or tamoxifen alone or combined. In the 88 ER-positive women who received tamoxifen for 2 weeks and underwent repeat Ki-67 assessment, a geometric mean percentage change in Ki-67 expression from baseline to 2 weeks of -59.5% (95% CI -68.5 to -47.9) was reported.[60] Importantly the IMPACT trialists have subsequently performed an exploratory analysis of their original data and have found that a higher Ki67 expression after 2 weeks of endocrine therapy was statistically significantly associated with a lower recurrence-free survival (P=0.004) whereas higher Ki67 expression at baseline was not.[61] In a study of the tumors from 104 postmenopausal women before and after 2 weeks treatment with anastrozole, luminal A and B tumors obtained similar benefit from therapy as measured by the proportional fall in Ki67 upon treatment. Tumors classified as basal and HER2-like showed poor reductions in Ki67 upon treatment.[62] Validation of these results in this study would be useful as future studies are planned.

Apoptosis and proliferation have also been examined in a HER2-overexpressing population. A neoadjuvant trial was performed in 35 patients with locally advanced HER2-overexpressing breast cancers who received weekly trastuzumab for the first 3 weeks, followed by a combination of trastuzumab and docetaxel for 12 weeks before surgery. Sequential core biopsies were assessed by immunohistochemistry for cell cycle and proliferation, apoptosis and survival, epidermal growth factor receptor, and total and p-HER-2. Apoptosis was significantly induced (median increase from 3.5% to 4.7) within week 1 consistent with a 35% increase above baseline. Tumors with high baseline Ki67 were less likely to respond.[63]

These results suggest that serial core biopsies can be collected from women with primary breast cancer who receive neoadjuvant hormonal therapy or chemotherapy and that early change in the apoptotic and proliferative indices in the tumors may predict response or resistance to the therapy. To our knowledge, early changes in markers of apoptosis and proliferation have not been evaluated in patients receiving dual anti-HER2 blockade with pertuzumab and trastuzumab. We will examine how baseline and change (day 15) in cleaved caspase 3 and Ki67 are associated with pCR.

5.5   Other Biomarkers of Response to Therapy

Ongoing discussions with our collaborators and Genentech will help us define other promising biomarkers for evaluation during this study, and may include correlation of changes in candidate gene expression or methylation with pCR.

5.5.1 Candidate Gene Expression Profile Analysis

The effectiveness of standard systemic therapies for breast cancer may vary among individuals. For example, the presence of the ER predicts response to endocrine therapy and perhaps relative resistance to chemotherapy. Overexpression or amplification of the HER2 oncogene may be associated with a relative resistance to endocrine manipulations and relative sensitivity to chemotherapy.[64] Review of the literature suggests that hundreds of individual genes or proteins may be associated with relative response or resistance to common chemotherapies in breast cancer. It is likely that other genes may confer relative resistance or sensitivity to specific treatments, but have not yet been identified. It is also possible that there are clusters of tens or hundreds of genes that may predict response or resistance to specific therapies.

Gene expression has been used by several investigators to classify breast neoplasms. It is also possible that chemosensitive vs. resistant tumors will have distinct gene profiles.[65,66] Baylor investigators evaluated molecular profiles of breast cancer responsive or resistant to 4 cycles of docetaxel administered every 3 weeks. These portraits were different than expected. [67] Similarly, MD Anderson investigators have developed a set of pharmacogenomic predictors to predict pCR to paclitaxel and anthracycline basal regimen.[68] Finally, the prognostic ability of the 70-gene Mammaprint assay was assessed in a HER2-positive population (heterogeneous for ER and node status) that did not receive adjuvant chemotherapy or trastuzumab (n=89), and 22% of them were classified as having as good prognosis (10-year DDFS, 84%) while the other 78% had a poor outcome (10-year DDFS 55%). [69]   It remains to be seen whether this assay may identify HER2postive patients that might be spared adjuvant chemotherapy and trastuzumab. Our hypothesis is that distinct baseline gene expression profiles can be correlated with response or resistance to preoperative chemotherapy. It is also possible that following therapy, distinct patterns can be observed in responsive or resistant tumors.

5.5.2 Methylation Profiles

In breast cancer, multiple genes are methylated, and thus silenced, compared to non-cancerous tissue. Gene methylation is likely related to cancer progression. HDAC inhibitors may also alleviate gene repression that is mediated through promoter hypermethylation. It is presumed that HDAC inhibitors can increase expression of the genes that are not methylated but may not induce the expression of hypermethylated genes. Some of the genes that are often methylated in breast cancer include growth promoting hormone receptors such as estrogen receptor alpha (ER), an important predictive factor of response to endocrine manipulations, and the retinoid receptor RARbeta, critical for cell differentiation. Other genes that are often hypermethylated in breast cancer include cyclin D, Twist, RASSF1A, APC, and HIN-1. Dr. Sukumar and other Johns Hopkins investigators have evaluated hypermethylation of a panel of seven genes using methylationspecific polymerase chain reaction (MSP) in a variety of breast tissues.[70-73] In invasive breast carcinomas, up to 100% of specimens contained at least one hypermethylated gene, 80%

contained two, and 60% contained three or more methylated genes.[73] In 44 ductal carcinoma in situ (DCIS) specimens, 95% had at least one methylated gene. In contrast, the percentage of women with benign breast disease having at least one methylated gene was only 15%. Only one of 8 reduction mammoplasty specimens contained hypermethylated genes. More recently, Dr. Sukumar and colleagues have developed a novel method, quantitative multiplex-methylation specific PCR (QMMSP) to accurately assess promoter hypermethylation for many genes simultaneously in small samples. QM-MSP is highly sensitive (1 in 10^4-10^5 copies of DNA) and linear over 5 orders of magnitude.[71] Because tumor samples may not be accessible in most patients with metastatic breast cancer, assays that can be conducted in blood samples are desirable.[71,72,74] Gene hypermethylation may be seen in circulating DNA and thus represent an attractive surrogate in the proposed study.[75]

5.5.3 Pharmacogenomics

The variability in response to drugs may be in part due to genetic polymorphism of receptors, transporters, or drug metabolizing enzymes. Single nucleotide polymorphisms (SNPs) have been identified in CYP450 enzymes and may be associated with a different ability to metabolize drugs. Due to the small sample size, we do not anticipate that correlation can be made between the presence of specific SNPs and response to therapy. However, if a SNP exists in an individual patient, it may help us to assess her individual response and toxicity. These results may also assist in design of future studies. To our knowledge no candidate genes are known which may predict response to anti-HER2 therapy at this time, with mixed data regarding the relevance of Fc receptor polymorphisms. At this time we plan to store samples obtained at baseline for potential future analyses in the event that further information becomes available from the literature and patients will be appropriately consented for same.

5.6   Study Rationale

The management of HER2-positive breast cancer has been revolutionized by the development of anti-HER2 therapies such as trastuzumab. Recent studies indicate that dual anti-HER2 therapy is more efficacious than single anti-HER2 therapy. NeoSphere was a Phase 2 randomized trial of preoperative systemic therapy comparing trastuzumab/docetaxel, trastuzumab/pertuzumab/docetaxel, trastuzumab/pertuzumab, and pertuzumab/docetaxel. The pCR rates were 29%, 46%, 17% and 24% respectively and all arms had acceptable toxicity. The fact that a large proportion of women treated with biological therapy alone can obtain a pCR has caused great excitement in the breast oncology community. There is a clear need to determine who these patients are upfront as they could potentially be spared the added toxicity of chemotherapy.

The NeoALLTO PET substudy has indicated that metabolic changes in the primary tumor of patients with invasive operable breast cancer treated with anti-HER2 therapies can be detected as early as 2 weeks of treatment. Pathologic CR rates were found to be twice as high in patients who are FDG-PET/CT responders compared to non-responders according to EORTC criteria. We expect, based on the NeoALLTO data, that early change in SUV on FDG-PET will be associated with response to pertuzumab/trastuzumab in patients with primary operable HER2-positive breast cancer. We propose to evaluate for the first time the correlation between early changes in SUV and pCR in men and women with ER-negative HER2-positive breast cancer receiving pertuzumab and trastuzumab alone. Based on the low pCR rate observed in those with ER-positive disease, we

propose to investigate this question in an ER-negative population alone. We anticipate that our findings will mirror the NeoALLTO PET substudy results but we hope to identify an SUV decline that more accurately predicts pCR. Data from TBCRC006 and the ongoing TBCRC023 trial indicate that the administration of dual anti-HER2 therapy alone is feasible in the pre-operative setting and is associated with pCR in a proportion of women. Our study will build on this experience by attempting to define early in the treatment paradigm the patients who are likely to obtain a pCR using changes in SUV on PET. The results from this phase 2 biomarker study, if promising, will be used to then plan a randomized study using a predefined cut point for SUV decline such that we can further attempt to identify a group of individuals with HER2-positive early breast cancer who do not require cytotoxic chemotherapy in addition to anti-HER2 agents. We believe this will be of great interest to breast cancer patients and this has been echoed by the TBCRC advocates in recent discussions.

In addition, we aim to identify plasma and tissue biomarkers that may predict response to pertuzumab and trastuzumab with a focus on the PI3K pathway. We propose to evaluate BEAMing as a technique to identify *PIK3CA* mutations in plasma in a HER2-positive, ER < 10% early breast cancer population at baseline and during therapy, and assess if a correlation exists between mutation status and pCR. We anticipate that in the future a combination of early changes in SUV on PET and tissue biomarkers will provide the optimal way of identifying those patients who do or not need chemotherapy or targeted therapies.

6. Patient Population

6.1 Inclusion Criteria

6.1.1 Female and male patients, 18 years old or older

6.1.2 Histologically proven infiltrating carcinoma of the breast on core needle biopsy that is:

- ER/PR ≤10% staining by IHC

- HER2 positive – IHC 3+, ISH ≥2.0, or average HER2 copy number ≥6.0 signals per cell or per current ASCO-CAP (American Society of Clinical Oncology – College of American Pathologists) or NCCN (National Comprehensive Cancer Network) guidelines.

Note: All histological diagnostic material should be reviewed at enrolling institution as required per local standards.

6.1.3 Unresected, untreated breast cancer that meets one of the following clinical stages (see Appendix A):

- T2, T3, or T4a-c lesion, any N, M0

Note: Patients with inflammatory breast cancer (T4d) are not eligible. Bilateral cancers are permitted with approval of the Protocol Chair. Participants with clinically evaluable disease will be followed for response by clinical examination; measurable disease is not required for participation.

6.1.4 ECOG performance status 0-1 (Appendix B)

6.1.5 Adequate organ function as follows:

- Absolute neutrophil count (ANC) $\geq$ 1,500/mm^3
- Platelet count $\geq$ 100,000/mm^3
- Hemoglobin $\geq$ 10 g/dL
- Creatinine $\leq$ 1.5 times the upper limit of normal with creatinine clearance $\geq$ 50 mL/min using the Modified Cockcroft-Gault method
- Bilirubin (total) $\leq$ 1.5 times upper limit normal (with exception of Gilberts syndrome)
- AST(SGOT), ALT(SGPT), and alkaline phosphatase $\leq$ 2 times the upper limit of normal

Note: Exceptions to lab parameters may be allowed with approval of the Protocol Chair

6.1.6 Adequate cardiac function as defined by LVEF $\geq$ 50% on echocardiogram or multi-gated acquisition scan (MUGA)

6.1.7 Able and amenable to baseline and follow-up PET/CT imaging and study-specific biopsy procedures.

> Note: If there are any imaging concerns that the patient may not be suitable for quantitative PET/CT (e.g., a metallic device directly overlies the breast), discussion with the local and central radiologists is required to confirm eligibility for the trial. Also, it is expected that subjects have all PET/CT imaging done on pre-qualified machines for the study; if baseline imaging done on another machine, please contact the Protocol Chair/designee for guidance prior to confirming eligibility.

6.1.8 The patient, if of childbearing potential, is willing to use effective, non-hormonal contraception while on treatment and for at least 6 months following the last dose of therapy.

6.1.9 Patient understands the study regimen, its requirements, risks, and discomforts, and is able and willing to sign an informed consent form.

6.2 Exclusion Criteria

6.2.1 Received prior or ongoing local (e.g radiation) or systemic treatment (chemotherapy or endocrine therapy) for the current breast cancer. Patients who received tamoxifen or raloxifene or another agent for prevention of breast cancer may be included as long as the patient has discontinued the treatment at least one month prior to baseline study biopsy.

6.2.2 Systemic treatment for prior cancer within the last 5 years, with the exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin.

6.2.3 Women who are pregnant or nursing

6.2.4 Current use of any investigational agents

6.2.5 Known hypersensitivity to trastuzumab or pertuzumab

6.2.6 Any medical condition that in the opinion of the investigator puts the patient at risk of potentially serious complications while on this therapy. Specifically, uncontrolled hypertension (systolic >150 and/or diastolic >100), unstable angina, congestive heart failure of any New York Heart Association (NYHA) classification, serious cardiac arrhythmia requiring treatment (exception: atrial fibrillation, paroxysmal supraventricular tachycardia), history of myocardial infarction within 6 months of enrollment.

6.3 Inclusion of Women and Minorities

Individuals of all races and ethnic groups are eligible for this trial. There is no bias towards age or race in the clinical trial outlined. This trial is open to the accrual of men and women.

7. Study Design and Treatment Plan

NOTE: If at any time the Protocol Chair is not available, the Protocol Co-Chair may provide oversight on study questions. The Study Liaison may also be contacted for guidance.

