MARGetuximab Or Trastuzumab (MARGOT):
A phase II study comparing neoadjuvant paclitaxel/margetuximab/pertuzumab to paclitaxel/trastuzumab/pertuzumab in patients with Stage II-III HER2-positive breast cancer

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IND Agent: Margetuximab (Margenza™) *supplied by TerSera Therapeutics LLC (formerly supplied by MacroGenics)*
Other Agents: paclitaxel, nab-paclitaxel, trastuzumab, pertuzumab, trastuzumab and pertuzumab FDC (PHESGO) *commercially supplied*

IND #: 145981
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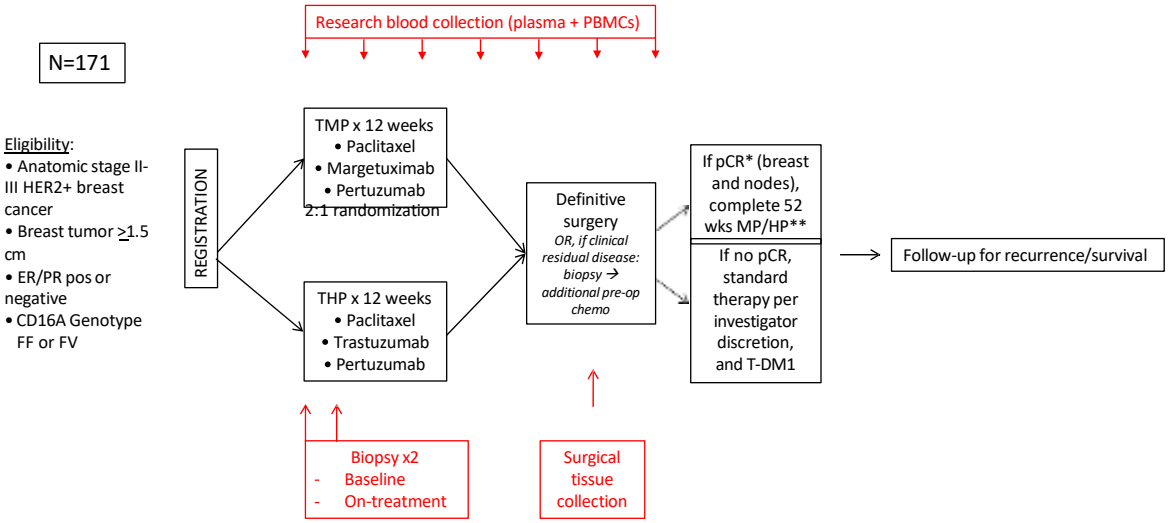
SCHEMA



**pCR defined as: RCB 0*

***concurrent endocrine tx allowed if HR+*

Adjuvant radiation per standard institutional procedure

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1. OBJECTIVES

1.1 Study Design

This is a randomized open-label phase II trial comparing paclitaxel/margetuximab/pertuzumab (TMP) to paclitaxel/trastuzumab/pertuzumab (THP) in patients with anatomic stage II-III HER2+ breast cancer who have the CD16A low affinity genotype (FF or FV). Eligible patients will have anatomic stage II-III (according to AJCC 8th edition anatomic staging table) HER2+ breast cancer. Patients may have any hormone receptor status, may be either pre- or post-menopausal, and must be treatment-naïve for this cancer. The FF or FV CD16A genotype will be confirmed by central testing in all patients.

At least 171 eligible patients will be randomized 2:1 to neoadjuvant TMP (Arm A, approximately 114 patients) or THP (Arm B: approximately 57 patients). All patients will receive 4 cycles of neoadjuvant therapy (1 cycle=21 days). Patients will be stratified by ER status (<10% vs ≥10%) and clinical anatomic stage (II vs III).

Arm A (n=114)

- paclitaxel 80 mg/m2 IV D1, D8, D15
- pertuzumab 840 mg initial, 420 mg subsequent IV D1
- margetuximab 15 mg/kg IV D1

Arm B (n=57)

- paclitaxel 80 mg/m2 IV D1, D8, D15
- pertuzumab 840 mg initial, 420 mg subsequent IV D1
- trastuzumab 8mg/kg initial, 6 mg/kg subsequent IV D1

Pathologic response will be assessed at surgery, per standard clinical practice. Patients with pCR (defined as Residual Cancer Burden (RCB) = 0) will complete one year of their assigned MP or HP therapy in the adjuvant setting, and additional chemotherapy is not recommended. Patients who do not achieve pCR will receive additional/alternative adjuvant therapy of the investigator's choice (four cycles of adjuvant adriamycin/cyclophosphamide (AC) chemotherapy followed by 14 cycles of adjuvant trastuzumab-emtansine (T-DM1) or 14 cycles of adjuvant T-DM1 alone, depending on extent of residual disease are recommended, but not mandated). In all patients with hormone receptor-positive (HR+) disease, any adjuvant hormonal therapy may be given at the investigator's discretion. All patients will be followed for recurrence and survival events.

Given that Margetuximab has not been previously combined with Paclitaxel or Pertuzumab, the first 6 patients randomized to TMP will be monitored and assessed for safety. Should ≥ 4 of the 6 patients experience a dose limiting toxicity (DLT) the TMP treatment regimen will be modified or discontinued. See Section 5.3 for more information.

1.2 Primary Objectives

To compare rate of pathologic complete response (pCR, defined as RCB 0) in patients with the

FF or FV CD16A genotype and anatomic stage II-III HER2+ breast cancer treated with 4 cycles of neoadjuvant TMP or THP

1.3 Secondary Objectives

- To compare rate of pCR (RCB 0) in patients treated with TMP or THP, according to hormone receptor-positive (HR+) or hormone receptor-negative (HR-) status.
- To assess Residual Cancer Burden (RCB) scores[1] in patients treated with TMP or THP, overall and according to HR+ or HR- status.
- To assess safety and tolerability of THP and TMP in the neoadjuvant and adjuvant setting.
- To measure event-free survival (EFS), recurrence-free interval (RFI), and overall survival (OS) in the overall study population.
- To compare EFS, RFI, and OS in the following subgroups:
    - Patients with pCR versus patients without pCR
    - Patients with RCB 0 or 1, versus patients with RCB 2 or 3
    - Patients randomized to neoadjuvant TMP, versus patients randomized to neoadjuvant THP

1.4 Correlative, Patient-Reported Outcomes, and Exploratory Objectives

- To describe patient and physician considerations and decisions in choice of adjuvant therapy following preoperative treatment with THP or TMP.
- To assess use of protocol-specified antibody doublet therapy only (MP or HP) without further cytotoxic chemotherapy in the adjuvant setting, among patients with stage II-III HER2+ breast cancer who achieve pCR following neoadjuvant TMP or THP.
- To assess humoral and cellular immune responses to TMP versus THP, in both tissue and blood.
- To assess radiographic response to neoadjuvant therapy in patients treated with TMP or THP, overall and according to HR+ or HR- status.
- To compare rate of pCR (RCB 0) in patients treated with TMP or THP, according to FF genotype or FV genotype.
- To determine the feasibility of sentinel lymph node mapping and biopsy after preoperative therapy in this cohort.
- To determine the incidence of additional nodal disease among patients in this cohort with positive sentinel lymph node(s), overall and stratified by size of largest sentinel lymph node metastasis.
- To describe symptoms and quality of life (both physical and mental health) during neoadjuvant TMP and THP.
- To compare mean changes in physical health, mental health and fatigue from baseline to the pre-operative visit at the end of neoadjuvant therapy with TMP or THP.
- To describe symptoms and QOL over time during therapy with TMP and THP.
- To explore the structure and function of the gut microbiome

2. BACKGROUND

2.1 Study Disease: HER2-positive breast cancer and neoadjuvant therapy

HER2, also known as *neu* and *c-erbB-2*, is an oncogene encoding a tyrosine kinase growth factor receptor in the family of the epidermal growth factor receptor (EGFR) and is amplified in approximately 20% of all human breast cancers. It is an independent predictor of time to relapse and overall survival in multivariable models, and is a marker of poor prognosis.[2] The functional significance of HER2 amplification in breast cancer prompted development of trastuzumab, a monoclonal anti-HER2 antibody that was the first agent developed to target the HER2 pathway. Addition of trastuzumab to standard chemotherapy produces an overall survival benefit in HER2-amplified metastatic breast cancer.[3] Similarly, trastuzumab plus chemotherapy is superior to chemotherapy alone in multiple large, randomized trials of adjuvant therapy in HER2-positive disease, resulting in a striking reduction in the risk of relapse and death by 50% and 30%, respectively.[4-7] These results led to the approval of trastuzumab for use in the early stage disease setting.

In addition to large registrational studies, clinical trials conducted in the preoperative setting have contributed to the understanding of the activity of anti-HER2 therapies with the ability to provide an early read-out of drug efficacy. In the "first generation" of neoadjuvant anti-HER2 trials, patients were randomized to receive chemotherapy with or without trastuzumab.[8-10] These seminal studies confirmed the superiority of neoadjuvant trastuzumab in combination with chemotherapy, showing pCR rates at least two times higher in the trastuzumab containing arms (summarized in Table 1). In the "second generation" of neoadjuvant anti-HER2 trials, patients were randomized to receive one of two different anti-HER2 agents or to receive trastuzumab with or without another anti-HER2 agent in an effort to further improve the clinical benefit achieved with trastuzumab alone (Table)[11-13].

Table: Representative first and second generation neoadjuvant trials of HER2-targeted therapies

Studies and sample size	Study design	pCR * rates, p values
MD Anderson [8] N = 64	1) T→FEC 2) T + H→FEC + H	26.3 60 (P = NR)
NOAH [9] N = 235	1) AT→T→CMF 2) AT +H→T+ H→CMF +H	19 38 (P = 0.001)
NeoALTTO [14] N = 455	1) H → T + H → surgery → FEC → H until w 52 2) L → T → surgery → FEC → L until w 52 3) H + L → T + H + L → surgery → FEC → H + L until w 52	29.5 24.7 (2 vs. 1; P = 0.34) 51.3 (3 vs. 1; P = 0.0001)

NeoSphere [15] N = 417	1) H + D → surgery → FEC → H until w 52	29.0
	2) H + PZ + D → surgery → FEC → H until w 52	45.8 (2 vs. 1; P = 0.0141)
	3) H + PZ → surgery → H + D → FEC → H until w 52	16.8 (3 vs. 1; P = 0.019)
	4) PZ + D → surgery → FEC → H until w 52	24.0 (4 vs. 2; P = 0.03)
NSABP B41 N = 529 [16]	1) AC → T + H → surgery → H until w 52	52.5
	2) AC → T + L → surgery → H until w 52	53.2 (2 vs. 1; P = 0.99)
	3) AC → T + H+ L → surgery → H until w 52	62.0 (3 vs. 1; P = 0.09)
CALGB 40601 N = 301 [17]	1) T + H → surgery → ddAC → H until w 52	46.0
	2) T + L → surgery → ddAC → H until w 52	37.0 (2 vs. 1; P =0.12)
	3) T + H + L → surgery → ddAC → H until w 52	56.0 (3 vs. 1; P = 0.12)
GeparQuinto [18] N = 620	1) EC + H → D + H → surgery	31.3
	2) EC + L → D + L → surgery	21.7 P < 0.05

Abbreviations: A = doxorubicin; C = cyclophosphamide; D= docetaxel; E = epirubicin; F = fluorouracil; H = trastuzumab; L = lapatinib; NR = not reported; PZ = pertuzumab; pCR = pathologic complete response; T = paclitaxel; X = capecitabine. Comments: MD Anderson and NOAH studies have chemotherapy-only arms compared with chemotherapy plus trastuzumab (first generation studies). NeoALTTO, NeoSphere, GeparQuinto, NSABP-B41 and CALGB 40601 represent the second generation of neoadjuvant studies with anti-HER2 regimens in all study arms. pCR definitions: MD Anderson – No evidence of invasive cancer in breast or axilla; NOAH – Total pCR in breast and axillary nodes; NeoALTTO – non invasive cancer in the breast or only non invasive in situ cancer; NeoSphere – pathologic complete response in the breast GeparQuinto – no microscopic evidence of residual viable cells in any specimen.