7.1   Recruitment

Patients will be recruited through the breast cancer clinics at each of the participating centers. Local institutions will assign a participant subject number at the time of consent which includes a site designation number and sequential subject number (e.g., 1-001, 1-002, etc.)

7.2   Determination of Eligibility

After eligibility is established at the participating institution, the study staff will register participants with the Coordinating Center at Johns Hopkins. The following is required to be submitted for successful registration:

- Registration forms
- Copy of subject consent
- Copies of the following source documents:
    - Diagnostic pathology report(s), including receptor status
    - Laboratory reports as per the eligibility criteria and pregnancy test, if applicable
    - Other documents, if requested.

Upon review of the registration documents, the Coordinating Center at Johns Hopkins will confirm successful registration by return email and/or fax to the local study team/designee.

Study treatment cannot begin until the patient is successfully registered with the Coordinating Center. In the event a subject's day 1 is delayed for unforeseeable reasons and a baseline assessment(s) fall outside the intended window, it is expected that these be repeated and reviewed by the Coordinating Center prior to treatment unless explicit approval is given by the Protocol Chair or her designee.

Subjects who sign a consent form, but do not initiate protocol treatment for any reason (e.g., subjects who are screen failures), will be replaced and will not count towards our accrual goal.

7.3   Preoperative Pertuzumab and Trastuzumab

7.3.1 Overview

A total of 4 cycles of combination pertuzumab and trastuzumab will be administered prior to definitive breast surgery.

A cycle where treatment is held may be made up if treatment is being tolerated. Treatment will be administered on an outpatient basis.

The doses of pertuzumab and trastuzumab do not need to be recalculated unless the body weight has changed by more than ±10% from baseline.

Note: Changes in the infusion times for pertuzumab and trastuzumab noted below may be made in the event of toxicity (e.g., extension of the infusion timing) or per institutional standard – without contacting the Protocol Chair.

7.3.2 Pertuzumab

Pertuzumab will be administered before trastuzumab every 3 weeks (840 mg as a loading dose, then 420 mg every 3 weeks) intravenously (IV) for 4 doses. The initial dose should be administered over about 60 minutes and subsequent administration may be infused over about 30-60 minutes.

Due to possibility of infusion-associated reactions (IARs) with pertuzumab, observation of the patient during and for 60 minutes after the first infusion and during and for 30 minutes following subsequent infusions is recommended (see Section 7.4.3.1).

7.3.3 Trastuzumab

Trastuzumab will be administered after pertuzumab every 3 weeks (8 mg/kg loading dose, then 6 mg/kg every 3 weeks) IV for 4 doses. The initial dose should be administered over 90 minutes. If this is well tolerated, subsequent administration may be infused over about 30 minutes. DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.

7.3.4 Premedications

Premedication regimens standard to each institution are suggested. This may include acetaminophen and diphenhydramine, for example. Each institution may employ the premedication regimens considered routine in their practices. The regimens used should be clearly noted in the medical record and research file.

7.3.5 Randomization and Blinding

This is an open-label, non-randomized phase 2 study.

7.3.6 Additional Preoperative Treatment

It is preferred that participants proceed to definitive surgery at the end of study therapy. The primary endpoint of this trial is correlation of SUVmax change with pCR. The option to administer additional treatment prior to surgery should be reserved for (a) disease progression on study treatment, or (b) cases where the investigator and/or patient is concerned about a possible incomplete clinical response AND residual disease is histologically confirmed as outlined in the study schema. **All cases should be discussed with the Protocol Chair prior to obtaining the post-study treatment biopsy (Section 8.0) and commencing additional therapy to ensure compliance with the protocol.**

In subjects who consider additional treatment, a tumor biopsy and blood sample will be required prior to initiating the treatment. **In scenario b), the biopsy must be reviewed both locally and by the Study Chair or designee to confirm presence of residual disease prior to initiation of**

additional treatment; it is requested that additional cores be collected at the same time for research purposes (Section 8.12). Documentation of residual disease by imaging to confirm response may be considered at the discretion of the treating team (standard of care procedure) to document need for additional treatment (Section 8.9).

It is recommended that additional preoperative treatment comprise a standard preoperative regimen of chemotherapy and HER2-directed therapy per NCCN guidelines. No other treatment options should be considered after study therapy and before surgery.

Any further monitoring and tests after completion of the study treatment and during the additional preoperative treatment are at the discretion of the treating team. A final toxicity check 30 days after the last dose of study treatment will be collected; however, only adverse events believed to be related to the study treatment or interventions are collected during this time period.

Upon completion of additional treatment, all post-treatment/surgical research tissue and blood samples are still required to be collected. Please see the Study Calendar (Section 8) for additional information.

7.4   Dose Modifications

Dose modification of pertuzumab and trastuzumab is not permitted.

If possible, symptoms should be managed symptomatically. In case of toxicity, appropriate medical treatment should be used (including anti-emetics for nausea/vomiting, anti-diarrheals for diarrhea, etc.). No dose escalation is planned for this study.

Toxicity assessments will be done using NCI Common Terminology Criteria for Adverse Events (CTCAE v 4.0) which is available at http://ctep.cancer.gov/reporting/ctc.html).

7.4.1 Re-Treatment Criteria

A new cycle of treatment may begin when the following are met:
- All non-hematologic toxicity improved to Grade ≤ 2 (or to baseline)
- ANC $\geq 1,000/mm^3$ and platelets $\geq 75,000/mm^3$

For any event, which is apparent at baseline, the dose delays will apply according to the corresponding shift in toxicity grade, if the investigator feels it is appropriate. (e.g. if a patient has grade 1 asthenia at baseline which increases to grade 2 during treatment, this will be considered as a shift of 1 grade and treated as a grade 1 toxicity for dose delay purposes).

A cycle may be delayed as noted below. A total of 4 cycles of treatment should be administered whenever possible.

For delayed or missed doses, if the time between 2 sequential infusions is less than 6 weeks, the 420 mg IV dose of pertuzumab should be administered. Do not wait until the next planned dose.

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

Pertuzumab should be withheld or discontinued if trastuzumab treatment is withheld or discontinued.

For delayed or missed doses, if the time between 2 sequential infusions is less than or equal to 4 weeks, the 6 mg/kg maintenance dose of trastuzumab will be administered over about 30 minutes. If the time between 2 sequential infusions is 4 weeks or more, the 8 mg/kg loading dose of trastuzumab will be administered over 90 minutes, followed every 3 weeks thereafter by a dose of 6mg/kg administered over 30 minutes.

7.4.2 Toxicity Management

NOTE: Dose delays and recovery windows refer to date of planned dose, not since last dose. For example: if cycle 2 was to start on January 1st, but is delayed due to grade 3 ANC; you may hold treatment up to additional 3 weeks from the originally planned cycle 2 day 1.

Figure 1: Actions to be Taken in Case of Pertuzumab and Trastuzumab Hematologic Toxicity

Hematologic toxicity related to study treatment	Action
ANC	Grade 1 or 2: Continue with study treatment Grade 3 or 4: Hold study treatment Toxicity resolves to Grade ≤2 within 3 weeks, resume study treatment Toxicity does NOT resolve to Grade ≤2 within 3 weeks, discontinue treatment, or discuss with Protocol Chair
Platelets	Grade 1: Continue with study treatment Grade 2: Consider holding treatment per grade 3 or 4 guidelines, or continue at treating physician discretion Grade 3 or 4: Hold study treatment Toxicity resolves to Grade ≤2 within 3 weeks, resume study treatment Toxicity does NOT resolve to Grade ≤2 within 3 weeks, discontinue treatment, or discuss with Protocol Chair

Hemoglobin	Grade 1 or 2: Continue with study treatment Grade 3 or 4: Hold treatment until resolution per below or consider transfusion Toxicity resolves to Grade ≤2 within 3 weeks, resume study treatment Toxicity does NOT resolve to Grade ≤2 within 3 weeks, discontinue treatment, or discuss with Protocol Chair.

Figure 2: Actions to be Taken in Case of Pertuzumab and Trastuzumab Non-Hematologic Toxicity

Non-hematologic toxicity related to study treatment, excluding cardiac toxicity	Action
1. Grade 1 or 2	Continue with study treatment
2. Grade 3 or 4	Hold study treatment
	Toxicity resolves to Grade ≤2 within 3 weeks, resume study treatment Toxicity does NOT resolve to Grade ≤2 within 3 weeks, discontinue treatment, or discuss with Protocol Chair
3. Recurrence of Grade 3 or 4 toxicity upon re-challenge	Discontinue treatment

Figure 3: Actions to be Taken in Case of Pertuzumab and Trastuzumab Cardiac Toxicity

Cardiac toxicity related to study treatment	Action
1. Asymptomatic drop in LVEF or symptomatic congestive heart failure	Hold study treatment. Monitor and continue per Figure 4
2. Other cardiac toxicities not covered by this figure.	Follow rules 1 and 2 above for non-hematologic toxicity

7.4.3 Important Toxicities

7.4.3.1 Infusion-Associated Reactions (IAR)

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

assessment should be based on the severity of the preceding reaction and response to administered treatment for the adverse reaction.

7.4.3.2 Serious Infusion-Associated Events

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

7.4.3.3 Respiratory Symptoms

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

7.4.3.4 Left Ventricular Dysfunction

Decreases in LVEF have been reported with drugs that block HER2 activity. Trastuzumab and pertuzumab both target HER2, thus there is a risk of cardiac dysfunction with these agents. In the CLEOPATRA pivotal trial, pertuzumab in combination with trastuzumab and docetaxel was not associated with increases in the incidence of symptomatic LVSD or decreases in LVEF compared with placebo in combination with trastuzumab and docetaxel.[2] Pertuzumab combined with trastuzumab and chemotherapy did not result in any significantly greater incidence of symptomatic LVSD or decreases in LVEF than trastuzumab and chemotherapy in patients with early breast cancer.[3] However, in the pivotal MBC trial a greater proportion of patients who developed symptomatic LVSD had received prior anthracyclines and/or radiotherapy compared to the proportion of patients receiving prior anthracyclines and/or radiotherapy in the overall pertuzumab-treated population.[2] Therefore, patients who have received prior anthracyclines or prior radiotherapy to the chest area may be at higher risk of decreased LVEF.

Pertuzumab has not been studied in patients with: a pretreatment LVEF value of $\leq 50\%$; a prior history of CHF; decreases in LVEF to <50% during prior trastuzumab adjuvant therapy; conditions that could impair left ventricular function such as uncontrolled hypertension, recent myocardial infarction, serious cardiac arrhythmia requiring treatment or a cumulative prior anthracycline exposure to > 360mg/m^2 of doxorubicin or its equivalent.

7.4.3.5 Management of Cardiac Safety

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

It is recommended that patients should have cardiac monitoring at regular intervals (e.g., every three months) during treatment with pertuzumab and trastuzumab. As part of this study, cardiac monitoring will occur at baseline and at conclusion of study treatment (i.e., after about 3 months); or additionally if required to monitor toxicity. ECHO or MUGA scans should be scheduled at the same radiology facility where the patient's baseline ECHO or MUGA was conducted. When a cardiac event occurs, the Cardiac Report Form must be submitted within 14 days of learning of the event.

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

Pertuzumab and trastuzumab should be discontinued in any patient who develops clinical signs and symptoms suggesting CHF. CHF should be treated and monitored according to standard medical practice. At present, there are inadequate data available to assess the prognostic significance of asymptomatic drops in LVEF.

Figure 4: Algorithm for Continuation/Discontinuation of Study Therapy in Asymptomatic Patients Based on LVEF Assessment
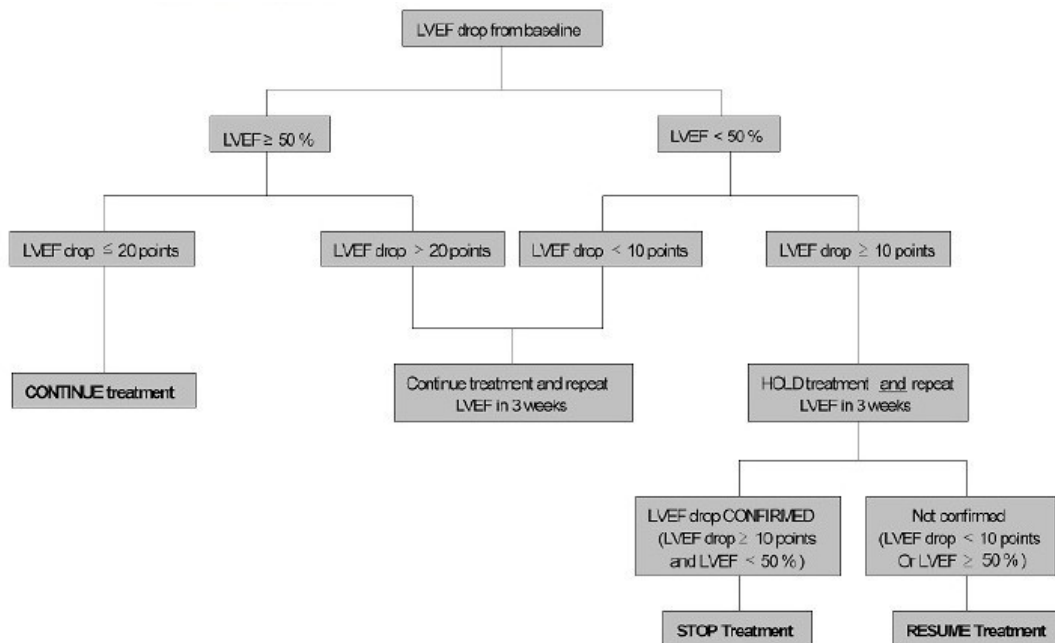
Note: The LVEF ≥50% and <50% refers to the current LVEF. For example: if the current LVEF is <50%, but did not drop at least 10 points from baseline, continue treatment and repeat LVEF in 3 weeks. If repeat LVEF still has not dropped at least 10 points, continue treatment and repeat LVEF at next scheduled time point. If LVEF has dropped ≥10 points on repeat, continue treatment and repeat

LVEF in another 3 weeks. If LVEF at that time is confirmed as having dropped ≥10 points AND LVEF is <50%, stop treatment.

The Protocol Chair must be notified if an assessment of LVEF is performed prior to the planned assessment after 12 weeks of therapy so guidance can be given if necessary.

7.4.3.6 EGFR-Associated Toxicities

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

7.4.3.7 Diarrhea

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

7.4.3.8 Rash

Rash has also been observed with EGFR inhibitors, mostly of mild to moderate intensity. Rash has been observed in approximately 17% of patients receiving pertuzumab in Phase 2 single-agent studies and up to 73% of patients in combination studies. The rash was generally of CTCAE Grade 1 or 2 in severity. Treatment recommendations for EGFR-associated rash include topical or oral antibiotics, topical pimecrolimus, topical or (for severe reactions) systemic steroids. These agents may be used in patients experiencing pertuzumab-related rash, as clinically indicated, although they have not been studied in this context.

7.4.4 Additional Preoperative Treatment

Standard institutional guidelines should be used for dose modifications for additional standard treatment received prior to surgery. It should be noted that for patients in whom taxane is added to pertuzumab/trastuzumab, a clinical ECHO or MUGA should be performed every 6 weeks while receiving that combination as was done in other preoperative studies using the combination.

7.4.5 Special Considerations

- For any grade 4 toxicity that recurs despite prophylaxis or dose reduction, study treatment should be discontinued.

- Any consideration to modification of the above dose delay guidelines should be discussed with the Protocol Chair for approval in advance.

7.5   Concomitant and Supportive Therapy

7.5.1 General

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the subject are allowed, provided their use is documented in the medical records. The administration of any other therapies intended to treat the primary condition including chemotherapy and biologic agents is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed.

7.5.2 Other

No other targeted therapy, chemotherapy, anti-estrogen therapy, radiation therapy, or investigational systemic therapy is allowed concomitant with study treatment. Subjects may continue on bisphosphonates. Initiation of bisphosphonate therapy while on study treatment must be authorized by the Protocol Chair.

7.6   Surgical Evaluations of Participants

7.6.1 Marker Placement

A titanium marker or "clip" should be placed at the tumor site(s) prior to starting treatment in patients who may be a candidates for breast conservation surgery. This will identify the tumor location(s) for the surgical procedure in the event that there is a complete response to preoperative treatment.

7.6.2 Evaluation of Axillary Lymph Nodes

7.6.2.1 Pre-Treatment

The standard of care for the institution should be followed (e.g., pre-neoadjuvant therapy sentinel lymph node mapping/lymph node biopsy for clinically node negative patients is generally recommended at Johns Hopkins and should be performed on all patients; such evaluations are not required for subjects at other sites).

• Clinical N0 Disease
    For patients with a clinical N0 disease, it is recommended that sentinel node mapping be done at baseline, but this is optional. Patients with negative lymph nodes should not require further evaluation at the time of definitive surgery. However, patients with a positive fine needle aspiration or a sentinel node should undergo an axillary node dissection at the time of definitive surgical procedure.

    At the time of sentinel lymph node mapping, the surgeon may obtain a biopsy of the sentinel node which will be snap frozen and be evaluated immediately by the pathologist for presence or absence of micrometastases. If the frozen biopsy specimen does not show micrometastases, the node will be removed and no further evaluation will be required unless the permanent section reveals micrometastases. If the frozen biopsy specimen reveals micrometastases, the surgeon will either 1) perform an axillary node dissection, or

2) will mark the node and no further evaluation will be done until the definitive surgical procedure when a lymph node dissection will be done.

- Clinical N1, N2 or N3 Disease

  For patients with a clinical N1, N2, or N3 disease, it is recommended that a fine needle aspiration (FNA) or a core biopsy is obtained prior to starting therapy to document the status of the node. Following the biopsy, decisions regarding sentinel node mapping for patients with N1 disease will be made at the discretion of the treating surgeon.

7.6.2.2 Post-Treatment

Patients with a positive sentinel lymph node or a positive sentinel lymph node biopsy prior to starting treatment, patients who underwent an unsuccessful sentinel node mapping, or those who did not undergo sentinel lymph node mapping at baseline should generally undergo axillary lymph node dissection at the time of the definitive surgical procedure.

Patients who had a positive FNA prior to treatment may undergo a sentinel node mapping at the time of breast surgery. Patients whose sentinel node is negative may or may not undergo node dissection at discretion of the surgeon. However, it is highly recommended that such patients will undergo node dissection. Patients whose sentinel node is positive should be recommended axillary lymph node dissection. If a patient with an FNA-positive node at baseline does not go axillary node dissection documentations should be made regarding the reason.

7.6.3 Breast Surgery

Following study therapy, patients will undergo breast conserving surgery or a mastectomy at the discretion of the treating surgeon. It is recommended that the definitive surgery take place 2-4 weeks after the last dose of study medication.

7.7   Additional Treatment

7.7.1 Chemotherapy, Pertuzumab and Trastuzumab

Additional standard preoperative treatment with pertuzumab, trastuzumab, and taxane per standard of care may be considered in cases of disease progression or incomplete clinical response to study treatment.

Postoperative chemotherapy and trastuzumab per standard of care (NCCN guidelines) is recommended for those who are medically fit for same and may be administered at the discretion of the treating team. It is preferred that an anthracycline-taxane based adjuvant chemotherapy be administered such as AC (adriamycin/cyclophosphamide) followed by paclitaxel plus trastuzumab per institutional preferences and per physician discretion; if not administered after the study treatment and prior to definitive surgery. An alternative for those with pre-existing cardiac risk factors is the docetaxel, carboplatin, trastuzumab regimen per the BCIRG006 study.[13] Trastuzumab should be administered to complete one year of therapy per standard practice.

7.7.2 Radiation Therapy

Radiation therapy will be administered at the conclusion of all protocol treatment, per institutional standards.

7.7.3 Hormonal Therapy

Adjuvant hormonal therapy will be administered to patients with ER 1-9% after completion of adjuvant chemotherapy and local therapy, per standard of care and physician discretion.

7.8   Evaluation of Long Term Outcomes

The subject's medical record will be reviewed approximately every 6 months from the start of therapy for information regarding recurrence of the breast cancer (local or systemic), development of a new cancer, or death. (Note: Only those subjects who initiate protocol treatment will be followed.)

In the event that a subject does not continue her post-study care at the institution, every attempt will be made to collect this information either by direct contact or through communication with her outside physician(s).

7.9   Discontinuation and Withdrawal of Subjects

All patients who initiate protocol treatment will be included in the overall evaluation of response (intent-to-treat analysis). All reasons for discontinuation of therapy should be documented clearly in the medical record.

If a subject discontinues or withdraws from the study, every attempt will be made to get a tissue/tumor biopsy and study bloods if the subject is able and willing to do so.

7.9.1 Discontinuation of Treatment

The reasons for discontinuation or protocol treatment include:

- Evidence of disease progression during study therapy at the discretion of the treating investigator and after discussion with the Protocol Chair (see Section 7.3.6).

- Non-compliance with the study protocol; including, but not limited to not attending the majority of scheduled visits. The Protocol Chair will determine when non-compliance should lead to removal from study.

- Unacceptable major toxicity.

- Intercurrent illness or condition that would, in the judgment of the treating investigator, affect assessment of clinical status to a significant degree or require discontinuation of study treatment.

- At subject's own request. Note: The reason for discontinuation from the study must be documented. The patients will be included in the overall evaluation of response (intent-totreat analysis) if any protocol therapy was administered prior to withdrawal.

- Study is closed for any reason (e.g. new information shows that the patient's welfare would be at risk if she continued study treatment).

7.9.2 Withdrawal from Study

The reasons for withdrawal from the study include:

- Subject withdraws consent for follow-up.

- Subject is lost to follow-up.

- Study is terminated for any reason.

7.10 Additional Information

Subjects may be given parking vouchers (if applicable) to cover parking costs during the study, depending on the preferences of each participating institution. No other subject remuneration is planned.

8. Study Calendar

Procedure	Baseline[1]	Cycle 1 Day 1	Cycle 1 Day 15 (-0/+3 days)	Every 3 weeks[&] (Cycles 2-4)	Post-Study Tx[2]	Post-Surgery
CLINICAL ASSESSMENTS/TESTS:						
Physical Exam	X			X	X	X
Clinical Tumor Measurements[3]	X			X	X	
ECOG PS, Vital Signs (Height, baseline)[4]	X			X		
CBC with diff, platelets	X[#]	X[#]		X	X	
Chemistries[5]	X[#]	X[#]		X	X	
Electrocardiogram (ECG)[6]	X					
MUGA/Echocardiogram	X				X	
Pregnancy Test (Blood or Urine)[7]	X[*]	X[*]				
Axillary Nodal Evaluation[8]	X					
Breast Imaging[9]	X				X	
TREATMENT:						
Trastuzumab[10]		X		X		
Pertuzumab[10]		X		X		
Additional preoperative treatment[11]					X	
CORRELATIVE BLOOD/TISSUE STUDIES:[12]						
Pharmacogenomics (whole blood)	X					
Serum, plasma	X		X		X	X
PET/CT Scan	X		X			
Image guided breast tumor biopsy	X		X		X	
Tumor sample- diagnostic/archival	X					
Tumor sample- from surgery					X	
OTHER ASSESSMENTS:						
Concomitant Medication Review	X			X	X	
Symptom/Toxicity Assessment[13]	X			X	X	X
Definitive Surgery[14]					X	
Follow-up/Survival (q6 months)[15]						X

[#] Required ≤ 14 days prior to first treatment (i.e., day 1); baseline labs may be used if within window. [*] Required ≤ 7 days prior to first treatment (i.e., day 1) unless otherwise noted. [&] All assessments for cycles 2-4 required ≤ 3 days prior to each specified treatment; the same window applies to the start of each cycle due to scheduling delays outside of those related to adverse events (exceptions may be made to extend this window, with approval by the Protocol Chair/designee).

Note: The schedule should be followed as closely as is realistically possible; however, the schedule may be modified due to problems such as scheduling delays or conflicts (e.g., clinic closure, poor weather conditions, vacations, etc.) with the guidance of the Protocol Chair/designee, as appropriate, and will not be reportable as a deviation unless the endpoints of the study are affected.

8.0 Study Calendar *(continued)*

1. Baseline assessments are required within 28 days of first treatment with exception of axillary nodal evaluation and breast imaging, if performed, where no window applies, and unless otherwise noted.
2. Post-study treatment assessments may be done as soon as 2 weeks, and preferably within 4 weeks, after the last dose of study treatment (pertuzumab/pertuzumab); these must take place prior to additional standard preoperative treatment (if applicable) or definitive surgery.
3. Any patient with clinically evaluable disease should have tumor measurements completed at baseline, prior to each cycle of treatment, and post-study treatment (including after additional standard preoperative treatment, if applicable).
4. Height at baseline only.
5. Chemistries to include measurement of sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total bilirubin, calcium, total protein, albumin, AST, ALT, and alkaline phosphatase.
6. ECG may be repeated at the discretion of the treating team at any time during or following treatment.
7. Pregnancy test (serum or urine) is required for women of childbearing potential; $\leqslant$ 7 days before initiating study therapy and must be negative to commence the study.
8. Axillary nodal evaluation is recommended prior to initiation of preoperative treatment and can be performed as per standard practice at each institution (no baseline window applies to this assessment).
9. Standard of care imaging may be performed at baseline and also to confirm response prior to additional preoperative treatment, and may include mammogram, ultrasound, magnetic resonance imaging (MRI) and/or other, at the discretion of the treating team.
10. Treatment will consist of Pertuzumab and Trastuzumab (both administered every 3 weeks, IV).
11. For cases of progression or residual clinical disease additional standard preoperative treatment should comprise of a standard preoperative regimen of chemotherapy and HER2-directed therapy per NCCN guidelines In these cases, all items under the "Post-Study Tx" must be done prior to start of additional treatment, including biopsy.
12. Correlative blood/tissue studies:
    - Blood for serum, plasma, and pharmacogenomics (baseline only) samples will be collected at baseline, on day 15 (window days 15-18), post-completion of study treatment (and prior to surgery/additional standard preoperative treatment), and about 1-4 weeks following the surgical procedure prior to initiation of any postoperative treatment. In patients who receive additional preoperative treatment, a sample is also requested at the completion of the additional treatment and prior to the surgical procedure.
    - PET scan should be performed prior to tumor biopsy at baseline and on day 15 (window days 15-18); additional window (>28 days) may be permitted for baseline PET with advance approval from Protocol Chair.
    - Tumor biopsy at baseline and on day 15 (window days 15-18). In patients who receive additional preoperative treatment, a biopsy is also required at the completion of study treatment and prior to the initiation of additional treatment. Note: A separate tumor biopsy is not needed on the day of surgery; tissue will be taken from the surgical specimen. Tissue from the original diagnostic and surgical procedures will also be collected for study tests.
    - All correlative blood/tissue and PET studies should be performed on the same day.
    - The diagnostic/archival tumor samples should only be submitted upon request from coordinating center.