While these second-generation neoadjuvant studies hold considerable promise, it must be acknowledged that the pCR definitions used across these trials were not homogeneous (pCR definitions are described in the Table), limiting cross-trial analysis.

Over the past years there have been significant improvements in our understanding of the biology of HER2-positive disease and several novel anti-HER2 drugs have been approved for the treatment of HER2-positive breast cancer.

Pertuzumab, another HER2-targeted humanized monoclonal antibody, represents the first of a novel class of drugs that have the ability to block the heterodimerization of HER2 with other members of the HER family (e.g., HER1, HER3). The resulting complementary and enhanced efficacy of HER2 blockade that is provided by the combination of trastuzumab plus pertuzumab has been demonstrated in both the preoperative, adjuvant, and metastatic settings where this combination of antibodies along with chemotherapy leads to improved outcomes compared with trastuzumab and chemotherapy.[19-21]

2.2 Mechanism of action of trastuzumab

Multiple mechanisms of action have been proposed for trastuzumab including activation of both innate and adaptive cellular immunity.[35] In vivo models have confirmed antibody-dependent

cell-mediated cytotoxicity (ADCC) as one mechanism of action with efficacy correlating with the presence of NK cells. [36,37] ADCC involves the Fc-gamma receptor (FcγR) on NK cells or macrophages cross-linking cell-bound antibodies (i.e. trastuzumab) leading to release of granzyme and perforin into the synapse, promoting apoptosis.

The Fc-gamma RIIIA (CD16) stimulatory receptor is encoded by two alleles that differ at amino acid 158: a V allele (valine; higher affinity) and an F allele (phenylalanine; lower affinity).[22] A retrospective analysis of the NSABP-B31 trial of adjuvant AC-T +/- trastuzumab demonstrated greater benefit for trastuzumab in patients with at least one high affinity (V) CD16A allele, compared to patients homozygous for the low affinity (F) allele.[39] This result suggests that those patients with at least one low affinity CD16A allele, which make up approximately 85% of the population, may not receive maximal efficacy from trastuzumab-based therapy and that they may benefit from use of an alternative agent.

2.3 IND Agent: Margetuximab

2.3.1 Background on margetuximab

Margetuximab is a monoclonal anti-HER2 antibody similar to trastuzumab but with five amino acid substitutions engineered into the IgG1 Fc domain to allow increased ADCC and decreased inhibition of immune effector cells, thereby optimizing tumor cell killing.[22]

Margetuximab is a mouse-human chimeric IgG1 anti-HER2 antibody based on the precursor to trastuzumab (mouse clone 4D5).[23] It was engineered to maintain the antigen-binding properties of the original antibody (4D5 V region), while optimizing its interactions with Fcγ receptors (FcγRs), important mediators of antibody function in vivo. MGFc0264, the engineered human IgG1 Fc domain of margetuximab, differs from that of wild type human IgG1 at 5 amino acid residues. The biochemical properties imparted by the engineered Fc domain have translated into favorable biological improvements compared with trastuzumab in terms of enhanced antitumor activity against HER2-expressing tumor cell lines in vitro and in mouse xenograft models.

The biochemical and biological properties of margetuximab were compared with those of RES120, a chimeric anti-HER2 mAb that is identical to margetuximab except for containing a wild type human IgG1 Fc domain, used here as a surrogate for trastuzumab. In selected in vitro studies, margetuximab was also compared to trastuzumab and pertuzumab.
In vitro studies were conducted to assess binding to recombinant human HER2 protein and to recombinant human, cynomolgus monkey, and murine Fcγ receptors. Studies were conducted with HER2-expressing tumor cell lines to assess margetuximab's ability to mediate Fc-independent activities (inhibition of proliferation; inhibition of HER2 extracellular domain (ECD) shedding) and Fc-dependent activities (ADCC activity; natural killer (NK) cell activation). Studies with human peripheral-blood mononuclear cells (PBMC) were also conducted to assess the potential for cytokine release.

As noted above, the Fc-gamma RIIIA (CD16) stimulatory receptor is encoded by two alleles that differ at amino acid 158: a V allele (valine; higher affinity) and an F allele (phenylalanine; lower affinity).[22] In vivo efficacy studies were conducted in a mouse xenograft model that utilized HER2-expressing tumor cells implanted in mCD16$^{-/-}$ (murine CD16 knockout) mice that were transgenic (Tg) for human CD16A-158F, which encodes the lower affinity allotype. The mCD16$^{-/-}$ hCD16A Tg mouse model was developed to overcome an inconsistency in differential binding between the wild type and engineered Fc domains to murine CD16 compared to human CD16A, and thus provide a setting for the enhanced properties of the engineered Fc domain of margetuximab, optimized for human FcγR interaction, to be assessed.

Single-dose and multi-dose toxicology/toxicokinetic (TK) studies were conducted in cynomolgus monkeys, which express both target antigen and FcγRs that are relevant for modeling margetuximab. The multi-dose studies were conducted under Good Laboratory Practice (GLP) compliance. These studies included TK parameters, assessments of subsets of circulating blood cells by flow cytometry, measurements of natural killer (NK) cell activity, and determination of serum cytokine levels.

Cross reactivity studies, conducted under GLP compliance, were performed with panels of human and cynomolgus monkey tissues. A study directly comparing the cross-reactivity of margetuximab and trastuzumab was included. The nonclinical pharmacology, TK, and toxicology data support the clinical development of margetuximab for the treatment of HER2-positive cancers. The antitumor activity and toxicokinetics analysis of margetuximab is summarized in a paper by Nordstrom et al.[24]

2.3.2 Clinical studies of margetuximab

As of a data cut-off date of 23 February 2019, margetuximab has been, or is currently being, evaluated in 4 TerSera-sponsored clinical studies (2 ongoing, 2 completed) and an ongoing Expanded Access Program (EAP) of individually approved, single-patient IND studies providing margetuximab in single, individually approved patients. Brief overviews of these studies and of the EAP are provided in the table, below.

Study No. Phase	Objective/Design	Population N (Treated)	Dosage/Frequency
Ongoing Studies			
CP-MGAH22-04 Phase 3	Randomized, comparator-controlled study to evaluate efficacy of margetuximab plus chemotherapy compared to trastuzumab plus chemotherapy Infusion sub-study cohort	Adult patients with advanced HER2+ breast cancer who have received at least 2 prior lines of anti-HER2 directed therapy, including pertuzumab, and 1-3 prior lines of therapy in the metastatic setting. (N = 530; 264 margetuximab + chemo; 266 trastuzumab + chemo) Infusion sub-study: non-randomized cohort to evaluate safety and tolerability of reduced infusion duration from 120 minutes in Cycle 1 to 30 minutes from Cycle 2 forward. Patients eligible for the sub-study must have received at least 4 prior lines of therapy for metastatic disease and progressed after the most recent therapy. (N = 42 of approximately 78 planned; 9 margetuximab monotherapy and 33 margetuximab + chemotherapy).	Margetuximab: 15 mg/kg IV Q3W Trastuzumab: IV Q3W; 8 mg/kg loading dose, then 6 mg/kg Chemotherapy (one of the following as per local regulation): • Capecitabine: 1000 mg/m^2, PO, BID 14 days Q3W cycle, • Eribulin: 1.4 mg/m^2, IV, Days 1 and 8 of Q3W cycle • Gemcitabine: 1000 mg/m^2, IV, Days 1 and 8 of Q3W cycle • Vinorelbine: 25-30 mg/m^2, IV, Days 1 and 8 of Q3W cycle
CP-MGAH22-05 Phase 1b/2	Open-label dose escalation and cohort expansion study to evaluate safety, tolerability, PK, PD, immunogenicity, and preliminary antitumor activity of margetuximab plus pembrolizumab	Adult patients with relapsed or refractory advanced HER2+ GEJ or gastric cancer (N = 95)	Margetuximab: 10 mg/kg (Cohort 1) and 15 mg/kg (Cohort 2) IV Q3W Pembrolizumab: 200 mg IV Q3W

Study No. Phase	Objective/Design	Population N (Treated)	Dosage/Frequency
EAP (NA)	Single-patient, individually approved Expanded Access, single-patient INDs	HER2+ metastatic breast cancer (N = 5)	15 mg/kg IV Q3W
Completed Studies			
CP-MGAH22-01 Phase 1	Single-arm, open-label, dose escalation and expansion study to evaluate safety, immunogenicity, PK, and potential antitumor activity	Adult patients with refractory HER2+ breast cancer or other HER2+ carcinomas (N = 66)	0.1, 0.3, 1.0, 3.0, 6.0 mg/kg IV QW for 4 weeks (Cycle 1); QW for 3 weeks and then every 4 weeks thereafter 10.0, 15.0, 18.0 mg/kg IV Q3W
CP-MGAH22-02 Phase 2	Single-arm, open-label evaluation of antitumor activity and toxicity	Adult patients with relapsed/refractory advanced HER2+ (2+ by IHC and lack HER2 amplification by FISH or 1+ by IHC and ≥10.5 by HERmark®) breast cancer (N = 25)	6.0 mg/kg IV QW (original protocol) 15 mg/kg IV Q3W (Amendment 2)

Abbreviations: BID = twice daily; DLT = dose-limiting toxicity; EAP = Expanded Access Program; FISH = fluorescence in situ hybridization; GEJ: gastroesophageal junction; HER2 = human epidermal growth factor; IHC = immunohistochemistry; IND = Investigational New Drug (application); IV = intravenous; MTD = maximum tolerated dose; PD = pharmacodynamics; PK = pharmacokinetics; PO = by mouth; QW = weekly; Q3W = every 3 weeks.

2.3.3 Phase III study of margetuximab in HER2+ breast cancer

The SOPHIA study, MacroGenics study CP-MGAH22-04, is a Phase 3 randomized, comparator-controlled study of margetuximab plus chemotherapy for the treatment of patients with HER2+ metastatic breast cancer who had received at least 2 prior lines of anti-HER2 directed therapy in the metastatic setting, or in case of having received (neo)adjuvant pertuzumab, at least 1 prior line of anti-HER2 directed therapy in the metastatic setting, and who had received at least 1,and no more than 3, lines of therapy overall in the metastatic setting. Eligible patients were randomized 1:1 to receive either margetuximab (15.0 mg/kg IV Q3W) or trastuzumab (8 mg/kg loading dose, 6 mg/kg subsequent doses, IV Q3W) to be administered in combination with chemotherapy (capecitabine, eribulin, gemcitabine, or vinorelbine) of the investigator's choice and as allowable per local regulations. Patients received treatment until disease progression, death, withdrawal of consent, or request by the treating physician to discontinue treatment. Following completion of (or discontinuation from) treatment, patients are followed for survival.