13. All subjects must have a final toxicity assessment at least 30 days following the last dose of study drug. In subjects who initiate additional preoperative treatment and/or have surgery during the 30 day time period, only toxicities believe to be related to the study treatment/interventions will be captured.

14. Definitive surgery consists of lumpectomy or mastectomy, with or without nodal evaluation (i.e., sentinel node and/or axillary node dissection) and will occur after completion of study treatment with or without additional preoperative treatment.

15. Subjects will be followed approximately every 6 months after completion of study treatment for disease status and survival.

9. Pharmaceutical Information

9.1   Pertuzumab (PERJETA®)

9.1.1 Classification

Pertuzumab is a recombinant, humanized monoclonal antibody based on the human IgG1 (κ) framework sequences and consists of two heavy chains (449 residues) and two light chains (214 residues). Like trastuzumab, pertuzumab is directed against the extracellular domain of HER2. However, it differs from trastuzumab in the epitope-binding regions of the light chain (12 amino acid differences) and heavy chain (29 amino acid differences). As a result, pertuzumab binds to an epitope within what is known as subdomain 2 of HER2 while the epitope for trastuzumab is localized to subdomain 4.

9.1.2 Formulation

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

9.1.3 Preparation and Administration

Prepare the solution for infusion, using aseptic technique, as follows:
- Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.
- Withdraw the appropriate volume of pertuzumab liquid concentrate from the vial(s).
- Dilute into the 250 mL 0.9% sodium chloride PVC or non-PVC polyolefin infusion bags. (Note: Dextrose (5%) solution should not be used to dilute

pertuzumab.) Mix diluted solution by gentle inversion. Do not shake.
Administer immediately once prepared.

9.1.4 Storage and Stability

Upon receipt, pertuzumab vials are to be refrigerated at 2°C–8°C (36°F–46°F) until use. Pertuzumab vials may not be used beyond the expiration date provided by the manufacturer. Because the formulation does not contain a preservative, the vial seal may only be punctured once. Any remaining solution should be discarded. Vial contents should be protected from light, and should not be frozen. The solution of pertuzumab for infusion, diluted in PVC or non-PVC polyolefin bags containing 0.9% Sodium Chloride Injection, USP, may be stored for up to 24 hours

prior to use. Diluted pertuzumab has been shown to be stable for up to 24 hours at a temperature range of 2°C–25°C. However, since diluted pertuzumab contains no preservative, the diluted solution should be stored refrigerated (2°C–8°C).

9.1.5 Supply

Pertuzumab will be supplied by Genentech/F Hoffman La Roche Inc., free of charge to subjects for the duration of participation in the study regimen. Sites will order supplies of pertuzumab directly from Genentech or a central distribution group, as directed by the Coordinating Center, during the study. NOTE: Pertuzumab supply will be commercial in participants who receive additional standard preoperative treatment.

9.1.6 Adverse Effects

The package insert should be consulted for the most current and complete information.

9.2   Trastuzumab (HERCEPTIN®)

9.2.1 Classification

Trastuzumab is a recombinant humanized monoclonal antibody, produced by Chinese hamster ovary cells in culture.

9.2.2 Formulation

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each vial of trastuzumab contains 400 mg of tratuzumab, 9.9 mg of L-histidine HCl, 6.4 mg of L-histadine, 400 mg of □,□-trehalose dihydrate, and 1.8 mg of polysorbate 20, USP.

9.2.3 Preparation and Administration

Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI) USP, containing 1.1% benzyl alcohol as a preservative, yields 21 mL of a multidose solution containing 21 mg/mL trastuzumab, at a pH of ~6..

9.2.4 Storage and Stability

Vials of trastuzumab are stable at 2□C–8□C (36□F–46□F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial.

9.2.5 Supply

Trastuzumab will be obtained from commercial supply and the patient or patient's insurance company will be responsible for the costs.

9.2.6 Adverse effects

Please see the trastuzumab Package Insert for more details on the known precautions, warnings, and adverse reactions of trastuzumab.

9.2.7 Other

The package insert should be consulted for the most current and complete information.

9.3   Docetaxel (Taxotere®)

Additional preoperative treatment with pertuzumab, trastuzumab, and docetaxel (or other taxane per institutional standard after discussion with Study Chair) will follow current package and institutional guidelines. The treatment and dose modifications guidelines will not be mandated by this protocol.

9.4   Drug Accountability

The research pharmacy will keep records for medication receipts, dispensation, and destruction as per standard practice at each institution for supplied agents.

10. Measurement of Effect

10.1 Pathologic Response Determination

We will evaluate pathological response in the breast in the axilla.

10.1.1 Endpoint Definitions for Pathologic Response
- Pathologic Complete Response (pCR): No viable invasive cancer in pathologic specimen (breast and axillary nodes), as determined by histological examination. We will report whether in situ disease only was present in the specimen versus no disease. We will also report for each woman whether she had a pCR in the breast only, in the lymph nodes only, or in both. We will also report the size of residual disease within the breast.

- Nodal Disease: We will report the number of subjects who had negative lymph node prior to starting treatment, or at the time of surgery: 0, 1-3, 4-10, or >10 lymph nodes.

10.2 Clinical Response Determination

We will evaluate clinical response in the breast, and when appropriate, in the axilla, infraclavicular and supraclavicular regions, and skin. At the time of study enrollment, patients will have a breast examination. Physical examination findings will be documented including clinical tumor size, character, mobility, and location of the breast mass. In addition, T4 features must be described. The patient will be examined by one of the treating investigators or his/her designee at each specified time point and after the final dose of treatment. Whenever possible, the same method (e.g., calipers or ruler) and investigator should be used to confirm consistency in the measurements.

10.2.1 Endpoint Definitions for Clinical Response (UICC criteria)
- Complete response (cCR) in the breast on physical exam will be defined as the absence of any palpable abnormality: i.e., no skin or breast thickening, mass, or associated skin or nipple changes. CR will be recorded separately for the breast and axilla.

- Partial response (cPR) in the breast will be defined as a 50% or greater decrease in the product of biperpendicular diameters as measured with a ruler, compared with the prechemotherapy measurement.

- Stable disease (cSD) in the breast will be defined as palpable disease which does not fit the definition of PR or PD.

- Progressive disease (cPD) will be defined as an increase in the product of biperpendicular diameters of 25% or greater compared to the original measurement.

- Inevaluable disease (cID) is defined as breast cancer that is not palpable in two dimensions.

11. Correlative Studies

11.1 Tumor Tissue Samples

In addition to the fresh tumor tissue samples below, sites may be asked to provide samples of routinely collected and processed tissues (e.g., formalin-fixed paraffin-embedded tissues) – including those from the original diagnostic and surgical procedures for the study tests. The samples will be shipped to Johns Hopkins for study as below, when requested. In the event of paraffin-embedded tissues where the blocks will not be released, 10-15, 5-10 micron, slides should be submitted for each applicable sample.

11.1.1 Collection

Up to 6 core-biopsies will be taken at each designated time point. The biopsies will be obtained at the surgical clinic, mammography suite, or the Oncology Center, by a skilled practitioner. These research biopsies may be obtained with mammography or ultrasound guidance as necessary. These core biopsies will be obtained in addition to diagnostic core needle biopsy material; however, whenever possible, and after a consent form has been signed, attempts will be made to coordinate diagnostic and study biopsies. Alternatively, patients who undergo sentinel node mapping or a lymph node biopsy may have study biopsy at the same time.

Biopsies will be obtained at the following time points:

- Prior to starting treatment: Attempts will be made to coordinate the baseline biopsy with the diagnostic biopsy or with the sentinel node mapping or lymph node biopsy. (Note: A titanium marker or "clip" may be placed at the discretion of the investigator performing the biopsy.)

- Day 15 (-0/+3 days): All day 15 blood/tissue and PET studies should be performed on the same day, with the PET scan taking place before the biopsy.

- Post-study treatment/Prior to additional preoperative treatment (14-28 days after last dose of study medication): In patients who receive additional preoperative treatment, a biopsy after completion of study medications and prior to initiation of additional treatment is required; samples at the time of surgery are still requested if tumor is present. The biopsy should be reviewed and reported to confirm presence of residual disease prior to initiation of treatment at the treating institution.

- Definitive surgery (i.e., mastectomy or lumpectomy): A separate tumor biopsy is not needed on the day of surgery; tissue will be taken from the surgical specimen.

Note: In any instance where a subject refuses a study biopsy after initiation of study treatment, it will not be considered a protocol deviation.

11.1.1.1 Specimen Handling, Transportation, Storage, and Processing

The study staff will be notified when a biopsy is taking place. The following procedures documented on the study tissues worksheet will be followed:

Up to three core-biopsy specimens will be rapidly frozen for future RNA extraction. Up to 3 core biopsy specimens will be suspended in 10% buffered formalin and after approximately 24 hours of suspension in formalin, the cores will be embedded in paraffin; a single slide should be cut and H&E stained for use by the study pathologist to evaluate presence of tumor. The block and slide will be shipped to Johns Hopkins for the appropriate studies listed below. Note: Cores to be placed in formalin specifically for the study at the time of definitive surgery are not required; a block or sections from the routine pathologic formalin-fixed paraffin-embedded (FFPE) specimens will be requested at the time the case if finalized, if available.

11.1.2 Methods

11.1.2.1 Immunohistochemistry

Serial biopsies will be stained using commercially available monoclonal antibodies including cleaved caspase 3 antibody and ki67. Other proteins related to the apoptotic process such as activated caspase 3, PARP, may be determined based on promising literature. Quantification will be performed by the study pathologist.

11.1.2.2 *PIK3CA* mutation status, PTEN expression, HER1-4 expression/activation

The study pathologist will retrieve specimens containing sufficient tissue for analysis; formalinfixed paraffin embedded (FFPE) and frozen tissues, if available, may be used. The specimens will then be processed in pathology and serial sections prepared and delivered to the Park Laboratory for PIK3CA analysis including hot spot mutation analysis and PTEN expression. DNA will be extracted from tissues and used for sequencing genes known to be altered in the PI3Kinase pathway including, but not limited to HER1, 2, 3 and 4, PIK3CA, PTEN, PIK3R and AKT1. If frozen tissue is available, additional proteomics analyses will be performed including, but not limited to, reverse phase protein array (RPPA) to examine the amount and phosphorylation status of key proteins in the HER2/PI3Kinase pathways including HER12,3 and 4. FFPE samples will also be used for in situ hybridization analyses of genes known to be amplified in the PI3Kinase pathway as well as immunohistochemical staining for genes that are altered in breast cancers that could also influence response to therapies (e.g. *TP53*).

11.1.2.3 RNA Extraction for Gene Expression Analysis

Tissues will be homogenized in Trizol (Gibco BRL) using a polytron homogenizer (Brinkman Instruments) and total RNA isolated according to the standard Trizol protocol. The total RNA obtained will be further subjected to an additional round of phenol chloroform extraction, precipitated and resuspended in RNAse free water. We will determine RNA concentrations by comparing the optical density ratios (OD260/OD280) obtained spectrophotometrically. Since

standard gel electrophoresis to assess RNA quality requires almost the entire RNA sample, we will use an Agilent 2100 analyzer and RNA 6000 LabChip kits (RNA microelectroseparation and analysis; Agilent Technologies, New Castle, DE).

RNA will be preserved for future studies of gene expression arrays. Gene expression analysis may be conducted for sets of genes currently suspected to be involved in the response of cells to pertuzumab and trastuzumab using quantitative reverse transcription PCR (RT-PCR).

The overall goal of these studies is to identify candidate markers for response and molecular profiles that might be relevant to an understanding of drug mechanisms. Although statistical methods will provide some indication of significance of particular molecular changes, validation will ultimately require additional testing in independent samples.

11.1.2.4 RNA Extraction for Immune Signature Endpoint

Tissue punches will be obtained from demarcated areas of invasive tumor from FFPE tissue using a 1 mm Biopsy Punch with Plunger (Fisher Scientific). Total RNA will be extracted from at least 1 mm tissue punch. In brief, a 1 mm tissue punch will be deparaffinized in Citrisolv ; Fisher Scientific) at room temperature for 30 minutes. The Citrisolv will be aspirated and the tissue was washed with 100% ethanol, vortexed, and centrifuged twice. Ethanol will be removed and the tissue dried at 37°C for 10 minutes. The sample will then be incubated in Proteinase K Digestion (PKD) buffer and proteinase K (1 µg/µl) for overnight (at least 8 hours) at 56°C. The digested tissue was incubated for 15 minutes at 80°C and centrifuged (14000 rpm) for 2 min at room temperature. The supernatant will be collected and the RNA extraction, including DNase I treatment, will be completed using the RNeasy FFPE kit on an automated QIAcube platform according to the manufacturer's instructions (QIAGEN, Valencia, CA). The concentration of the purified RNA will be determined using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies; Wilmington, DE). Purified total RNA was stored at -80°C. Samples will be analyzed using a Nanostring custom codeset that includes immune function genes associated with RFS in N9831.[59]

11.2 Blood Samples

11.2.1 Collection

Blood samples will be drawn as per the time points identified on study calendar and include:

- Prior to starting treatment.