Primary results from the SOPHIA study were reported at the American Society for Clinical Oncology Annual Meeting in June 2019.[25] The study met its primary endpoint of progression-free survival (PFS) in the intention to treat population, with PFS 5.8 months in the margetuximab + chemotherapy arm versus 4.9 months in the trastuzumab + chemotherapy arm (hazard ratio 0.76, p=0.033). Of note, the benefit of margetuximab appeared confined to the patients with the low-affinity CD16 allele; the hazard ratio was 0.68 (95% CI, 0.52–0.90) in the 86% of patients with either a FF or FV genotype, and 1.78 (95% CI, 0.87–3.62) in the 14% of patients with the VV genotype. Similar allele-based differences in margetuximab benefit were apparently observed in an interim analysis of overall survival, although these results are not yet publicly available. This differential activity is consistent with the preclinical data suggesting that superiority of margetuximab over trastuzumab is most apparent in the context of low affinity Fc receptors that do not bind trastuzumab well.

In the SOPHIA study, the toxicity of margetuximab was acceptable, and generally similar to trastuzumab, with an increased incidence of low-grade infusion-related reactions in patients receiving margetuximab (though notably, all patients in the trial had previously received trastuzumab).

2.4 Other Agents

2.4.1 Trastuzumab and Biosimilars

Refer to the Full Prescribing Information for trastuzumab or biosimilars for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ in addition to information provided in Sections 5 and 8 of the protocol.

2.4.2 Paclitaxel

Paclitaxel is a chemotherapy agent in the taxane class.

Refer to the Full Prescribing Information for paclitaxel for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ in addition to information provided in Sections 5 and 8 of the protocol.

2.4.3 Pertuzumab

Pertuzumab (Perjeta ®) is a recombinant monoclonal antibody that binds to the dimerization domain of HER2.
Refer to the Full Prescribing Information for Pertuzumab for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ in addition to information provided in Sections 5 and 8 of the protocol.

2.4.4 Trastuzumab and pertuzumab FDC SC (PHESGO)

A subcutaneous fixed dose combination of trastuzumab and pertuzumab (PHESGO) may be used instead of intravenous HP in the adjuvant setting for Arm B patients who achieve pCR at time of surgery.
Refer to the Full Prescribing Information for pertuzumab, trastuzumab, and hyaluronidase-zzxf injection (PHESGO) for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ in addition to information provided in Sections 5 and 8 of the protocol

2.4.5 Nab-paclitaxel (Abraxane)

Protein-bound paclitaxel is paclitaxel bonded to albumin as a delivery vehicle.
Refer to the Full Prescribing Information for abraxane for complete safety information: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/ in addition to information provided in Sections 5 and 8 of the protocol.

2.5 Rationale

2.5.1 Use of margetuximab in the neoadjuvant setting

Margetuximab is a monoclonal anti-HER2 antibody similar to trastuzumab but with five amino acid substitutions engineered into the IgG1 Fc domain to allow increased antibody-dependent cell-mediated cytotoxicity (ADCC) and decreased inhibition of immune effector cells, thereby optimizing tumor cell killing.[22] Recent results from the randomized phase III SOPHIA study (NCT02492711) of margetuximab plus chemotherapy versus trastuzumab plus chemotherapy for patients with refractory metastatic HER2+ breast cancer demonstrated a significant improvement in progression-free survival for patients receiving margetuximab (see protocol Section 2.2.3 for more details).[25] The improved clinical activity of margetuximab compared to trastuzumab in patients with metastatic HER2+ breast cancer raises the question of whether margetuximab will also demonstrate improved activity in non-metastatic HER2+ breast cancer. This possibility is of particular interest given that other agents acting through immunologic mechanisms appear to generally have greater activity in earlier stages of disease.

2.5.2 De-escalation of adjuvant therapy following pathologic complete response in HER2+ breast cancer

In this protocol, we plan to examine de-escalation of therapy from a multi-agent to a single-agent chemotherapy backbone plus HP or MP in patients with stage II-III HER2+ breast cancer, based on pCR as a prognostic biomarker. At present, standard-of-care neo/adjuvant therapy for stage II-III HER2+ breast cancer involves either adriamycin/cyclophosphamide followed by taxane plus trastuzumab (AC-TH) or taxane/carboplatin/trastuzumab concurrently (TCH), with consideration of pertuzumab in all patients.[26] These multi-agent chemotherapy regimens are associated with multiple short-and long-term toxicities including alopecia, fatigue, neuropathy, cytopenias, cardiomyopathy, and secondary leukemia,[6] with impacts ranging from common but temporary reduction in quality of life to rare and life-threatening conditions.

Though HER2+ breast cancer was historically associated with a worse prognosis than other types of breast cancer,[2] in recent years there has been explosive development and FDA approval of numerous effective HER2-targeted therapies. In 2017 the large majority of patients with non-metastatic HER2+ disease is cured with modern regimens incorporating chemotherapy plus HER2-directed therapy. In the recently published APHINITY trial of adjuvant multi-agent chemotherapy plus trastuzumab with or without pertuzumab, 3-year invasive-disease-free survival was 93-94% for all patients, and 90-92% in patients with node-positive disease.[21] Clearly, ongoing efforts must strive to understand the patients for whom HER2+ breast cancer recurs despite these highly active regimens, and additional treatment options are needed for those patients. At the same time, the excellent long-term outcomes of most patients treated with modern regimens for non-metastatic HER2+ disease mean that we must begin to thoughtfully identify patients who can be cured with less toxic, less intensive therapy.

We plan to investigate pCR-based de-escalation: selecting patients who are likely to have excellent long-term outcomes with less treatment, on the basis of pCR following an effective neoadjuvant regimen. De-escalating therapy on the basis of pCR makes sense for a number of reasons: Pathologic complete response at surgery provides real-time, concrete evidence of response to a given therapy for a given patient; and it is widely recognized as a positive prognostic biomarker in breast cancer.[27-29] If pCR-based de-escalation is found to be a feasible strategy that maintains excellent long-term outcomes for patients with HER2+ breast cancer, it has the potential to spare a select group of breast cancer patients the short- and long-term toxicities of multi-agent chemotherapy, without compromising their long-term survival.

Supporting the safety of a de-escalation strategy for patients who achieve pCR on this trial, prior evidence demonstrates excellent outcomes following pCR to neoadjuvant therapy in HER2+ breast cancer, regardless of neoadjuvant regimen received. For example, in the phase III KRISTINE study of neoadjuvant T-DM1 plus P for HER2+ breast cancer (a non-standard and entirely chemotherapy-sparing regimen), the 3-year IDFS event rate was similar and excellent among patients who achieved pCR following neoadjuvant T-DM1/P (96.7% 3 year IDFS) versus those patients who achieved pCR following neoadjuvant TCHP (docetaxel/carboplatin/HP—a standard, chemotherapy-based regimen; 3 year IDFS 97.5%). These excellent outcomes post-pCR were seen in the chemotherapy-sparing regimen despite the fact that only a small minority (9.1%) of those patients who achieved pCR after T-DM1+P received additional adjuvant chemotherapy.[30] Similarly, in the I-SPY2 trial of a variety of chemotherapy-sparing neoadjuvant regimens for HER2+ tumors,

3 year distant disease-free survival was 98% among patients who achieved pCR following neoadjuvant therapy, in a combined analysis that incorporated many heterogeneous chemotherapy-sparing neoadjuvant regimens.[31]

2.5.3 Choice of regimen: neoadjuvant paclitaxel/trastuzumab/pertuzumab (THP) or paclitaxel/margetuximab/pertuzumab (TMP)

On this prospective protocol, de-escalated therapy patients (i.e. those that achieve pCR) will receive only single-agent chemotherapy with paclitaxel (T) in addition to trastuzumab/pertuzumab or margetuximab/pertuzumab. The rationale for incorporation of margetuximab into the experimental arm is addressed above. THP is an optimal de-escalated regimen for a number of reasons. First, regimens incorporating doublet HER2-directed monoclonal antibodies (trastuzumab plus pertuzumab) show high levels of activity in both metastatic and non-metastatic HER2+ breast cancer, and improved outcomes compared to H alone.[20,21,32] Indeed, in the phase II NeoSphere trial, 16.8% of patients treated with HP alone in the neoadjuvant setting had pathologic complete response (pCR) at surgery.[20] Second, in stage I HER2+ breast cancer, long-term outcomes are very favorable with paclitaxel plus trastuzumab, and this single-agent chemotherapy backbone is now standard-of-care in that setting.[33]

Although the taxane chemotherapy backbone used with HP in the NeoSphere and TRYPHAENA studies (both evaluating neoadjuvant taxane plus HP) was docetaxel, national guidelines support the use of paclitaxel over docetaxel.[26] Safety data from a prospective clinical trial of metastatic HER2+ breast cancer patients treated with paclitaxel plus HP suggest that it is associated with less toxicity than a docetaxel-based regimen.[34]

2.5.4 Screening for CD16 genotype and restricting study to those patients with the low affinity allele (FF or FV)

As noted above, subgroup analysis of the clinical data from the phase 3 SOPHIA trial suggest that the superiority of margetuximab over trastuzumab is restricted to those patients who have a low affinity CD16 genotype (FF or FV), and that margetuximab may even be inferior to trastuzumab in those patients with the high affinity genotype (VV). While this differential activity of margetuximab based on genotype has not been validated yet in another study and may be spurious, given that this trial will enroll a population in which trastuzumab has clear benefit, it is felt prudent to only include patients in whom the available data suggests margetuximab is most effective. Therefore, in this study, all patients will be assessed for CD16 genotype during screening, and only those with the low affinity genotype (FF or FV) will be enrolled.

2.6 Correlative Studies Background

2.6.1 Examining tissue and blood markers of ADCC during neoadjuvant therapy with TMP or THP

Multiple mechanisms of action have been proposed for trastuzumab or biosimilars including ADCC as well as activation of adaptive cellular immunity.[35] Small pilot studies evaluating patients with HER2+ operable breast cancer have confirmed ADCC as a likely mechanism of trastuzumab therapy and have suggested a correlation between the presence of Fc receptors (CD16+) on the surface of NK cells and macrophages with efficacy. To date, limited work has been done to determine the role of peripheral NK cell activity (i.e. function vs. phenotype) or the presence of NK cells in the tumor microenvironment on the response to trastuzumab therapy. And while ADCC has been demonstrated as a mechanism of action of pertuzumab in in vivo models[38], less is known regarding the impact of NK cell presence and activity on response to pertuzumab (or the combination of trastuzumab and pertuzumab) in patients. Furthermore, margetuximab is a monoclonal anti-HER2 antibody similar to trastuzumab but with five amino acid substitutions engineered into the IgG1 Fc domain to allow increased ADCC and decreased inhibition of immune effector cells, thereby optimizing tumor cell killing.[22] Thus, studies in human tumors exploring the effects of these HER2-directed antibodies, particularly margetuximab, are of significant clinical relevance.

2.6.2 Rationale for on-study tumor biopsy

Currently, there are limited data available on the ability of margetuximab (and limited data with trastuzumab) to activate ADCC or other immune mechanisms in human cancers. This study thus provides a unique opportunity to obtain mechanistic data on the immune effects of margetuximab and trastuzumab in human tumor tissue. Because a substantial percentage of patients will have a pCR, and these patients' cancers will undoubtedly be biologically distinct from those who do not have a pCR, it is necessary to obtain a biopsy at Cycle 2, rather than rely solely on the surgical sample, in order to ensure there is tissue available from all patients.