- Day 15 (-0/+3 days): All day 15 blood/tissue and PET studies should be performed on the same day, whenever possible.

- Post-study treatment/Prior to additional preoperative treatment (14-28 days after last dose of study medication): In the event that a subject receives additional preoperative treatment, every attempt should be made to get a blood sample after completion of the study treatment and prior to additional treatment, as well as after additional treatment and prior to surgery.

- Definitive surgery (i.e., mastectomy or lumpectomy): This sample should be collected after completion of all treatment and prior to surgery (collection on the day of surgery preferred, if feasible).

- About 1-4 weeks after the definitive surgery: This blood sample should be drawn prior to initiation of any planned postoperative treatment (e.g., additional chemotherapy, radiation therapy).

11.2.2 Specimen Handling, Transportation, Storage, and Processing

The following samples will be collected and studied as outlined in the methods:

- Serum samples

About 20-30 milliliters of blood will be collected at each time point.

- Plasma samples

About 30 milliliters of blood will be collected at each time point.

- Pharmacogenomic samples

About 10 milliliters (mL) of blood will be collected at baseline only.

The samples will be processed and stored at -70°C or below until transfer for analysis; some samples may be batched/shipped ambient for processing by Johns Hopkins as outlined in the separate study bloods worksheet.

11.2.3 Methods

11.2.3.1 BEAMing and digital PCR for ptDNA

There is a great need to devise improved methods for screening patients for *PIK3CA* mutations and following responses to therapies. Based upon preliminary data, Dr. Ben Park and his research team has already demonstrated that BEAMing (Beads, Emulsification, Amplification and Magnetics) can reliably and accurately detect *PIK3CA* mutations from cell-free, plasma-derived tumor DNA (ptDNA) in metastatic breast cancer patients.[53] Similar to studies by others, they have also shown that *PIK3CA* mutational status can change or convert with recurrent metastatic disease. Pre-surgical plasma will be obtained from patients to assess both the presence and amount of mutant *PIK3CA* ptDNA by BEAMing. In addition, blood samples post-surgery and during followup will also be studied from patients identified with *PIK3CA* mutations. ptDNA will be isolated from these samples and also screened for the presence and amount of *PIK3CA* mutations by BEAMing. Mutant *PIK3CA* levels before and after surgery will be compared in order to assess whether changes in disease burden correlate with changes in ptDNA. Preliminary sample processing will be performed in Dr. Park's laboratory as per Inostics Standard Operating Procedures. These samples will then be batch-shipped to Inostics laboratory, Germany where ctDNA and BEAMing techniques will be performed on each sample per Inostics standard operating procedures. Every sample will be tested for the two *PIK3CA* hotspot mutations on Exon 9 (E542K, E545K) and a further hotspot mutation on Exon 20 (H1047R).

In addition to PIK3CA mutational status, newer next generation sequencing technologies will enable the research team to discover somatic mutations (e.g.TP53 mutations) and genomic rearrangements which are unique for the patient's cancer cells relative to their non-cancerous cells. Although such cancer specific alterations may or may not play a role in drug sensitivity, they do serve as cancer specific "biomarkers" for each individual. As this technology matures, we will be able to identify and measure these additional genomic alterations for every patient, not just those with PIK3CA mutations, thus broadening the scope and applicability of using ptDNA as biomarkers for disease burden and response to therapies. We therefore propose the following: before and after therapy, a tube of blood is drawn from the patient and genomic DNA from plasma is extracted and then banked. A portion of the patient's tumor is obtained to extract genomic DNA. The genomic DNA is then subjected to next gen sequencing to identify at least 10 to 20 genomic rearrangements/mutations that are present only in cancer cells. Having several unique rearrangements ensures that we can follow the tumor over time as genetic instability could, in theory, lead to the loss of one or more of these personalized markers. Once markers have been identified, we will then query the pre- and post-therapy blood specimens for these markers and use BEAMing/digital PCR to quantitate the amount of ptDNA at these two time points.

11.2.3.2 Methylation

We expect that over 70% of patients will have circulating DNA at baseline. DNA methylation studies will be performed at the Breast Cancer Research Laboratories at Johns Hopkins under the direction of Dr. Sara Sukumar. QM-MSP studies will be done on DNA extracted from serum or plasma obtained from each subject. Primers and probes for Q-MSP analysis have been designed to specifically amplify hypermethylated promoter sequences of Cyclin D2, RAR-beta, APC-1, Twist, RASSF1A, HIN-1, and ER-alpha. PCR will be performed in separate wells for each primer/probe set. Fluorogenic PCR amplification will be carried out using a 7900 Sequence detector (Perkin-Elmer Applied Biosystems) as previously described.

11.2.3.3 Pharmacogenomics

Genomic DNA isolated from whole blood at baseline will be used to genotype candidate genes that may affect pharmacodynamic effects of response and toxicity. We will store the sample obtained at baseline for potential future analyses in the event that further information becomes available from the literature about promising candidate genes that may predict response to anti-HER2 therapy, and patients will be appropriately consented for same.

11.3 Positron Emission Tomography (PET) Imaging

11.3.1 Collection

FDG PET/CT will be performed at baseline and on day 15 (window days 15-18); it is preferred that imaging be performed prior to biopsy.

Sites are asked to administer oral contrast per local standards, if applicable; unless otherwise contraindicated in the subject. The preferred oral contrast is READI-CAT 2 (barium sulphate suspension, 2.1% w/v); though site specific preferences should prevail with the caveat that the

contrast to be used does not contain any additives (e.g., flavoring) or glucose. The dose of oral contrast to be administered should be administered with enough water to provide for a total liquid volume of 500 milliliters at each administration – half 15 minutes prior to injection and the remaining half 15 minutes prior to scanning.

The FDG dose will be calculated based on the weight (kg) of the patient as follows: Dose (mCi) = Weight (kg) * 0.22(mCi/kg), where the maximum dose is 25mCi with weights greater than 114kg or 250lbs. (NOTE: A range of FDG dosing may be used of 0.1-0.25 mCi/kg after approval from the nuclear medicine collaborators at Johns Hopkins.)

A full description of timing of FDG administration and scan image acquisition, copies of which will be provided electronically (e.g., CD) to Johns Hopkins for central analysis, is provided in Appendix D. Note: As stated in the manual, the guidelines are recommendations only; sites should not contravene from site-specific procedures and should contact the Protocol Chair/her designee with any questions or concerns.

11.3.2 Methods

A PET scan produces a three-dimensional image of the sugar's distribution throughout the body. Since malignant cells use more sugar than normal cells, the radioactive sugar tends to find its way to and concentrate in cancerous tissue. Tumor standardized uptake values (SUV), defined as the ration of tumor FDG concentration (mCi/kg) to whole body concentration (injected dose divided by weight of the patient in kg) will be used to assess treatment response.

The presence and location of the primary tumor and any metastases will be qualitatively assessed. There will be no qualitative scoring in this study. The normal liver SUV corrected for lean body mass (SUL) will be measured as outlined in the PERCIST criteria.[36] For the purposes of the primary endpoint, maximal SUV (SUVmax) will be correlated with pCR, however, we may also explore other criteria per PERCIST in an exploratory fashion.

Prior to imaging of the first study participant, all sites are required to comply with the Quality Assurance Manual process outlined in Appendix C. This will allow the best and most comparable images for central analysis.

11.4 Leftover Samples

Any leftover study blood and tissue samples will be stored in the Breast Cancer Laboratory at Johns Hopkins for future research studies. These samples may be released for use in future studies after approval by the Protocol Chair and other regulatory bodies, as appropriate.

In addition, the study PI and collaborators have approval by the TBCRC to use all research biospecimens collected during the conduct of this trial to address the research questions described in the protocol document. All future use of residual or repository specimens collected in this trial for purposes not prospectively defined will require review and approval by the TBCRC according to its established policies, whether the specimens are stored in a central site or at a local institution in a virtual repository.

Subjects will be asked to consent to the future use of samples in the consent document.

11.5 Additional Information

The study coordinator will keep a log (separate logs will be kept for the blood and tissue samples, and radiology/imaging exams) that includes the study number and identifying participant information. The technicians will keep a log with the collection conditions, processing and storage information.

The laboratory and radiology investigators will be blinded to the subject identifiers and clinical data while generating the research data; additionally, the reported results will not disclose any unique patient identifiers.

A separate study tissues and study bloods worksheet will be provided to all participating sites outlining specific procedures for collection and processing of samples.

12. Adverse Events

12.1 General

This study will use the descriptions and grading scales found in the revised National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 for adverse event reporting that can be found at http://ctep.cancer.gov/reporting/ctc.html.

Information about all adverse events, including those volunteered by the subject, discovered by investigator/study personnel questioning, or detected through physical examination, laboratory test or other means, will be collected, followed, and reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the study drug(s)/intervention(s), and actions taken.

All adverse events experienced by subjects will be collected from the time of first dose of study medication, throughout the study and until the final assessment as outlined in the Study Calendar (Section 8). Subjects continuing to experience toxicity after discontinuation of the study drug may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible. Any adverse event experienced during additional preoperative treatment or after the surgical procedure that the investigator feels is related to study treatment will be captured.

12.2 Definitions

12.2.1 Adverse event (AE)

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

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment (any procedures specified in the protocol).

Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy.

12.2.2 Serious adverse event or reaction

An AE should be classified as an SAE if the following criteria are met:
- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

Note: Any occurrence of pregnancy must also be reported in the same time frame as a serious adverse event.

12.2.3 Other information:

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

  In addition per IRB regulations of the Coordinating Center, any adverse event that is not included in the informed consent, but is believed to be considered related to the study intervention, is also considered "unexpected".

- Expected (known) adverse event: An adverse event, which has been listed or characterized in the I.B. or P.I.

In addition per IRB regulations of the Coordinating Center, an adverse event is considered "expected" only if it is included in the informed consent document as a risk.

12.3 Relationship

To ensure consistency of AE and SAE causality assessments, the causality/relationship of all events to study medication(s) will be assessed by an investigator and assigned as follows:

12.3.1 "Yes" to the question of causality:

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

The causality in this case may also be classified as follows:

- Definitely: An adverse event which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, for which no alternative cause is present.
- Probably: An adverse event, which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, but for which a potential alternative cause may be present.
- Possibly: An adverse event, which has a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, but a potential alternative cause does not exist.

12.3.2 "No" to the question of causality:

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

The causality in this case may also be classified as follows:

- Unlikely: An adverse event which does not have a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, does not reappear or worsen after re-administration of the investigational drug/agent (if applicable), and for which there is evidence that it is related to a cause other than the investigational drug/agent.
- Unrelated: An adverse event, for which there is evidence that it is definitely related to a cause other than the investigational drug/agent. In general, there is no timely relationship to the administration of the investigational drug/agent, or if there is a timely relationship, the event does not follow a known pattern of response, and there is an alternative cause.

12.4 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs, while avoiding colloquialisms and abbreviations.

12.4.1 Diagnosis vs. Signs and Symptoms

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

12.4.2 Deaths

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

12.4.3 Preexisting Medical Conditions

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

12.4.4 Hospitalizations for Medical or Surgical Procedures

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

Hospitalizations for the following reasons do not require reporting:
- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions;
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study; or,
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

12.4.5 Post-Study Adverse Events

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

12.5 Reporting Procedures

12.5.1 General

All adverse events will be captured on the appropriate study-specific case report forms (CRFs), sponsor-provided, or in a designated database.

All serious adverse events, regardless of causality to study drug, will be reported to the Coordinating Center.

12.5.2 Adverse Events of Special Interest

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

The following adverse events are of **special interest** for pertuzumab and should be reported in an expedited manner:

- Left Ventricular Dysfunction ☐ Infusion-associated reactions
- Hypersensitivity reactions/anaphylaxis
- Embryo-fetal toxicity or birth defects
- Pregnancy: If a female subject becomes pregnant while receiving investigational therapy or within 6 months after the last dose of study drug, a report should be completed and expeditiously submitted to the Coordinating Center and to Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject should be reported as an SAE.

All serious adverse events must be reported to the Coordinating Center within 1 business day after the investigator becomes aware of the event. Events should be reported using a MedWatch form (3500) as available on the FDA website (see link below).

Follow-up information must also be reported within 1 business day of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding serious adverse events to the participating sites within 5 days of review of the information by the Protocol Chair (or her designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e.,

possibly, probably, or definitely) related to the study medication. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

12.5.3 Institutional Review Board

All adverse events and serious adverse events will be reported to the IRB per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification to the study protocol, these modifications will be provided to the IRB as soon as is possible.

12.5.4 Genentech

12.5.4.1 Reconciliation

Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

12.5.4.2 SAE Reporting

The Coordinating Center will report all SAEs to Genentech within the timelines described below. The completed Medwatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

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

- Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available.
- Serious AE reports that are related to pertuzumab and AEs of Special Interest (regardless of causality) will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date.
- Serious AE reports that are unrelated to pertuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- Additional Reporting Requirements to Genentech include the following:
- Any reports of pregnancy following the start of administration with pertuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- All Non-serious Adverse Events originating from the Study will be forwarded in a quarterly report to Genentech.
- The Genentech-provided "SAFETY REPORTING FAX COVER SHEET" for Genentechsupported research should be used for all correspondence.

Initial report: The report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:
- Protocol description (and number, if assigned)

- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

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

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the patient for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at:
http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm

For questions related to safety reporting, please contact Genentech Drug Safety at (888) 835-2555 (phone) or fax (see above).