2.6.3 Microbiome Analysis

The gut microbiome modulates immune system development,[49] but the microbial populations of healthy individuals vary markedly in composition.[50,51] The diversity of intestinal microbiota represents a careful balance between immune tolerance of beneficial microbes and inflammatory responses against pathogens. Alterations in the gut microbiota and their resulting interactions with intestinal epithelium and the host immune system are associated with many diseases, including cancer.[52] Preclinical studies have provided strong evidence for the role of gut microbiota in modulating response and resistance to immune checkpoint inhibitors, raising the possibility that stool microbiota could be used as a response biomarker for immunotherapy.[53,54] Interestingly, postmenopausal women with breast cancer have altered composition and low diversity of their gut microbiota compared to healthy controls, as measured by the Shannon index.[55]

Emerging evidence suggests that the gut microbiome may influence response to checkpoint inhibitors in a number of malignancies.[56-59] Preclinical studies in murine models have demonstrated that microbiome composition is associated with response to PD-L1 inhibitors, with mice exhibiting a "favorable" microbiome having a greater likelihood of

responding to treatment as compared to mice with an "unfavorable" microbiome. Studies also demonstrate that transplanting feces from mice with favorable microbiota to those with unfavorable microbiota increases response rates, suggesting that modification of the microbiome could have a therapeutic effect in patients receiving checkpoint inhibitors.[60] Translational work demonstrates that fecal transplants from patients responding to checkpoint inhibitors to nude mice increases the likelihood of response to immunotherapy in these animals, whereas transplants from human non-responders leads to a lower likelihood of response.[57,58] The evidence linking the gut microbiome to checkpoint inhibitor efficacy in humans has been relatively limited to date, but a number of recent publications have demonstrated a relationship between composition of the microbiome and response to immunotherapy in melanoma, non-small cell lung carcinoma and renal cell cancer.[56-59] Notably, the bacterial species associated with a favorable response to treatment have differed across disease sites and studies.[56,59]

Microbiome composition is influenced by many factors, including age, genetics, and use of antibiotics and probiotics.[60-62] Studies demonstrate that dietary composition is one of the primary drivers of microbiome diversity and taxa.[60,61] Other factors related to energy balance, the balance of calories ingested versus expended, such as physical activity and body weight also impact microbiome composition.[61,62] Limited data in humans suggest that factors impacting microbiome composition may also be related to checkpoint inhibitor response. An analysis of 113 patients with metastatic melanoma found significant associations between dietary factors (ingestion of red meat [p=0.006], sugar-sweetened beverages [p=0.048] and fruits/vegetables [p=0.049]) and microbiome composition.[63] Use of antibiotics (p=0.05) and probiotics (p=0.02) was also associated with lower microbiome α-diversity. Exploratory analyses of 46 of these patients who were initiating treatment with anti-PD-1 therapy suggested that individuals in this cohort with a higher consumption of dietary fiber had a 5-fold likelihood of response to anti-PD-1 therapy as compared to individuals with low fiber consumption (personal communication, Wargo). These findings are provocative but were based on a dietary screening tool that provides a relative crude assessment of dietary intake. The study also did not assess other modifiable factors that can influence microbiome composition, such as physical activity, precluding identification of an optimal strategy of lifestyle modification to enhance immunotherapy response.

The identification of bacterial species associated with response to therapy could open new strategies to maximize the clinical benefit of cancer treatments through the modulation of gut microbiota.

In an effort to identify characterize the microbiome in patients with newly diagnosed breast cancer and correlate with response to therapy, stool samples will be collected to determine baseline characteristics of the structure of the gut microbiome. Additionally, to identify potentially modifiable drivers of microbiome diversity and composition, participants will undergo assessment of dietary composition, physical activity and body mass index at the same timepoints.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Stage II or III (according to AJCC cancer staging manual anatomic staging table, 8th edition) histologically confirmed invasive carcinoma of the breast. A minimum tumor size of 1.5 cm (in breast mass or axillary lymph node) determined by physical exam or imaging (whichever is larger) is required. Patients with inflammatory breast carcinoma (T4d) are NOT eligible.

3.1.2 Centrally confirmed to have a low affinity CD16 germline genotype (FF or FV)

3.1.3 HER-2 positive by 2018 American Society of Clinical Oncology/College of American Pathologists criteria, as assessed by standard institutional guidelines (*central testing is not required*).

3.1.4 ER/PR determination is required. ER- and PR-assays should be performed by immunohistochemical methods according to standard institutional guidelines

3.1.5 Bilateral breast cancers are allowed as long as both cancers are HER2-positive (as defined in 3.1.2), or the contralateral cancer is a $\leq$1 cm, ER+ tumor.

3.1.6 Patients with multifocal or multicentric disease are eligible if the treating investigator has determined the patient should be treated as HER2-positive.

3.1.7 Breast imaging should include dedicated ultrasound of the ipsilateral axilla. For subjects with a clinically positive axilla based on exam or imaging, a fine needle aspiration or core biopsy procedure will be performed to determine the presence of metastatic disease in the lymph nodes (though lymph node sampling procedure need not be resulted prior to patient's registration on trial, as long as all other eligibility are met).

3.1.8 Men and women (with any menopausal status) ≥ 18 years of age are eligible.

3.1.9 ECOG performance status 0 or 1

3.1.10 Required laboratory values demonstrating adequate organ function:

- ANC ≥ 1000/mm^3
- Hemoglobin ≥ 9 g/dl
- Platelets ≥ 100,000/mm^3
- Serum creatinine $\leq$ 1.5 x ULN (institutional) OR calculated GFR ≥60mL/min
- Total bilirubin ≤ 1.5 x ULN (institutional). For patients with Gilbert Syndrome, the direct bilirubin should be within the institutional normal range OR total bilirubin ≤

2.0 mg/dL.

- AST and ALT ≤ 2.5x ULN (institutional)

3.1.11 Left ventricular ejection fraction (LVEF) ≥ 50%.

3.1.12 Women of childbearing potential must have a negative serum pregnancy test within 14 days of treatment start. Childbearing potential is defined as: those who have not been surgically sterilized and/or have had a menstrual period in the past 12 months

3.1.13 Women of childbearing potential and men with partners of childbearing potential must be willing to use one highly effective form of non-hormonal contraception or two effective forms of non-hormonal contraception by the patient and/or partner and continue its use for the duration of the study treatment and for 7 months after the last dose of study treatment (See Section 5.10.1).

3.1.14 Patients with a history of ipsilateral or contralateral DCIS or LCIS are eligible

3.1.15 Patients undergoing breast conservation therapy (i.e. lumpectomy) must not have any contraindications to radiation therapy.

3.1.16 Non-English-speaking patients are eligible but will be exempt from patient-completed questionnaires.

3.1.17 Willing and able to sign informed consent.

3.1.18 Willing to undergo breast biopsy for research purposes.

3.2 Exclusion Criteria

3.2.1 Pregnant or nursing women due to the teratogenic potential of the study drugs.

3.2.2 Active, unresolved infection requiring intervention

3.2.3 Receipt of intravenous antibiotics for infection within 7 days prior to registration.

3.2.4 Uncontrolled hypertension (systolic >180 mm Hg and/or diastolic >100 mm Hg) or clinically significant (i.e. active) cardiovascular disease: cerebrovascular accident/stroke or myocardial infarction within 6 months prior to first study medication, unstable angina, congestive heart failure (CHF) of New York Heart Association (NYHA) Class II or higher, or serious cardiac arrhythmia requiring medication.

3.2.5 Significant symptoms (Grade ≥ 2) from peripheral neuropathy.

3.2.6 Other concurrent serious diseases that may interfere with planned treatment, including severe pulmonary conditions/illness, uncontrolled infections, uncontrolled diabetes.

3.2.7 Any prior treatment for the current breast cancer, including chemotherapy, hormonal therapy, radiation, or experimental therapy.

3.2.8 Patients with any prior history of invasive breast cancer within the past 5 years are not eligible. Non-metastatic invasive breast cancers diagnosed more than 5 years ago and any other type of prior non-metastatic cancer is allowed.

3.3 Inclusion of Women and Minorities

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

3.4 Central Testing for CD16 Genotype

Central Testing for the CD16 genotype will be performed by Flagship Biosciences, Inc. (formerly Cancer Genetics Incorporated and formerly Interpace Pharma Solutions). The CD16 genotype will be assessed using a validated PCR based assay. Participants with samples determined to have low affinity for CD16 genotype (FF or FV) will be eligible for registration.

Flagship Biosciences, Inc. will supply kits containing sample collection (EDTA) tubes and all necessary supplies and instructions to submit samples for central testing. This will include: sample submission forms (with a unique barcode for each site), shipping box, gel packs, sleeves, appropriate labels for shipment and a pre-printed FedEx label.

Submit the following:

- One 10 mL EDTA tube
- Completed sample submission form

Label tubes appropriately according to the instructions in the lab manual with patient name with the Protocol Number, Specimen ID, and collection date (preferably all on a printed label). Documentation of the patient ID, site, and contact info at the site should be included with the shipment. Ship using the shippers provided in the kit to:

Flagship Biosciences, Inc.
[redacted]

Notify Flagship Biosciences, Inc. Sample Management Team when samples have been submitted by emailing the air bill/tracking number, Study ID, Sample ID, and Site ID to [redacted] Results of central review will be provided directly by email to sites in approximately 7 business days after receipt of sample.

Kit reorder/resupply information is found in the Flagship Biosciences, Inc. Lab Manual. Please contact the DFCI Project Management team at [redacted] if additional assistance is needed beyond the instructions in the lab manual.

4. REGISTRATION AND RANDOMIZATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

DF/HCC institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy

or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

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

The eligibility checklist(s) and all pages of the consent form(s) will be faxed to the ODQ at [redacted] The ODQ will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant.

Randomization can only occur during ODQ business hours (8:30am - 5pm Eastern Time, Monday through Friday excluding holidays).

An email confirmation of the registration and/or randomization will be sent to the study coordinator(s) from the registering Site, treating investigator and registering person immediately following the registration and/or randomization.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the DF/HCC Sponsor-Investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at Dana-Farber Cancer Institute by the Project Manager. To verify slot availability, participating sites may email or call the Project Manager. The required forms in section 4.4 should be emailed to the Project Manager.

Following registration, participants should begin protocol therapy within 7 business days. Issues that would cause treatment delays should be discussed with the DF/HCC Sponsor-Investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.4 Registration Process for Other Investigative Sites

To register/randomize a participant, the following documents should be completed by the research nurse or data manager and emailed [redacted]

- Completed and signed DF/HCC Eligibility Checklist
- Signed participant consent form
- HIPAA authorization form

- Required laboratory test results including: Hematology (CBC with differential), serum chemistries (creatinine and/or creatinine clearance, bilirubin, ALT, and AST), and pregnancy test (if applicable)
- Baseline imaging reports with tumor measurements (MRI/Mammogram/Ultrasound)
- CT (chest/abdomen/pelvis) scan report for patients with Stage III disease
- Pathology report confirming diagnosis and ER/PR/HER2 status
- Results of central testing demonstrating low affinity for CD16 genotype (FF or FV)
- Clinic visit note with medical history, physical exam, vital signs and ECOG

To complete the registration process, the Project Manager will

- follow DF/HCC policy (REGIST-101) and register the participant on the protocol
- Email the study team at the participating site with the participant study number, treatment assignment and to confirm registration

NOTE: Participants MUST be registered prior to the start of protocol treatment. Registration can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Time Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Project Manager.