12.5.5 Food and Drug Administration (FDA)
The Coordinating Center will be responsible for reporting to the FDA as follows:

- For Investigator-Sponsored IND Studies, some additional reporting requirements for the FDA apply 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/Fax Report: The 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 trastuzumab and pertuzumab. An unexpected adverse event is one that is not already described in the trastuzumab and pertuzumab 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 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 trastuzumab and pertuzumab. An unexpected adverse event is one that is not already described in the trastuzumab and pertuzumab investigator brochure.

Written IND Safety reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the investigator 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 to the FDA, Genentech, 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).

NOTE: Any study report(s) submitted to the FDA should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. All FDA/IND correspondence should be faxed to Genentech Drug Safety at (650) 225-4682 or (650) 225-5288; whereas copies of literature articles should be mailed to the assigned Clinical Operations contact for the study.

13. Data and Safety Monitoring

13.1 Data Management

All information will be collected on study-specific case report forms by the study staff at each institution. The necessary forms will be provided to each site by the Coordinating Center.

The completed forms will be forwarded to the Coordinating Center for central review and inclusion in the study dataset with relevant source documentation as outlined in the case report forms. The data submission schedule is as follows:

At the time of registration:

- Registration Form
- Informed Consent Form (signed by the subject)
- Eligibility Checklist
- Source documents related to eligibility

Within 2 weeks after registration:

- Baseline study case report forms

- Pertinent source documents

Within 2 weeks after each cycle is completed:

- Per-cycle case report forms
- Pertinent source documents

Every 6 months during follow-up:

- Follow-up case report forms
- Pertinent source documents (if necessary)

All study data will be reviewed for completeness and accuracy by the Protocol Chair. The Principal Investigator (or his/her designee) at each respective institution is responsible for review, and ensuring the completeness and accuracy, of the data generated by his/her institution. The study data will also be periodically reviewed by the Sidney Kimmel Comprehensive Cancer Center Clinical Research Office.

13.2 Meetings
The Coordinating Center will schedule teleconferences to take place as needed depending on the rate of accrual, and will include the Protocol Chair and Principal Investigators from each site. The following study team members involved with the conduct of the trial will be included as appropriate: study coordinators, data managers, research nurses, sub-investigators, collaborators (if applicable), and statistician.

During these meetings matters related to the following will be discussed: enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), validity and integrity of the data, safety data, analysis of samples by Drs. Park, Perez, Thompson and Sukumar's labs, and progress of data for objectives.

At these meetings, the data pertaining to clinical response and toxicity will also be reviewed, dependent upon rate of accrual, as outlined in Section 16.4.

A summary of the items discussed at each teleconference will be prepared by the Coordinating Center and forwarded to each participating site. In addition, study investigators meet 2-3 times each year in regularly scheduled TBCRC meetings.

13.3 Monitoring
As an IND is required for this study, it will be conducted in accordance with the data and safety monitoring program established by the Clinical Research Office of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (SKCCC CRO). As required, a monitoring plan will be established for the study with monitoring to be conducted by the SKCCC CRO Quality

Assurance staff or other group, if agreed upon to be appropriate. A medical expert committee or formal data safety monitoring board are not planned or required at this time.

As above, all data will be sent to the Coordinating Center for central collation and review. Subject cases from sites will be chosen for a thorough review; it is unclear how often this may occur; however, in the event that a case is chosen for central review by the Coordinating Center, each site will be asked to provide copies of all source documents and to ensure that all data is current. There are no formal on-site evaluations planned by the Coordinating Center; however, these may occur depending on site accrual rate, identified problems or concerns, or other reasons, as appropriate.

In addition, a Data Monitoring Committee (DMC) comprising of breast cancer experts and the study statistician will be constituted to review all cases where progressive disease is confirmed or suspected during the neoadjuvant setting. Please see Section 16 for additional information regarding this review.

14. Administrative Procedures

14.1 Protocol Amendments
Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB before implementation. The Protocol Chair (or her designee) is responsible for the coordination and development of all protocol amendments, and will disseminate this information to the participating centers.

14.2 Informed Consent
An investigator will explain to each subject the nature of the study, its purpose, procedures involved, expected duration, potential risks and benefits. Each subject will be informed that participation in the study is voluntary and that she may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment. This informed consent will be given by means of a standard written statement and will be submitted for IRB approval prior to use. No patient will enter the study before her informed consent has been obtained. In accordance with the Health Insurance Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

14.3 Ethics and Good Clinical Practice
This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

14.4 Regulatory Authorities

14.4.1 Institutional Review Board

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards of each participating center.

14.4.2 Food and Drug Administration (FDA)

This trial falls under Investigational New Drug (IND) application #117913 and is held by the Protocol Chair. All reporting required with regards to the clinical trial outlined will comply with FDA regulations and be handled by the Protocol Chair and Coordinating Center.

15. Coordinating Center & Site Responsibilities

15.1 Protocol Chair

The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.

- Assuring that all participating institutions are using the correct version of the protocol.

- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.

- Reviewing and ensuring reporting of Serious Adverse Events (SAE).

- Reviewing data from all sites.

15.2 Coordinating Center

The Coordinating Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.

- Managing central patient registration.

- Collecting and compiling data from each site.

- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.

- Facilitating audits by securing selected source documents and research records from participating sites for audit, or by auditing at participating sites.

15.3 Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).

- Submitting data to the Coordinating Center.

- Registering all patients with the Coordinating Center by submitting patient registration form, and signed informed consent promptly.

- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.

- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.

- Collecting and submitting data according to the schedule specified by the protocol.

Additional information for participating sites:

15.3.1 Staffing

The participating sites will provide experienced staff, and adequate equipment and facilities to support this clinical trial. The participating sites will also be responsible for research staff training in computer applications, human subjects research, and HIPAA compliance, as well as the continuing education in these areas as required by local institutional standards.

15.3.2 Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Coordinating Center. The required documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures and recruitment material, etc.), IRB membership rosters, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Coordinating Center will provide each participating site with a comprehensive list of the necessary documents. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Coordinating Center.

The requirements for data management, submissions, and monitoring are outlined below.

15.3.3 Confidentiality

All unpublished information that the Coordinating Center gives to the investigator shall be kept confidential and shall not be published or disclosed to a third party without the prior written consent of the Protocol Chair (or her designee).

15.3.4 Record Retention

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed. In general, the FDA guidelines will be followed and communicated to site by the Coordinating Center; guidance may be found here: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.62.

15.3.5 Publication

It is understood that any manuscript or releases resulting from the collaborative research will be circulated to all participating sites prior to submission for publication or presentation. The Protocol Chair will be the final arbiter of the manuscript content.

15.3.6 Additional Information

Each participating site is responsible for submitting additional information as requested by the Protocol Chair (or her designee). The Coordinating Center may terminate the study at a participating site in the event that these conditions are not followed.

16. Statistical Considerations

16.1 Overall

This is a single arm phase 2 study in the neoadjuvant setting aimed to correlate early change (day 15) in maximal standardized uptake values (SUVmax) on FDG-PET with pathological response to pertuzumab/trastuzumab (PT) in patients with primary operable HER2-positive and ERnegative breast cancer. We hypothesized that PT will have an impact on SUVmax and changes in SUVmax on FDG-PET as early as 15 days after treatment are associated with response to PT. Our ultimate goal would be to determine the predictive ability of early changes in SUVmax and identify an optimal cut point in SUVmax % reduction that accurately predicts pathological complete response (pCR) to PT such that this information can be used in future studies to guide therapy for a similar patient population.

16.2 Study Design

Eligible patients will receive 12 weeks of PT in the pre-operative setting. An FDG-PET scan will be performed at baseline and 15 days (window days 15-18) after commencement of therapy. Tumor biopsies will also be performed at baseline, after 2 weeks of therapy and at surgery to assess biomarkers with a particular focus on the PI3K pathway and novel predictive gene-expression profiles, and response or resistance to therapy. We choose to undertake this study in ER-negative disease alone in that 1) a considerable distinction in metabolic activity has been observed between the different subtypes of breast cancer, 2) the NeoALLTO PET study also indicated that there was not a strong association between change in SUV and response in the ER-positive group, and 3) the pCR rate for ER-positive group was low based on the data from the NeoALLTO PET substudy, which requires a much larger sample size to reach a reasonable number of responders. We anticipate that our findings will mirror the NeoALLTO PET substudy results and meanwhile we hope to identify a cut point in SUVmax decline through a Receiver operating characteristic (ROC) analysis that more accurately predicts pCR rather than using pre-specified thresholds as in previous studies.

16.3 Sample Size and Accrual

Eligible patients will need to have both baseline and day 15 FDG-PET scan performed and SUVmax data collected as well as surgery specimen successfully evaluated to be considered evaluable for the primary analysis. A total of 80 evaluable patients will be accrued onto this study.

It is expected that early change in SUVmax has good discriminating potential for predicting pCR to PT. We chose to power the study to detect a significant difference from a null value based on AUC, a measure used to quantify the overall predictive power of the biomarker. Specifically, we are interested in finding that percent SUVmax reduction predicts response to PT with an AUC significantly greater than 0.65, a predictive power that is deemed minimally acceptable under the scope of this study. For a test of the hypothesis H0: AUC ≤0.65 vs. H1: AUC > 0.65, a total of 80 evaluable patients with a projected pCR rate of 25% (i.e., 20 responders and 60 non responders) will have 81% or greater power to detect a true AUC of 0.80 at one-sided type I error rate of 0.10. The power calculation is based on the asymptotic z-test and standard error of AUC estimated using the Hanley-McNeil method.[76] Given 20 responders and 60 non-responders, precision of the sensitivity and specificity estimates will be no wider than ±20% and ±12%, respectively. The table below presents, for several observed levels of sensitivity or specificity, the 90% confidence intervals and their half widths, which were constructed using exact binomial procedures. For observed sensitivity and specificity of 80%, the 90% confidence intervals will be 60% to 93% (± 16%) and 70% to 88% (± 9%), respectively. We will be fairly confident that the decline in SUVmax has sensitivity of at least 70% if the observed estimate reaches 90%. Similarly, we will be fairly confident that the test has specificity of at least 80% if the observed value is 90%.

Table: Precision (indicated by half width of exact 90% CI) for estimating sensitivity and specificity for a sample size of 80 patients and a pCR rate of 25% (i.e., 20 pathological responders and 60 non-responders).

Observed sensitivity	Exact 90% CI	Half width of 90% CI	Observed specificity	Exact 90% CI	Half width of 90% CI
0.60	0.39 – 0.78	0.19	0.60	0.49 – 0.71	0.11
0.70	0.49 – 0.86	0.18	0.70	0.59 – 0.80	0.10
0.80	0.60 – 0.93	0.16	0.80	0.70 – 0.88	0.09
0.90	0.72 – 0.98	0.13	0.90	0.81 – 0.96	0.07

The anticipated accrual rate for this group of patients is approximately 3-5 per month and the accrual period is expected to be 24 months. We anticipate accruing additional 10% (i.e., 8 patients) to account for major protocol violation as well as potential missing data due to incomplete information on decline in SUVmax and pCR. Therefore, the maximum overall accrual of this study will be 88 patients.

16.4 Interim Analysis and Early Stopping Guidelines

There are no plans to stop the study early for futility with respect to the primary objective (i.e., association of change in SUVmax with pCR) considering the small number of pathological

responders (~20). Meanwhile, preliminary data of change in SUVmax with PT are not available and it would be difficult to presume a priori what % reduction in SUVmax would be a meaningful decline before we correlate it with treatment response. However, there is concern that patients may not derive sufficient benefit (i.e., the lesion shows clear signs of progression) from receiving antiHER2 agents (PT) without chemotherapy during the neoadjuvent setting, and thus we will carry out interim futility analyses based on proportion of clinical progression by cycle 1. Clinical response will be assessed at every cycle by clinical breast examination during neoadjuvant treatment (Cycles 1-4). Chemotherapy may be administered pre-operatively if evidence of disease progression during PT, at the discretion of the treating investigator and after discussion with the Protocol Chair (see Section 7.3.6). A Data Monitoring Committee (DMC) comprising of breast cancer experts and the study statistician will be constituted to review all cases where progressive disease is confirmed or suspected during the neoadjuvant setting, or incomplete response after 12 weeks PT,. All available data pertaining to these scenarios will be transmitted to the DMC, to aid with assessment of disease progression. If it becomes evident that the proportion of progression convincingly exceeds 10%, the accrual will be suspended and the study may be terminated. A fully amended protocol will be submitted with treatment modifications (e.g., adding chemotherapy to PT for all patients).

The following early stopping rules will serve as guidelines for the DMC to hold enrollment if the posterior probability of progression by cycle 1 during the neoadjuvant setting being greater than 10% is 75% or higher. The prior for this monitoring rule is beta (0.5, 7.5), assuming the proportion of progression is 6.3%[3] with 90% probability that this proportion is between 0.3% and 23%.

Stop if # clinical progressions	2	3	4	5	6	7	8	9	10	11
Out of N	2-6	7-14	15-22	23-31	32-39	40-48	49-57	58-66	67-75	76-80

The following table shows the operating characteristics of the stopping rules based on 5000 simulations.

Underlying risk of clinical progression	0.05	0.10	0.15	0.20
Probability of early stopping	7.3%	43.5%	83.0%	97.6%
Average sample size	75.2	56.0	33.6	19.4

While there is not a formal independent data safety monitoring board, toxicity will be monitored, and information will be reviewed with investigators and appropriate staff from all participating sites at routine study teleconferences (Section 13.2). If any safety concerns arise with the presentation of further results from the NeoSPHERE or CLEOPATRA trials we may amend the protocol to incorporate formal safety monitoring plans.