5. TREATMENT PLAN

Data will be collected and maintained on study specific case report forms (CRFs). See Section 10 Study Calendar for additional details. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents of therapies other than those described below may be administered with the intent to treat the participant's malignancy.

5.1 Treatment Regimen

In this study, patients will be randomized to treatment on one of two arms (paclitaxel/margetuximab/pertuzumab, TMP, or paclitaxel/trastuzumab/pertuzumab, THP). On both arms, one cycle is defined as 3 weeks, and study treatment will be administered in 21-day (3-week) cycles. Treatment and study visits will be administered within a +/- 3 day window. Biosimilars may be used for trastuzumab on Arm B of this study.

Treatment will typically be administered on an outpatient basis. Sites may use their institutional standards (e.g., the Dubois formula) for calculating the doses of paclitaxel and trastuzumab/biosimilar.

Arm A Regimen Description**					
Agent*	**Pre-Medications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Paclitaxel	The following premedication regimen is recommended, but may be	80 mg/m^2	IV per local standard	Days 1, 8, and 15 of	21 days (3 weeks)

	altered per institutional standards and/or physician discretion: • Prior to 1st and 2nd paclitaxel infusion: 12 mg dexamethasone, 50 mg IV diphenhydramine, 20 mg IV famotidine • Prior to subsequent doses of paclitaxel: Steroid should be eliminated if no infusion-related reaction during 1st and 2nd infusion. Other premeds per investigator discretion/institutional standard.		operating procedures	each 21-day cycle	
Pertuzumab	No routine pre-medications recommended	840 mg for initial dose; 420 mg for subsequent doses	IV per local standard operating procedures	Day 1 of each 21-day cycle	
Margetuximab	No routine pre-medications recommended	15 mg/kg	IV over 120 minutes for first infusion; IV over 30 minutes for subsequent infusions, if first is well tolerated	Day 1 of each 21-day cycle	
*Required order of infusion for the three medications is as follows: (1) Paclitaxel, then (2) Pertuzumab, then (3) Margetuximab. Alterations to order of drug administration are not permitted. **Further administration details follow in Section 5.3.					

Arm B Regimen Description**					
Agent*	**Pre-Medications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Paclitaxel	The following premedication regimen is recommended, but may be altered per institutional standards and/or physician discretion: • Prior to 1st and 2nd paclitaxel infusion: 12 mg dexamethasone, 50 mg IV diphenhydramine, 20 mg IV famotidine	80 mg/m^2	IV per local standard operating procedures	Days 1, 8, and 15 of each 21-day cycle	21 days (3 weeks)

	• Prior to subsequent doses of paclitaxel: Steroid should be eliminated if no infusion-related reaction during 1st and 2nd infusion. Other premeds per investigator discretion/institutional standard.				
Pertuzumab	No routine pre-medications recommended	840 mg for initial dose; 420 mg for subsequent doses	IV per local standard operating procedures	Day 1 of each 21-day cycle	
Trastuzumab or biosimilar	No routine pre-medications recommended	8 mg/kg for initial dose; 6 mg/kg for subsequent doses	IV per local standard operating procedures	Day 1 of each 21-day cycle	
*Required order of infusion for the three medications in each regimen is as follows: (1) Paclitaxel, then (2) Pertuzumab, then (3) Trastuzumab. Alterations to order of drug administration are not permitted. **Further administration details follow in Section 5.3.					

5.2 Pre-Treatment Criteria

The following laboratory criteria should be met prior to proceeding with treatment at cycle 1 day 1 and on Day 1 of each subsequent cycle. Eligibility criteria do not need to be re-met at cycle 1 day 1.

Guideline criteria to treat at cycle 1 day 1, and subsequent cycle day 1 treatment days:

- Absolute neutrophil count $\geq$1000/mm^3
- Platelets $\geq$100,000/mm^3
- Total bilirubin $\leq$ 1.5 x ULN (institutional).
- AST and ALT $\leq$2.5 x ULN (institutional)
- Serum creatinine $\leq$ 1.5 x ULN (institutional) OR calculated GFR $\geq$60mL/min

5.3 Agent Administration

5.3.1 Paclitaxel

Paclitaxel will be administered in clinic on Days 1, 8, and 15 of each 21-day cycle (with window of +/- 3 days, see Section 5.1) per local standard operating procedures. The dose for the initial treatment with paclitaxel is 80mg/m^2 IV per week. Treatment will typically be administered on an outpatient basis. It is permissible for day 8 and/or day 15 infusions

to occur locally along with appropriate laboratory assessments, per local standard operating procedures. Dose modifications and toxicity management for paclitaxel are described in Section 6.

Paclitaxel will be administered first of the three drugs in the regimen.

Preparation and administration of paclitaxel, including pre-medications (e.g. dexamethasone), should follow institutional guidelines; suggested pre-medications are found in Section 5.1 Table. Anti-emetics typically should not be administered prophylactically before initial treatment with paclitaxel, though this may be modified based on the investigator's discretion for a given patient.

Protocol therapy will consist of 12 doses (4 cycles) of paclitaxel delivered in the neoadjuvant setting. Following surgery, it is not expected that patients will receive additional paclitaxel in the adjuvant setting.

For patients who experience a hypersensitivity reaction to paclitaxel, nab-paclitaxel (Abraxane) may be administered for subsequent doses, at the discretion of the treating investigator. The starting dose of nab-paclitaxel should be 125 mg/m2 weekly (if an investigator wishes to use an alternative starting dose based on tolerability of prior weekly paclitaxel doses, this will require approval of the principal investigator).

5.3.2 Trastuzumab or Biosimilar

Trastuzumab (or biosimilar) will be administered on Day 1 of each 21-day cycle (with window of +/- 3 days, see Section 5.1), per institutional guidelines. The loading dose for the initial treatment with trastuzumab (or biosimilar) is 8 mg/kg IV. All subsequent doses are 6 mg/kg IV every 3 weeks. For delayed or missed doses, if the time between 2 sequential infusions is less than 6 weeks, the 6 mg/kg IV dose may be administered. If the time between 2 sequential infusions is 6 weeks or more, the initial dose of 8 mg/kg may be re-administered followed every 3 weeks thereafter by a dose of 6 mg/kg.

Premedication for nausea and infusion reactions are not commonly required but may be given at the investigator's discretion.

Protocol therapy for patients randomized to trastuzumab will consist of 4 doses of trastuzumab (4 cycles) or biosimilar delivered in the neoadjuvant setting. Following surgery, patients who achieved pCR and received THP in the neoadjuvant setting will receive 13 doses of additional trastuzumab (or biosimilar), for a total of 17 doses. Subcutaneous HP (PHESGO) may be administered in the adjuvant setting in patients who achieve pCR on THP.

5.3.3 Pertuzumab

Pertuzumab will be administered on Day 1 of each 21-day cycle (with window of +/- 3 days, see Section 5.1), per institutional guidelines. The initial loading dose of

Pertuzumab is 840 mg. The pertuzumab dose in subsequent cycles is 420 mg. For delayed or missed doses of Pertuzumab, if the time between 2 sequential infusions is less than 6 weeks, the 420 mg IV dose of Pertuzumab may be administered. If the time between 2 sequential infusions is 6 weeks or more, the initial dose of 840 mg Pertuzumab may be re-administered followed every 3 weeks thereafter by a dose of 420 mg IV.

Premedication for nausea and infusion reactions are not commonly required but may be given at the investigator's discretion.

Protocol therapy will consist of 4 doses of pertuzumab (4 cycles) delivered in the neoadjuvant setting. Following surgery, patients who achieved pCR will receive 13 doses of additional pertuzumab, for a total of 17 doses. Subcutaneous HP (PHESGO) may be administered in the adjuvant setting in patients who achieve pCR on THP.

5.3.4 Margetuximab

Margetuximab will be administered on Day 1 of each 21-day cycle (with window of +/- 3 days, see Section 5.1). The dose of margetuximab is 15 mg/kg.

Premedication for nausea is not commonly required but may be given at the investigator's discretion.

Protocol therapy for patients randomized to margetuximab will consist of 4 doses of margetuximab (4 cycles) delivered in the neoadjuvant setting. Following surgery, patients who achieved pCR and received TMP in the neoadjuvant setting will receive 13 doses of additional margetuximab, for a total of 17 doses. However, if a patient decides to receive standard of care trastuzumab instead of margetuximab in the adjuvant setting, it will not be considered a protocol violation.

5.4 Dose-Limiting Toxicity (DLT)

The phase III SOPHIA study[25] suggested that the safety profile of margetuximab is globally acceptable and similar to the safety profile of trastuzumab. However, as margetuximab has not been previously combined with paclitaxel or pertuzumab in a prospective trial, dose limiting toxicity (DLT) will be closely monitored.

A DLT monitoring plan for the first 6 patients enrolled to the TMP arm of the trial follows below, and additional information on supplemental DLT monitoring for the first one third (N=40) of patients enrolled to the TMP arm follows in Section 14 (Statistical Considerations).

Once 6 patients assigned to the experimental arm (TMP) have received at least one cycle of assigned treatment, a safety review will be conducted before additional patients are enrolled. Based on the monitoring criteria, if ≥4 of 6 patients (or before 6, if 3/3, ≥3/4, ≥4/5 patients) experience DLT within the first cycle of treatment, then the treatment regimen will be modified or discontinued. The review will include a per-patient listing of all reported AEs to date, including

actions required for dosing, to more fully review the nature, frequency, severity and timing of the events. This information combined with fewer DLTs may also result in modification of the experimental treatment regimen.

A DLT is defined as an adverse event or abnormal laboratory, enumerated (1) through (8) below, value assessed as suspected to be trial treatment related (possible, probable or definite) and occurs within the *first cycle of treatment*. Toxicities and lab values will be categorized and graded according to CTCAE v5.0. See Section 6 for guidance on dose holds and modifications.

1. Any grade ≥ 3 non-hematologic, non-hepatic adverse event with the following exceptions:
   - Grade 3 nausea that resolves in ≤ 3 days,
   - Grade 3 fatigue that resolves to ≤ grade 2 in ≤ 5 days,
   - Grade 3 fever (>40C for ≤ 24 hrs),
   - Grade 3/4 lab values deemed by the investigator to be clinically insignificant,
   - Grade 3 rash that resolves to ≤ grade 2 in ≤ 7 days with therapy equivalent to prednisolone 10 mg/day or less,
   - Grade 3 arthralgia that can be managed with supportive care that resolves to ≤ grade 2 within 7 days,
2. Grade ≥ 4 neutropenia (ANC < 500uL) lasting > 7 days.
3. Grade ≥ 3 febrile neutropenia.
4. Grade ≥ 4 anemia.
5. Grade ≥ 4 thrombocytopenia or grade 3 with clinically significant bleeding.
6. Grade ≥ 3 or more elevation of serum hepatic transaminase (ALT or AST) lasting > 7 days.
7. ≥ Grade 3 elevation in AST or ALT AND total bilirubin > 2 x ULN
8. Grade ≥ 2 (symptomatic) heart failure.

5.5 Additional Pre-Surgical Therapy

In participants with evidence of disease progression on neoadjuvant THP or TMP, additional pre-surgical therapy is allowed (and constitutes a reason for taking a patient off of the treatment protocol, see Section 5.14). The selected additional treatment regimen will be at the discretion of the treating investigator. It would also be acceptable for a patient with disease progression on THP or TMP to proceed directly to surgery, if deemed to be in the patient's best interest per investigator discretion.

In participants with obvious residual disease as determined by physical examination or imaging, if performed, following completion of neoadjuvant THP or TMP, additional systemic therapy may be given in the neoadjuvant setting at the investigator's discretion (and, if given, should be recorded in the CRF); four cycles of adriamycin and cyclophosphamide is the recommended

regimen in this case. In this case, a biopsy of the presumed residual disease is required both for clinical purposes (to confirm presence of malignant cells) **and** for research purposes (see Section 9). Participants who receive additional pre-surgery therapy will continue to be followed, as described in Section 5.16. All patients who receive additional pre-surgery therapy will be considered as non-pCR for purposes of the primary endpoint, regardless of whether they had a biopsy to confirm the presence of residual disease.

5.6 Physical Examinations

At the initial study visit, physical examination should include examination of breast and local-regional lymphatics. Clinical T and N staging according to AJCC cancer staging manual anatomic staging table 8th edition should be documented. At subsequent visits with medical or surgical providers (or more frequently, if clinically indicated), breast examination and evaluation of local-regional lymphatics should be performed, with breast tumor measurements performed and documented in the medical record or research chart (for examinations performed prior to surgery). Additional physical examinations should be focused on organ systems related to adverse events. Physical examinations will occur at least approximately once every 3 weeks (i.e. at day 1 of each cycle). Weight is to be measured at Pre-Study (≤ 28 days of registration) and on Day 1 of the specified cycles. Vital signs will include measurements of pulse rate, systolic and diastolic blood pressures, and temperature.

5.7 Tumor Staging

5.7.1 Breast mammogram, ultrasound, MRI

All subjects are required to have a mammogram and diagnostic breast ultrasound (with or without breast MRI*) performed at screening (with at least one of these imaging studies falling within 42 days of registration) and closely preceding or following the last dose of neoadjuvant therapy (from up to one week before to a maximum of up to 4 weeks after the last dose of neoadjuvant therapy, which is C4D15 paclitaxel), to facilitate surgical planning. Baseline breast imaging should include imaging of the ipsilateral axilla. The same imaging modality must be used at screening and prior to surgery to assess tumor response (with the exception that breast MRI, if performed at screening, is strongly recommended but not required to be repeated prior to surgery).

* Breast MRI is strongly recommended in patients in whom breast conserving therapy is under consideration, although not required if practical or financial considerations preclude MRI, as long as the target lesion can be adequately measured by mammogram and/or ultrasound.

5.7.2 CT scans

Subjects with Stage III disease according to AJCC Staging Manual anatomic staging table, Edition 8, will have CT scans of chest, abdomen and pelvis with or without a bone scan (PET scan as an alternative is allowed but not preferred) performed during screening to rule out metastatic disease.

5.8 Surgical Assessment

All subjects will be seen and examined by the treating surgeon during screening and at the Pre-Operative Visit. Each visit will include a clinical breast and lymph node examination and review of the imaging studies (mammogram, MRI, and any other radiographic method) of the breast. After examining the subject and reviewing the pertinent radiographic studies at the screening and pre-operative visits, the surgeon will determine whether the subject is eligible for breast conserving surgery. If the subject is not a breast conservation candidate, the reason(s) will be documented in the CRF (multicentric tumor, tumor location, extensive calcifications on imaging, other).

5.9 Axillary Assessment and Axillary Surgery

An axillary assessment will be performed as part of screening. Ipsilateral axillary lymph nodes will be assessed as clinically normal or clinically suspicious by physical examination. In all patients, ipsilateral axillary lymph nodes will also be assessed as normal or suspicious independently by dedicated axillary ultrasound. Axillary ultrasound and/or biopsy do not need to be repeated if performed prior to the screening period.

For patients with clinically node negative disease both at baseline and following preoperative therapy, sentinel lymph node (SLN) biopsy will be performed after neoadjuvant systemic therapy. Mapping technique (i.e. single versus dual tracer) will be at the discretion of the attending surgeon.

For patients with clinically node positive disease at presentation who become clinically node negative by physical examination following completion of their neoadjuvant systemic therapy, SLN biopsy will be performed after preoperative therapy, and the following procedures will apply:

- Dual tracer with both radioisotope and blue dye will be employed.
- For any patient in whom mapping fails, an axillary lymph node dissection (ALND) will be performed at the time of the initial operation.
- For patients without a clipped positive node in whom fewer than 3 SLNs are retrieved, an ALND will be performed at the time of the initial operation.
- For patients with a clipped positive node, the clipped positive node plus at least 2 additional SLNs must be retrieved. Otherwise, an ALND will be performed at the time of the initial operation.
    - The clip may be localized with a radioactive seed at surgeon discretion. Other types of localization are also allowable per surgeon discretion.
- Identified SLNs from any patient eligible for omission of ALND at the time of the initial operation, based on the above criteria, will be sent for intraoperative frozen section evaluation.
    - If the SLN(s) are positive on this intraoperative frozen section evaluation, then a completion ALND will be performed.
- Pathologic evaluation of SLN(s) by immunohistochemistry (IHC) staining for cytokeratin is required in any patient who has not undergone ALND.
- If the SLN(s) are positive by either hematoxylin & eosin (H&E) staining or by IHC staining for cytokeratin, then a completion ALND will be performed.

- Note that positivity on final pathologic evaluation will include the finding of any amount of nodal disease (isolated tumor cells, micromet, or macromet), regardless of how detected, and will warrant completion ALND.

For patients with clinically node positive disease at presentation who remain clinically node positive by physical examination following completion of their neoadjuvant systemic therapy, or for any patient who becomes clinically node-positive by physical examination following completion of their neoadjuvant systemic therapy, an ALND will be performed.

Deviation from this algorithm will be considered a protocol violation.

5.10 General Concomitant Medication and Supportive Care Guidelines

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, herbal/homeopathic remedies, nutritional supplements) used by a patient from 7 days prior to registration through the end of treatment. All concomitant medications should be reviewed by the study staff and recorded in the medical record.

The following treatments are permitted throughout the duration of the study treatment phase and during follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy in section below. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion.
- Hematopoietic growth factors (e.g., G-CSF, granulocyte macrophage colony stimulating factor) may be used at investigator's discretion for the primary prophylaxis and/or management of treatment-emergent neutropenia and/or for secondary prophylaxis as per NCCN/European Society for Medical Oncology guidelines[42,43] or local standard practice.
- Bisphosphonate or denosumab therapy to be used in accordance with the approved labeled indication and/or nationally recognized treatment guidelines.

Explicitly prohibited therapies prior to the end of treatment visit include:

- Anti-cancer therapies other than those administered in this study, including cytotoxic chemotherapy, radiotherapy (except for adjuvant radiotherapy for breast cancer after completion of chemotherapy), immunotherapy, biological, hormonal (except for adjuvant hormonal therapy for breast cancer after completion of chemotherapy) or targeted (e.g., lapatinib, neratinib) anti-cancer therapy. GnRH agonist used for purposes of fertility preservation is allowed.
- Any systemically active oral, injected, or implanted hormonal method of contraception except for progesterone coated intrauterine devices (IUDs) that had been previously implanted
- Estrogen-replacement therapy
- Any investigational agent, except those used for this study

5.10.1 Timing and Use of Contraception

The agents used on this study may have teratogenic effects on a developing fetus. If, for any reason, a woman should become pregnant or suspect that she is pregnant while participating in this study, she should inform her treating physician immediately.

Women of childbearing potential and men with partners of childbearing potential must be willing to use one highly effective form of non-hormonal contraception or two effective forms of non-hormonal contraception by the patient and/or partner and continue its use for the duration of the study treatment and for **7 months** after the last dose of study treatment

Highly Effective Non-Hormonal Contraception
Methods of birth control which result in a low failure rate (i.e., less than 1% per year) when used consistently and correctly are considered highly-effective forms of contraception.
The following non-hormonal methods of contraception are acceptable:

- True abstinence when this is in line with the preferred and usual lifestyle of the participant. [Periodic abstinence (e.g., calendar, ovulation, symptothermal post-ovulation methods) and withdrawal are not acceptable methods of contraception].
- Male sterilization (with appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). For female participants, the vasectomized male partner should be the sole partner (partner's post-vasectomy documentation of the absence of sperm in the ejaculate is not required).

Effective Non-Hormonal Contraception
Alternatively two of the following effective forms of contraception may be used instead:

- Placement of non-hormonal intrauterine device (IUD) or intrauterine system (IUS). Consideration should be given to the type of device being used, as there is higher failure rates quoted for certain types, e.g., steel or copper wire.
- Condom with spermicidal foam/gel/film/cream/suppository.
- Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.
- The use of barrier contraceptives should always be supplemented with the use of spermicide. The following should be noted:
- Failure rates indicate that, when used alone, the diaphragm and condom are not highly effective forms of contraception. Therefore, the use of additional spermicides does confer additional theoretical contraceptive protection.
- However, spermicides alone are ineffective at preventing pregnancy when the whole ejaculate is spilled. Therefore, spermicides are not a barrier method of contraception and should not be used alone.

It should be noted that two forms of effective contraception are required. A double barrier method is acceptable, which is defined as condom and occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream /suppository.

5.11 Definitive Breast Surgery

Definitive breast surgery (lumpectomy and/or mastectomy) can be performed as soon as 14 days but no later than 42 days following administration of the last dose of protocol therapy i.e. C4D15 paclitaxel administration (unless an additional preoperative systemic therapy regimen is administered, as described in Section 5.4). If contralateral mastectomy is performed concurrently, the pathology report from the contralateral breast must be recorded in the CRF if invasive disease is identified.

For guidance regarding axillary surgery, see Section 5.8 above.

5.12 Post-operative Radiotherapy

Decisions regarding post-surgical radiotherapy will be made by the treating team and should be recorded in the CRF.

5.13 Post-operative Adjuvant Systemic Therapy

Note that assessment of pCR must occur after the final breast cancer surgery is performed (i.e. if patient returns to the operating room for ALND, pCR status cannot be ascertained until ALND pathology is reviewed).

5.13.1 Patients who achieve pCR at the time of surgery (defined as RCB 0): These patients should go on to receive antibody-only therapy with trastuzumab (or biosimilar) and pertuzumab (IV or FDC SC HP) if that was their preoperative regimen, or margetuximab and pertuzumab (MP) if that was their preoperative regimen, for 13 doses in the post-operative setting, for a total of 17 doses of HP/MP overall (4 doses pre-operatively and 13 doses post-operatively). However, if a patient decides to receive standard of care trastuzumab instead of margetuximab in the adjuvant setting, it will not be considered a protocol violation. For patients with HR+ disease, any regimen of adjuvant hormonal therapy may be given at the investigator's discretion. Receipt of adjuvant bone modifying agents is allowed and will not be considered in the assessment of escalated or de-escalated adjuvant therapy. Receipt of additional adjuvant chemotherapy is not recommended but will not be considered a protocol violation if administered. If a patient achieves pCR with less than the intended regimen (e.g. less than 11 paclitaxel doses and/or less than 4 HP or MP doses), they may still be treated in accordance with the above adjuvant therapy guidelines, or alternative therapy may be offered at the discretion of the treating investigator with input from sponsor.