16.5 Definition of Variables

Clinical and pathologic responses are dichotomous variables which were defined in section 10.1.1 and 10.2.1. For each of the variables (e.g., apoptosis, cell proliferation, SUV metrics, gene expression levels, gene methylation), we will look at how each change from baseline to the first follow-up, and from baseline to surgery. Apoptosis and proliferation will be measured as the percentage (%) of cells that stain for the marker. Change will be defined as a percent change from baseline. For PET imaging, standardized uptake values (SUV), defined as the ratio of tumor FDG concentration (mCi/kg) to whole body concentration (injected dose divided by weight of the patient in kg) will be measured per PERCIST and corrected for lean body mass (SUL). The SUVmax, defined as the maximum single voxel SUV measured within a defined ROI or VOI, will be used for the primary analysis. For gene expression profiles, we will be using cDNA arrays and expression levels will be in the form of log ratios comparing samples to a reference sample (see below for description of measures for proteomic measures). For gene methylation, we will be using the QM-MSP to measure promoter hypermethylation of the genes and the relative amount of methylation will be calculated as percent methylation defined by Dr. Sukumar's lab.

16.6 Data Quality and Normalization

Standard approaches will be used to evaluate the quality of the data. For cell proliferation and apoptosis as well as for SUV metrics, histograms and boxplots will be generated to look for skewness and outliers. Appropriate transformations will be taken to symmetrize the data if needed.

16.7 Analysis Plan

16.7.1 Primary Endpoint

Change in SUVmax at day 15 will be calculated as a % reduction from baseline SUVmax. Results will be summarized with descriptive statistics (e.g., mean, standard deviation, median and range), classified by pathological responders and non-responders. Distributions of % reduction in SUVmax will be compared between the two groups using a t test or nonparametric Wilcoxon rank sum test where appropriate. The discriminative and predictive ability of early changes in SUVmax will be evaluated by constructing ROC curves. The ROC curve combines the information of the true positive rate and true negative rate. In order to identify the most useful criterion of early change in SUVmax, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall accuracy and area under the curve (AUC) along with 90% confidence intervals will be estimated for the entire range of threshold values in SUVmax % reduction. As our long term goal is to identify a group of individuals with HER2-positive early breast cancer who could potentially be spared the added toxicity of chemotherapy in addition to anti-HER2 agents and meanwhile to direct other patients towards an aggressive or investigational approach, we aim to identify a cut point that has the right balance in sensitivity (1 – false negative rate) and specificity (1 – false positive rate). PET scan will be considered a positive test if the resulting % reduction in SUVmax >= optimal cut point and a negative test if the resulting % reduction in SUVmax < optimal cut point. We are generally more concerned about getting a false positive, meaning a woman may end up omitting additional chemotherapy when she does need it. Without prior knowledge of predictive ability of change in SUVmax on PET for response to anti-HER2 therapy, however, explicit criteria for choosing an optimal cut point are difficult to construct a priori as 'optimality' ultimately depends on practical constraints and clinically relevant trade-offs. We will

evaluate the ROC curves for sensitivity and specificity combinations that would be appropriate for clinical decision. As a general rule, an optimal cut point will be defined by maximizing the sum of sensitivity and specificity while giving more weight to specificity than sensitivity in the sense that we would like to have a lower false positive rate than a false negative rate. A final decision will be carefully discussed among the team constituted by the study PI, PET imaging experts and the study statistician. A bootstrap re-sampling approach will be used for obtaining a point estimate and a confidence interval for the cut point as well as an interval validation. Additionally, logistic regression will be used to determine if change of SUVmax predicts response independent of standard clinicopathologic factors such as age, tumor size, grade, and proliferation index. A potential of baseline SUVmax as a predictor of pCR will be assessed in a similar fashion. Imputation for missing data is not planned in this study due to unverifiable assumptions. Patients who progress prior to surgery may receive additional preoperative therapy. These patients along with death related to disease prior to surgery will be considered as pathological non-responders (failures on PT) in the primary analysis. A sensitivity analysis will be performed with the exclusion of these patients and results will be reported as well. We will explore SUV metrics using other criteria per PERCIST in a similar fashion.

16.7.2 Secondary Endpoints

PIK3CA mutation in peripheral tumor DNA obtained by blood sample will be evaluated both qualitatively (present/absent) and quantitatively (amount of the abnormal DNA in circulation). Results will be summarized using descriptive statistics including mean, standard deviation, median, range (for quantitative measure), frequencies and proportions (for qualitative measure). Measurements will be performed at baseline, D15, post treatment/pre surgery and post surgery. Paired comparisons between baseline and other time points will be made using paired t test for quantitative measures and McNemar's test for qualitative measures. The time course of quantitative markers will be investigated graphically depicting aggregate and subject-level trends over time. Patterns of change in the values over time and how the patterns differ by response status will be evaluated using linear mixed effects models. Mutation status and percentage change from baseline will be compared between pathological responders and non-responders using Fisher's exact test and t-test and, respectively. We will further use random-effects models that allows for estimation of the subject-specific effects on the repeated observations. Relevant covariates (e.g., standard clinicopathologic factors) will be included in the models to control for confounding effects. ROC analysis described as above will be performed as appropriate. Concordance of the mutation status between ptDNA and tumor specimens will be assessed by kappa statistic with a 95% CI or by Lin's correlation coefficient for continuous data. Somatic mutations and additional genomic alterations will be further sought using next generation sequencing data analysis. Pre and post changes will be analyzed in a similar fashion. Markers identified will be coupled with PIK3CA mutation data and correlated with pCR using logistic regression approach and ROC analysis.

Key proteins involved in the PI3K pathway activation (e.g., PTEN low and/or PIK3CA mutation, HER 1-4 expression and/or phosphorylation) and other secondary outcomes (novel gene expression profiles, baseline and change in Ki67) will be correlated with pCR in a similar fashion as described above. The expression of the genes that we identified from the N9831 analysis will be measured using RNA from formalin-fixed, paraffin-embedded biopsy samples. A modified

surface mapping tool will be applied to assign tumors to immune enriched and not-enriched groups.[59] We will use Fisher's exact test to evaluate the association of this immune function signature with pCR and estimate the odds ratio along with the 95% confidence interval via logistic regression. pCR rate to preoperative PT + taxane will be estimated with the 95% confidence interval in the setting of histologically confirmed residual cancer after 12 weeks of preoperative PT. Long term outcomes (e.g., recurrence-free survival and overall survival) will be summarized using the Kaplan-Meier method. Results from the secondary analyses will be largely exploratory and will provide valuable preliminary data for future studies.

16.7.3 Exploratory Endpoints

Baseline biomarkers (e.g., KI67, serum methylation, gene expression profiles) will be summarized using descriptive statistics. Histograms and plots will be generated to assess the distribution of the data for each marker. Suitable transformations will be performed on skewed marker data as appropriate to achieve normality. The significcae of each molecular marker as predictors of response to anti-HER2 therapy will be initially assessed using the t test or Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables. Mantel-Haenszel chisquare test will be used for ordered levels of measurements. The significance of multiple molecular markers as predictors of response or resistance to anti-HER2 therapy will be evaluated using the multiple logistic regression approach. ROC analysis described as above will be performed as appropriate, and sensitivity, specificity, PPV, NPV, AUC and overall accuracy will be estimated as well. The above analyses of multiple molecular markers will be considered exploratory in this small Phase 2 trial and other statistical approaches may be applied during the course of the exploration. Furthermore, the multiple testing of individual markers will warrant caution in the interpretation of significance tests.

Changes in biomarkers (e.g., PIK3CA, ER, Ki67, serum methylation markers, gene expression profiles) through serial samples collected at different time points will be summarized with descriptive statistics and described using graphical presentation (e.g., scatterplots, histograms, or boxplots). We will create plots that display changes in serial samples over time with tumor tissue and blood samples overlaid and blocks of treatment window (e.g., PT, neoadjuvant chemotherapy, and definitive surgery) indicated. Standard statistical tests will be used to explore whether or not the data shows evidence of changes in patients who are responsive or resistant to anti-HER2 therapy. The analyses will be exploratory, aimed to provide preliminary data for subsequent studies.

Additional exploratory analyses include correlating baseline and change (day 15) in SUV on FDG PET with pCR among patients with histologic confirmed residual disease after 12 weeks PT and subsequent addition of standard therapy per investigator discretion (Section 7.3.6), using Wilcoxon rank sum test and logistic regression model as well.

16.7.4 Safety

All subjects will be evaluable for toxicity from the time of their first dose of study medication. The assessment of safety will be based mainly on the frequency of adverse events. Adverse events will be summarized in each arm based on NCI CTCAE Version 4.0. Frequencies and percentages of

patients having any adverse event will be presented. Safety profiles will contribute to decisions whether to move the regimen forward in further studies.

16.8 Reporting and Exclusions

16.8.1 Enrollment

A sample size of up to 88 subjects is planned (accounting for 8 inevaluable patients). In the event that a subject does not initiate study treatment after registration, that subject will not count towards the accrual goal.

16.8.2 Evaluation of Toxicity

All subjects will be evaluable for toxicity from the time of their first dose of therapy.

16.8.3 Evaluation of Response

Analysis will be by intention to treat. All subjects included in the study must be assessed for response to treatment even if there are major protocol treatment deviations or if they terminate treatment early. All subjects who meet the eligibility criteria and receive a first dose of study medication will be included in the main analysis of the response rate.

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APPENDICES

A   American Joint Commission on Cancer Staging
B   ECOG Performance Status
C   PET/CT Quality Assurance Manual
D   PET/CT Imaging Guidelines

APPENDIX A: American Joint Committee on Cancer Staging

T – Primary Tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	Ductal carcinoma in situ
Tis (LCIS)	Lobular carcinoma in situ
Tis (Paget)	Paget's disease of the nipple with no tumor Note: Paget's disease associated with a tumor is classified according to the size of the tumor.
T1	Tumor $\leq$ 2 cm in greatest dimension
T1mic	Microinvasion $\leq$ 0.1 cm in greatest dimension
T1a	Tumor > 0.1 cm but not > 0.5 cm in greatest dimension
T1b	Tumor > 0.5 cm but not > 1 cm in greatest dimension
T1c	Tumor > 1 cm but not > 2 cm in greatest dimension
T2	Tumor > 2 cm but not > 5 cm in greatest dimension
T3	Tumor > 5 cm in greatest dimension
T4	Tumor of any size with direct extension to (a) chest wall or (b) skin, only as described below
T4a	Extension to chest wall, not including pectoralis muscle
T4b	Edema (including peau d'orange" or ulceration of the skin of the breast, or satellite skin nodules confined to the same breast
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma

N – Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed (e.g., previously removed)
N0	No regional lymph node metastasis
N1	Metastasis in movable ipsilateral axillary lymph node(s)
N2	Metastases in ipsilateral axillary lymph nodes fixed or matted, or in clinically apparent ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastasis
N2a	Metastasis in ipsilateral axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastasis only in clinically apparent ipsilateral internal mammary nodes and in the absence of clinically evident axillary lymph node metastasis

N3	Metastasis in ipsilateral infraclavicular lymph node(s), or in clinically apparent ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
N3a	Metastasis in ipsilateral infraclavicular lymph node(s) and axillary lymph node(s)
N3b	Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastasis in ipsilateral supraclavicular lymph node(s)

APPENDIX A (CONTINUED): American Joint Committee on Cancer Staging

PN – Regional lymph nodes	
pNX	Regional lymph nodes cannot be assessed (e.g., previously removed or not removed for pathologic study)
pN0	No regional lymph node metastasis histologically, no additional examination for isolated tumor cells
pN0(i-)	No regional lymph node metastasis histologically, negative IHC
pN0(i+)	No regional lymph node metastasis histologically, positive IHC, no IHC cluster > 0.2 mm
pN0(mol-)	No regional lymph node metastasis histologically, negative molecular findings (RT-PCR)
pN0(mol+)	No regional lymph node metastasis histologically, positive molecular findings (RT-PCR)
pN1mi	Micrometastasis (> 0.2 mm, none > 2.0 mm)
pN1	Metastasis in one to three axillary lymph nodes and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN1a	Metastasis in one to three axillary lymph nodes
pN1b	Metastasis in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN1c	Metastasis in one to three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN2	Metastasis in four to nine axillary lymph nodes, or in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis
pN2a	Metastasis in four to nine axillary lymph nodes (at least one tumor deposit > 2.0 mm)
pN2b	Metastasis in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis
pN3	Metastasis in 10 or more axillary lymph nodes, or in infraclavicular lymph nodes, or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes
pN3a	Metastasis in 10 or more axillary lymph nodes (at least one tumor deposit > 2.0 mm), or metastasis to the infraclavicular lymph nodes

pN3b	Metastasis in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN3c	Metastasis in ipsilateral supraclavicular lymph nodes

M – Distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

APPENDIX A (CONTINUED): American Joint Committee on Cancer Staging

Stage Grouping	T	N	M
0	Tis	N0	M0
I	T1	N0	M0
IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

APPENDIX B: ECOG Performance Status Scale

Score	Definition
0	Asymptomatic
1	Symptomatic, fully ambulatory
2	Symptomatic, in bed less than 50% of day
3	Symptomatic, in bed more than 50% of day, but not bedridden
4	Bedridden

APPENDIX D: PET/CT Imaging Guidelines

PET/CT Imaging Quality Assurance Manual

A Phase 2 Clinical Trial Assessing the Correlation of Early Changes in Standardized Uptake Value (SUV) on Positron Emission Tomography (PET) with Pathological Complete Response (pCR) to Pertuzumab and Trastuzumab in Patients with Primary Operable HER2-Positive Breast Cancer

Protocol: TBCRC026

Introduction

We have every confidence that your PET scanner is working just fine. However, we need evidence that confirms this. Furthermore, we need to produce this evidence in a standardized way. All sites involved in this study are, therefore, asked to:

1) Provide sample clinical studies to the coordinating site;

2) Perform the following phantom experiment on each on-site camera which may potentially be used in the acquisition of studies on protocol TBCRC026.