5.13.2 Patients who do not achieve pCR at the time of surgery: All patients who do not achieve pCR are recommended to receive therapy with trastuzumab emtansine (T-DM1) for 14 doses in the post-operative setting. Patients with substantial residual disease (i.e. RCB III) or particularly high risk should also receive additional adjuvant chemotherapy prior to T-DM1. In this case, four cycles of adjuvant adriamycin/cyclophosphamide (AC) is recommended as a preferred adjuvant chemotherapy regimen. Patients with a small to moderate burden of residual disease (i.e. RCB I-II) may opt to receive T-DM1 alone but

additional adjuvant chemotherapy can also be used. For patients with HR+ disease, any regimen of adjuvant hormonal therapy may be given at the investigator's discretion. Receipt of adjuvant bone modifying agents is allowed and will not be considered in the assessment of escalated or de-escalated adjuvant therapy.

5.13.3 Post-operative hormonal therapy: Specific regimen and duration of post-operative hormonal therapy should be delivered at the investigator's discretion.

5.13.4 Recording of systemic adjuvant chemotherapy planned: At the post-operative visit, all efforts should be made for the patient and provider to review surgical pathology results and discuss a plan for adjuvant chemotherapy. pCR status and planned adjuvant chemotherapy (or lack thereof) should be recorded in the CRF and the questionnaire regarding planned adjuvant chemotherapy (see Questionnaire Packet) must be completed by a member of the study team and signed by the investigator. If for any reason the discussion of planned adjuvant chemotherapy is not completed at the post-operative visit, CRF recording of planned non-hormonal adjuvant systemic therapy and completion of the questionnaire may be delayed until a final decision is made regarding planned adjuvant chemotherapy, though it is strongly encouraged that this decision be made within 28 days (and as soon as possible) after the post-operative visit. Every effort must be made to complete adjuvant chemotherapy decision-making at an in-person visit. This determination of planned adjuvant chemotherapy is necessary for real-time administration of patient questionnaires. Additional information regarding assessment and questionnaires around adjuvant systemic therapy decision-making can be found in Section 9.

5.13.5 Recording of all systemic adjuvant therapy received: Over the course of adjuvant systemic therapy, the patient record should be regularly reviewed and adjuvant systemic therapy received (including hormonal therapy, targeted therapy, and bone modifying agents) should be recorded and updated as of the most recent review timepoint. As in Section 5.16 "Duration of Follow-up," follow-up assessments should occur at least yearly until 10 years after surgery, or until patient death (if occurring sooner than 10 years).

5.14 End of Treatment Visit

For patients who DO NOT achieve pCR following neoadjuvant THP or TMP, the post-surgery follow-up visit at which a final decision is made about whether or not the patient will receive further adjuvant chemotherapy will be considered the end of treatment visit (regardless of whether or not the patient goes to surgery immediately following protocol therapy or receives additional preoperative therapy). It is expected that this will constitute the first post-surgery follow-up visit for the vast majority of patients (see Section 5.12.2). If breast surgery is not performed the participant's end of treatment visit should be within 28 days after the last dose of protocol-specified therapy.

For patients who achieve pCR following neoadjuvant THP or TMP, and therefore go on to receive additional HP or MP in the adjuvant setting, the end of treatment visit will occur with the final infusion of adjuvant antibody therapy. Patients who achieve pCR following neoadjuvant

THP or TMP + additional neoadjuvant therapy may also stay on study to receive HP or MP in the adjuvant setting, and the end of treatment visit for such patients will occur with the final infusion of antibody therapy. However, if such patients (who received additional neoadjuvant therapy) choose to make the post-operative visit be their end of treatment visit on-study, that will not be considered a protocol violation.

5.15 Criteria for Taking a Participant Off Protocol Therapy

Participants will be removed from the protocol therapy when one of the following criteria applies:

- Completion of protocol-mandated therapy
- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF).

In the event of unusual or life-threatening complications, treating investigators must immediately notify the PI.

When a participant is removed from protocol therapy and/or is off of the study, the participant's status must be updated in OnCore in accordance with REGIST-OP-1.

5.16 Duration of Follow-Up

Follow-up assessments should occur at least yearly (+/-2 months) until 10 years after surgery (with time measured from the date of protocol registration), or until patient death (if occurring sooner than 10 years).

Comprehensive information about adjuvant therapy received must be recorded for all patients regardless of whether or not they receive adjuvant antibody therapy on study.

Event-free survival (EFS) will be defined from the time of registration until the occurrence of the first of the following events:

- Local/regional recurrence: a recurrent or new invasive ipsilateral breast cancer, invasive breast cancer in the axilla, regional lymph nodes, chest wall, or skin of the ipsilateral breast.
- Contralateral invasive breast cancer,
- Distant recurrence: metastatic disease that has either been biopsy confirmed or clinically

diagnosed as recurrent invasive breast cancer. A single new lesion on a bone scan without evidence of lytic disease on x-ray and without symptoms does not in and of itself constitute distant recurrence, but multiple new bone lesions, or increased isotope uptake associated with new bone symptoms are more likely due to metastases. Bone metastases may also be documented with x-rays and clinical description.

- Death from any cause

In situ cancer is not included as EFS event. If a patient has in situ breast cancer (on the ipsilateral or contralateral side) diagnosed during follow-up before any of the EFS events above, then the patient should continue to be followed for EFS on study (even if she is given hormonal therapy or undergoes additional surgery after the in-situ diagnosis). These patients will be followed for survival.

If a patient is diagnosed with a non-melanoma skin cancer or a vaginal carcinoma in situ, she will continue on this study and continue to be followed for EFS.

Disease-free survival (DFS) will be defined for patients who undergo surgery for breast cancer as the interval from the time of surgery until the occurrence of EFS events noted above.

Recurrence-free interval will be defined for patients who undergo surgery for breast cancer as the interval from the time of surgery until the occurrence of invasive local/regional recurrence (as defined in EFS events, above), distant recurrence (as defined in EFS events, above), or death from breast cancer.

It is recommended that any disease-free survival event is biopsied to confirm recurrent disease. Every effort will be made to collect information on breast cancer status, new anti-cancer therapy, and new onset malignancy diagnoses via simplified CRFs. Following a DFS event, survival information (i.e., date and cause of death or last known alive date if not deceased and new onset malignancy information) should be collected until the completion of 10 years of follow-up.

5.17 Criteria for Taking a Participant Off Study

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

- Completion of follow-up
- Lost to follow-up
- Withdrawal of consent
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure the participant's status is updated in OnCore in accordance with REGIST-OP-1

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following section. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for

Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Patients will be assessed for toxicity prior to each dose. Patients will be instructed to notify their physician immediately for any and all toxicities. Assessment of causality (chronology, confounding factors, concomitant medications, medical history, diagnostic tests, and previous experience with the study treatment) should be conducted by the investigator prior to dose modification and/or delay whenever possible.

As a general approach, it is suggested that all AEs be managed with supportive care when possible at the earliest signs of toxicity. If supportive care is ineffective, a dose delay or dose reduction may be considered to avoid worsening toxicity. Dosing will occur only if the clinical assessment and laboratory test values are acceptable.

If there are dosing delays for any reason, all study assessments are to be delayed in the same fashion, such that planned assessments occur in conjunction with cycles of treatment.

During study treatment, a given toxicity may be attributable to any one, or a combination, of the study drugs. It is important to evaluate the possible cause of toxicity and weigh risk versus benefit for each agent to determine the schema of dose delays/modifications (e.g., which agent to prioritize for maintaining dose timing/level). Determination of attribution is up to investigator discretion.

General guidelines are provided in the following subsections based on the known safety profile of study drugs. Toxicity management is organized by most likely causative agents but guidelines may be applied to management of toxicity regardless of causative agent. General guidelines for dose delay and dose reduction are outlined below. The dose delay and reduction instructions provided in these sections are intended to serve as guidelines to allow ongoing treatment for patients without signs or symptoms of progression while ensuring patient safety. In addition to the guidelines provided below, drug holds and dose reductions for the management of adverse events are permitted at the discretion of the treating physician.

Though the standard neoadjuvant regimen as written in this protocol has a 12-week duration, it will be acceptable to make up infusion of missed doses of any component of the regimen through 14 weeks from cycle 1 day 1 (meaning, up to 3 weeks of delay from what was intended to be the final infusion date is acceptable).

In the event that any of the individual study drug(s) in the regimen is delayed as a result of toxicity, the administration of other agent(s) may be continued based on the following guidelines:

- If pertuzumab alone is held, all other agents may continue. Do not make up missed pertuzumab dose if pertuzumab alone is held (i.e. pertuzumab is only to be administered in combination with trastuzumab or margetuximab).
- If trastuzumab or margetuximab is held, pertuzumab should be held as well. Paclitaxel may continue as per investigator discretion.

- If paclitaxel is held, other agents (HP or MP) should continue on schedule.

Trastuzumab, margetuximab, and pertuzumab may be delayed as a result of toxicities (no dose reductions are allowed for these agents). Paclitaxel doses may be delayed and/or reduced for toxicities.

6.1 Paclitaxel and nab-paclitaxel Dose Modifications

If a patient requires a dose reduction for paclitaxel, dosing will be reduced by one dose level per Table below. Dose re-escalation is not allowed after a dose reduction.

Table: Dose reductions for Paclitaxel

Dose Level	**Dose**
Starting dose	80mg/m^2
First reduction	64mg/m^2
Second reduction	48mg/m^2
Indication for further dose reduction	Off Study treatment

For patients receiving nab-paclitaxel on trial who require dose modifications, dosing will be reduced by one dose level per Table below. Dose re-escalation is not allowed after a dose reduction.

Table: Nab-paclitaxel dose reduction levels

Dose Level	**Nab-paclitaxel Dose**
Starting dose	125 mg/m^2 weekly
First reduction	100 mg/m^2 weekly
Second reduction	75 mg/m^2 weekly
Indication for further dose reduction	Off study treatment

6.2 Management of Infusion-Related Reactions

Infusion-related reactions are known to occur with the administration of monoclonal antibodies as well as with paclitaxel, and may be seen with trastuzumab, margetuximab, pertuzumab, or paclitaxel on this protocol.

Guidelines for management of infusion-related reactions are below. Institutional standard protocols for initial management of infusion-related reactions may also be applied. If an infusion-related reaction occurs to an agent *other than margetuximab* at its first infusion on protocol, investigator discretion may be used regarding premedication for subsequent doses of the agent (though it should be noted that given the potential importance of immunologic mechanisms to the efficacy of trial therapy, steroids should be minimized if deemed safe per investigator).

For infusion-related reactions *attributed to margetuximab* at its first infusion, the following

guidance must be followed regarding subsequent dosing:

- Grade 1 infusion reaction at initial margetuximab infusion: no premedication recommended prior to second margetuximab infusion
- Grade 2 infusion reaction at initial margetuximab infusion: premedication is recommended prior to second margetuximab infusion. Specific premedication regimen is up to investigator discretion.
- Grade 3 infusion reaction at initial margetuximab infusion: Premedication is required prior to second margetuximab infusion. For additional guidance, see table below.