Sample Clinical Studies

In addition to the following phantom study, we are requesting two (2) representative ^{18}F-FDG PET/CT Whole Body Breast CA studies, acquired on each camera which may be used in this study, to be sent to the study coordinating center in the format and fashion prescribed below. If the data is not de-identified upon receipt by the study coordinating center, we will de-identify it for you and dispose of the PHI before inclusion of the data into the quality assurance database for this study.

Phantom

The phantom to be used is a 20 cm diameter cylinder phantom and should be already available at the site. It should be a fillable phantom, as opposed to a ^{68}Ge resin phantom, and should have no inserts. Ideally the phantom should be around 20 cm long, although cylinders of other lengths are acceptable.

Before performing the experiment, take time to determine the exact volume of water inside the phantom. This information can be obtained in a variety of ways including: documentation from the phantom manufacturer; carefully measuring the volume of water required to completely fill the phantom; weighing the phantom before and after filling with water (if sufficiently accurate scales are available); measuring the internal dimensions with a ruler (water volume = 3.14 × internal radius × internal radius × internal length).

In addition to the volume, measure the internal diameter of the cylinder using a ruler to the nearest millimeter. *Please answer the following questions:*

1) *Volume of water within phantom:*

2) *How was this determined?*

3) *Internal diameter of the phantom:*

Phantom Preparation

Ensure that the time on all clocks in the lab is accurate and in agreement with the scanner's clock. If the discrepancy is more than 1 minute, reset the clocks.

APPENDIX D: PET/CT Imaging Guidelines

Fill the phantom with water, leaving a large air bubble.

Prepare a syringe with 1-3 mCi of FDG. Measure the activity in a dose calibrator and record the activity and the measurement time.

Inject the activity into the phantom and flush several times to ensure there is minimal residual activity in the syringe. Record the residual activity and the time of the measurement.

Seal the phantom and invert a few times to ensure the activity is well mixed. Top up the phantom with water so that the phantom is completely full.

Dry the outside of the phantom and check carefully for leaks. Do not proceed if you think the phantom may be leaking. *Please answer the following questions:*

4) *Pre-injection ^{18}F activity:*

5) *Time of measurement:*

6) *Residual activity left in the syringe after adding to phantom:*

7) *Time of measurement:*

Phantom Positioning

Immediately transfer the phantom to the scanner and position using a phantom holder where available or by securing the phantom directly onto the patient bed. Orient the phantom so that it appears as a disk in the transverse plane.

Adjust the bed height and the phantom position so that it is centered perfectly within the transverse field-of-view, using the positioning lasers as necessary.

Check the phantom once again for leaks and remove from scanner immediately if a leak is suspected.

Data Acquisition

Scan the phantom using the PET/CT protocol that will be used for patient studies. Ensure that the axial scan range entirely covers the phantom using 2 or more bed positions as necessary. Adjust the protocol (for this phantom experiment) so that emission data are acquired for at least 10 minutes per bed position.

When configuring the scan, enter the activity in the phantom and the measurement times.

Convert the volume of the water component of the phantom to kg. For example if the water inside the phantom had a volume of 5600 mL, the weight would be 5.6 kg. Because the phantom is much lighter than a patient multiply this by 10 and enter the result as the patient weight. For example, for a 5600 mL phantom enter 56 kg.

Image Reconstruction

Once the scan is complete, reconstruct the images using the protocol that will be used for patient studies. All parameters should be exactly as they are for patients with one exception: the reconstructed pixel size should be approximately 1 mm. This can be achieved by increasing the matrix size and/or decreasing the diameter of the reconstruction. For example, changing the diameter from 60 cm to 25.6 cm and changing the matrix size from 128 to 256 will change the

APPENDIX D: PET/CT Imaging Guidelines

pixel size from 4.687 mm to 1.0 mm. Please make sure that no other parameters inadvertently change when you alter the diameter or the matrix size. *Please answer the following questions:*

8) *Type of Scanner (manufacturer & model):*

9) *Scanner Accredited / Qualified: (Y / N)*

    a. *Accrediting / Qualifying Organization:*

    b. *Date of Accreditation / Qualification:*

10) *Reconstruction algorithm:*

11) *Iterations:*

12) *Subsets:*

13) *Smoothing filter:*

14) *Other filter parameters:*

15) *Reconstruction diameter:*

16) *Matrix size:*

Data Transfer

Please write all PET, CT and CT scout image data in DICOM Part 10 format to a CD. Please send this CD, along with a copy of this completed **PET/CT Imaging Quality Assurance Manual** and the requested sample clinical cases, to the coordinating center at the following address:

Stacie Jeter, CCRP
Senior Clinical Research Program Manager
Breast Cancer Research Program
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Bunting-Blaustein Cancer Research Building
1650 Orleans Street, CRB-1 186
Baltimore, MD 21231-1000
410 614-6138 (phone)
410 614-9421 (fax)
sjeter1@jhmi.edu

Questions

If you have any questions about this phantom experiment, please contact Martin Lodge at mlodge1@jhmi.edu or (410) 614-2416.

APPENDIX D: PET/CT Imaging Guidelines

PET/CT Imaging Appendix

A Phase 2 Clinical Trial Using Early Changes in Standardized Uptake Value (SUV) on Positron Emission Tomography (PET) to Guide Anti-HER2 Therapy with Perfuzumab and Trastuzumab Alone or in Combination with Paclitaxel for Women with Primary Operable HER2-Positive Breast Cancer

Protocol: TBCRC026

Acquisition Protocol Guidelines for ^{18}F-FDG-PET/CT Scans

The following ^{18}F-FDG PET imaging protocol recommendations should be followed as much as possible, without contravening any site specific protocol procedures, in order to strive for a high-level of consistency between multi-site data. At sites, or in situations, where significant deviation from the recommended protocol is required, please document such deviations on the **PET/CT Exam Information Form** (found at the end of this appendix) and aim for consistency between baseline and follow-up examination procedures above all other considerations.

1) Image Acquisition
   a) Patient Preparation
      i) Prior to imaging appointment
         (1) Patient should be instructed to fast for a minimum of 4 hours prior to imaging
         (2) Patient should be instructed to refrain from exercise for 24 hours prior to imaging
         (3) Patient should be encouraged to drink water
         (4) Claustrophobic patients should be instructed to bring appropriate medications with them to the imaging center. Document any sedative medication taken, or self-reported to have been taken, by the patient in the comments section of the **PET/CT Exam Information Form**.
         (5) If patient is diabetic, then consult with a Nuclear Medicine Physician prior to the PET study to coordinate site-specific requirements/procedures for scheduling and imaging of diabetic patients. Recommended procedures follow (though at sites with different policies already in place, consistency in policy practice between baseline and follow-up studies should take precedence).
            (a) If taking insulin, consult with physician prior to PET study
            (b) NPO after midnight
            (c) Check blood sugar in AM to verify below 200 mg/dL
            (d) Diabetic patient should preferentially be given early AM appointment
      ii) At imaging appointment, prior to administration of radiotracer
         (1) Obtain a blank **PET/CT Exam Information Form** (found at the end of this appendix) which should be filled-in by the individual responsible for the administration of the imaging exam.
         (2) Patient should change into gown and pants, removing all metal objects (including bra)
         (3) Patient height / weight should be measured and recorded.
         (4) Patient serum glucose concentration should be measured and recorded. If found to be greater than 150 mg/dL, or less than 50 mg/dL, immediately notify on-site Nuclear Medicine Physician for guidance before proceeding with imaging.

APPENDIX D: PET/CT Imaging Guidelines

(5) Female patients younger than 55 years of age should be asked if they are pregnant or if breastfeeding. Site specific policies should be followed for patients answering in the affirmative.

(6) Patient should be placed in a comfortable position.

(7) If the referring physician has ordered an IV contrast exam, follow on-site protocols and procedures. Document administration of IV contrast and any steroidal agent the patient may have taken in advance of the imaging procedure on the **PET/CT Exam Information Form.**

(8) Insert an angiocatheter
   (a) Where indicated, insert angiocatheter contra lateral to primary tumor
   (b) Maintain IV access through to study completion

(9) Administer Oral Contrast (unless otherwise contra-indicated)
   (a) Preferred oral contrast agent is READI-CAT 2 (barium sulphate suspension, 2.1% w/v), though site specific preferences shall prevail.
   (b) Total dose shall be halved and administered along with enough water to provide for 500ml of liquid at each administration
   (c) Each administration shall occur at the following time points:
      (i) 15 minutes prior to injection
      (ii) 15 minutes prior to scanning
   (d) Oral contrast, as well as any additives (e.g. flavoring), shall not contain glucose.

iii) Administration of ^{18}F-FDG radiotracer
   (1) Dosage
      (a) A standard dose of 0.22 mCi/kg, with a maximum dose of 25 mCi, is recommended. An allowed range of dosing of 0.1 - 0.25 mCi/kg is acceptable following consultation with a Johns Hopkins Nuclear Medicine collaborator. Deviations from the recommended dosage should be documented on the **PET/CT Exam Information Form.** Consistency in dosing regimen between baseline and follow-up scans should be followed (± 20% between baseline and follow-up study).

iv) Uptake
   (1) Instruct patient to sit quietly and to remain still for the duration of the uptake phase
   (2) Dim room lights
   (3) Allow 45 minutes for ^{18}F-FDG uptake
   (4) Administer final dose of oral contrast
   (5) Ask patient to empty bladder just prior to imaging
   (6) Imaging should begin between 50-70 minutes post-injection of radiotracer.

v) Scan Protocol (Site and/or camera specific requirements may differ from these recommendations. In those cases, consistency in scan protocols between baseline and follow-up scans should take precedence)
   (1) Recommended patient positioning guidelines
      (a) Position patient head first and supine with arms above head (if possible).
      (b) Landmark at patient's forehead and iso-center of scanner

APPENDIX D: PET/CT Imaging Guidelines

PET/CT Imaging Appendix

    (2) Acquisition
        (a) Acquire images using site-specific camera acquisition protocols for standard whole body Oncologic imaging. Use identical imaging protocol, including identical scanner, for both baseline and follow-up imaging procedures. Document PET emission acquisition mode (2D or 3D) on **PET/CT Exam Information Form**.
        (b) The Imaging FOV should include the area from mid-thigh to skull base.
        (c) PET Emission imaging should proceed in the direction of mid-thigh up to skull base.
    (3) Image Reconstruction
        (a) Reconstruct PET Emission images both with and without attenuation correction.

2) Image Analysis
  a) An Image Data Set shall be defined as including the following (additional acquisitions or data sets should be included if site-specific acquisition and/or reconstruction protocols provide for them):
    i) CT Modality Series
      (1) Scout image
      (2) Attenuation Correction Map / Anatomical localization map images
      (3) Diagnostic images
    ii) PET Modality Series
      (1) Attenuation corrected images
      (2) Non-Attenuation corrected images
    iii) Non-image data required for PET SUV calculations (This information should be recorded on the **PET/CT Exam Information Form** and may also be included in the image headers)
      (1) Radioisotope dose information, including
        (a) Dose Assay (required)
        (b) Time of Dose Assay (required)
        (c) Time of Dose Administration (required)
        (d) Time of Image Scan (required)
        (e) Post-injection (Residual) Dose Assay
        (f) Time of measurement of Post-Injection Dose Assay
      (2) Patient Height (measured at time of imaging)
      (3) Patient Weight (measured at time of imaging)
      (4) Serum Blood Glucose level (measured at time of imaging)
  b) Format
    i) Image data shall be in the DICOM Part 10 format
    ii) CDs meant for distribution to patients with included image viewers are generally not acceptable as they usually do not include the data in the original DICOM Part 10 format but instead use proprietary or screen capture formats. **Original (transaxial) data is required for quantitative analysis at study coordinating center.**
  c) Demarcation
    i) Image data and accompanying materials shall be scrubbed of PHI (Protected Health Information) and re-coded with study specific identifiers as specified by the study coordinator. If it is not possible to de-identify the data before distribution to the study

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APPENDIX D: PET/CT Imaging Guidelines

coordinating site, the study coordinating center will de-identify the data and destroy the original PHI before accepting the data set into the study database.

d) Data Transmission

    i) For each study, a copy of the above described image data shall be burned to a CD in the specified format and, along with a completed **PET/CT Exam Information Form**, sent to the following address for inclusion and analysis in this study:

Stacie Jeter, CCRP
Senior Clinical Research Program Manager
Breast Cancer Research Program
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Bunting-Blaustein Cancer Research Building
1650 Orleans Street, CRB-1 186
Baltimore, MD 21231-1000
410 614-6138 (phone)
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sjeter1@jhmi.edu
ATTN: TBCRC026