Event	Intervention	Action to be Taken
Infusion-related symptoms Grades 1–2.	Supportive care with oxygen, β-agonists, antihistamines, antipyretics, or corticosteroids may be used as appropriate at the investigator's discretion. Premedication with corticosteroids, antihistamines, and antipyretics may be used before subsequent infusions at the investigator's discretion (exception: specific premedication requirements for margetuximab, as above). Patients should be monitored until complete resolution of symptoms.	Decrease infusion rate by 50% or interrupt infusion for patients who experience any other infusion-related symptoms (e.g., chills, fever). When symptoms have completely resolved, infusion may be restarted at ≤ 50% of prior rate and increased in 50% increments every 30 minutes as tolerated. Infusions may be restarted at the full rate at the next cycle, with appropriate monitoring.
Grade ≥ 3 allergic/hypersensitivity reaction	Supportive care with oxygen, β-agonists, antihistamines, antipyretics, or corticosteroids may be used, as appropriate, at the investigator's discretion. Patients should be monitored until complete resolution of symptoms.	Stop infusion; the causative agent (trastuzumab, margetuximab, pertuzumab, or paclitaxel) should be permanently discontinued or, if continued, it is suggested that this be done in the context of close observation and/or co-management with allergy specialist colleagues. If continued, it is also suggested that infusion is started at ≤ 50% of prior rate and increased in 50% increments every 30 minutes as tolerated. Alternatively, for a grade ≥3 infusion reaction to margetuximab, subsequent use of trastuzumab in place of margetuximab is allowed.

6.3 Management of Neuropathy, Hepatotoxicity, and Neutropenia

Neuropathy can occur associated with paclitaxel. Guidance for paclitaxel dose reductions in case of neuropathy is in the Table below. As paclitaxel is a standard of care drug being administered in standard fashion, investigator discretion or institutional standards may also be used in management of paclitaxel-associated neuropathy.

Paresthesia/Dysesthesia	**Paclitaxel: Action to be Taken**
(If persistent for > 7 Days or Causing the Next Cycle to be Delayed)	
Grade 1 paresthesias/dysesthesias that do not interfere with function	Maintain dose
Grade 2 paresthesias/dysesthesias that interfere with function, but not activities of daily living	Maintain dose
Grade 3 paresthesias/dysesthesias with pain or with function impairment that interfere with activities of daily living	Withhold therapy dose until neuropathy ≤Grade 2 Reduce one dose level
Grade 4 Persistent paresthesias/dysesthesias that are disabling or life-threatening	Discontinue therapy

Hepatic toxicity manifesting as elevated transaminases may be observed with paclitaxel. Guidelines for management are below. As paclitaxel is a standard of care drug being administered in standard fashion, investigator discretion or institutional standards may also be used.

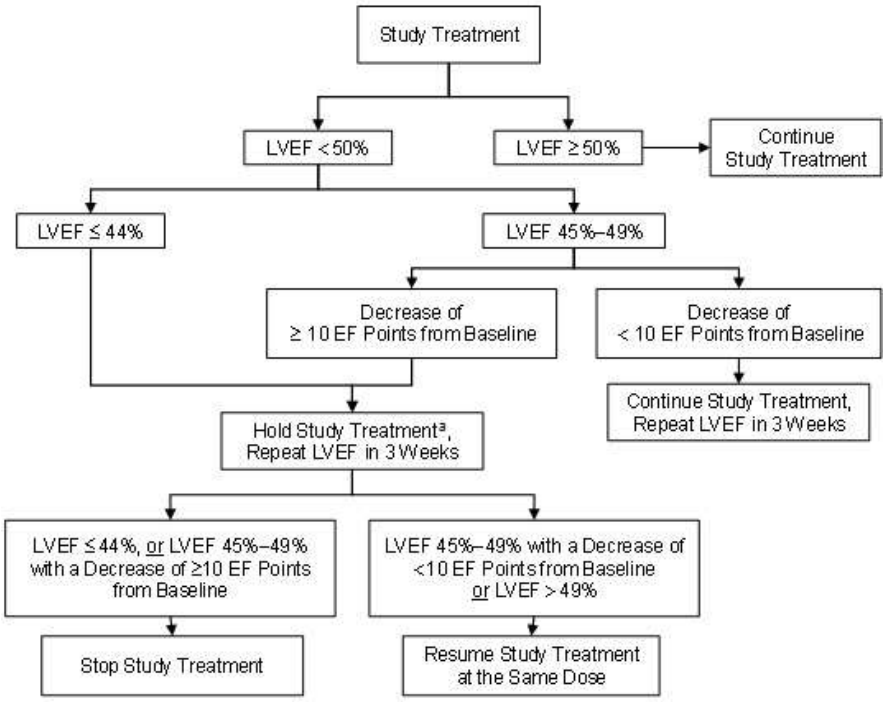
Hepatic Toxicity	**Grade**	**Paclitaxel: Action to Be Taken**
AST/ALT elevated	Grade 3 (>5.0-20.0 x ULN) on the laboratory evaluation for the planned day of dosing.	Hold paclitaxel until recovered to ≤ Grade 1. Resume with dose reduction by one level.
	Grade 4 (>20.0 x ULN)	Discontinue paclitaxel. Repeat lab evaluation (within 24 hours) may be done to exclude lab error prior to discontinuing study treatment.

Hematologic Toxicity	**Grade**	**Paclitaxel: Action to Be Taken**
Cytopenias, particularly neutropenia, may be observed with paclitaxel. Guidelines for management are below. As paclitaxel is a standard of care drug being administered in standard fashion, investigator discretion or institutional standards may also be used.		
Neutrophil count decreased	Grade 3/4 (ANC <1000) on the laboratory evaluation for the planned day of dosing.	Hold paclitaxel until ANC ≥1000, and restart paclitaxel at same dose. If dose held more than 2 times for grade 3/4 neutropenia, dose reduce paclitaxel by one level or add growth factor, per investigator discretion.

6.4 Management of Cardiotoxicity

Decreased ejection fraction (EF) may be seen in association with trastuzumab, margetuximab, or pertuzumab. Management of decreased EF with or without heart failure symptoms is addressed below.

Ejection fraction decreased	**Asymptomatic decrease in LVEF**	**See Figure for dose modifications**
	Grade 3 or 4	Discontinue all HER2-directed treatment.
Heart Failure	Grade 3 or 4	Discontinue all HER2-directed treatment.
Heart Failure Accompanied by LVEF <45%	Grade 2-4	Discontinue all HER2-directed treatment.



LVEF = left ventricular ejection fraction; EF = ejection fraction % points

Note: Baseline refers to the screening LVEF. Study treatment refers to trastuzumab, margetuximab, or pertuzumab; paclitaxel may be continued at investigator's discretion.

[a] Three intermittent holds of study treatment will lead to discontinuation.

6.5 Toxicity Attributed to Pertuzumab

Diarrhea and rash are considered EGFR-related risks based on the mechanism of action of pertuzumab. To prevent dehydration, early treatment of diarrhea with anti-diarrheal medication should be considered, and patients should be treated with fluids and electrolyte replacement, as clinically indicated. Treatment recommendations for EGFR-associated rash include topical or oral antibiotics, topical pimecrolimus, and 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. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

7.1 Expected Toxicities

7.1.1 Adverse Event List for Margetuximab

Sixty of 91 (65.9%) patients treated with single-agent margetuximab in clinical studies have experienced at least one AE considered to have possible, probably, or definite relationship to margetuximab treatment. The most frequently reported treatment-related AEs, regardless of severity, included infusion related reaction (n=17 [18.7%]), nausea (n=14 [15.4%]), and fatigue (n=12 [13.2%]). Including the term CRS (n=4), treatment-related infusion-related reactions have occurred in a total of 21 (23.1%) patients administered single-agent margetuximab.

The majority of treatment-related AEs occurring among patients administered single-agent margetuximab were Grade ≤2 in severity. Treatment-related AEs with a maximum severity of Grade ≥3 were experienced by 8 (8.8%) of 91 patients and included the following:

- Grade 3: blood amylase increased (n=2 [2.2%]); and vomiting, infusion related reaction, lymphocyte count decreased, blood alkaline phosphatase increased, ejection fraction decreased, and hypertension (n=1 [1.1%] each)
- Grade 4: lymphocyte count decreased and lipase increased (n=1 [1.1%] each)
- Grade 5: none.

Treatment-related AEs (regardless of severity) occurring in ≥5% of all patients participating in single-agent studies of margetuximab are presented by decreasing frequency for all patients overall and by dose/regimen and study in the table below.

Table 17 **Treatment-Related [a] Adverse Events Reported Among ≥5% of Patients Receiving Margetuximab Single Agent in Studies CP-MGAH22-01 and CP-MGAH22-02 Regardless of Severity as of 23 February 2019**

	Cohort n (%)										
System Organ Class Preferred Term [b]	H22-01 0.1 mg/kg q1w (N=3)	H22-01 0.3 mg/kg q1w (N=3)	H22-01 1.0 mg/kg q1w (N=3)	H22-01 5.0 mg/kg q1w (N=6)	H22-01 6.0 mg/kg q1w (N=19)	H22-01 10.0 mg/kg Q3W (N=6)	H22-01 15.0 mg/kg Q3W (N=20)	H22-01 18.0 mg/kg Q3W (N=6)	H22-02 6.0 mg/kg q1w (N=14)	H22-02 15.0 mg/kg Q3W (N=11)	ALL (N=91)
AT LEAST ONE EVENT	3 (100)	2 (66.7)	3 (100)	5 (83.3)	11 (57.9)	5 (83.3)	9 (45.0)	5 (83.3)	12 (85.7)	5 (45.5)	60 (65.9)
General disorders and administration site conditions	3 (100)	1 (33.3)	1 (33.3)	3 (50.0)	6 (31.6)	2 (33.3)	0	2 (33.3)	3 (21.4)	1 (9.1)	22 (24.2)
Fatigue	2 (66.7)	1 (33.3)	0	0	4 (21.1)	1 (16.7)	0	1 (16.7)	2 (14.3)	1 (9.1)	12 (13.2)
Chills	0	0	0	0	3 (15.8)	1 (16.7)	0	0	2 (14.3)	0	6 (6.6)
Pyrexia	1 (33.3)	0	1 (33.3)	0	3 (15.8)	0	0	1 (16.7)	0	0	6 (6.6)
Gastrointestinal disorders	1 (33.3)	1 (33.3)	0	2 (33.3)	4 (21.1)	1 (16.7)	2 (10.0)	0	8 (57.1)	1 (9.1)	20 (22.0)
Nausea	1 (33.3)	1 (33.3)	0	0	4 (21.1)	0	0	0	8 (57.1)	0	14 (15.4)
Vomiting	0	1 (33.3)	0	2 (33.3)	1 (5.3)	0	1 (5.0)	0	2 (14.3)	1 (9.1)	8 (8.8)
Diarrhoea	0	0	0	1 (16.7)	1 (5.3)	1 (16.7)	1 (5.0)	0	3 (21.4)	0	7 (7.7)
Injury, poisoning and procedural complications	0	1 (33.3)	0	1 (16.7)	3 (15.8)	0	5 (25.0)	2 (33.3)	2 (14.3)	4 (36.4)	18 (19.8)
Infusion related reaction	0	1 (33.3)	0	1 (16.7)	3 (15.8)	0	5 (25.0)	2 (33.3)	2 (14.3)	3 (27.3)	17 (18.7)
Investigations	0	0	0	3 (50.0)	3 (15.8)	3 (50.0)	2 (10.0)	2 (33.3)	2 (14.3)	2 (18.2)	17 (18.7)
Lymphocyte count decreased	0	0	0	2 (33.3)	1 (5.3)	0	0	1 (16.7)	1 (7.1)	0	5 (5.5)
Metabolism and nutrition disorders	0	1 (33.3)	0	0	3 (15.8)	0	0	0	1 (7.1)	1 (9.1)	6 (6.6)
Decreased appetite	0	1 (33.3)	0	0	2 (10.5)	0	0	0	1 (7.1)	1 (9.1)	5 (5.5)

Abbreviations: H22-01 = CP-MGAH22-01; H22-02 = CP-MGAH22-02; QW = weekly; Q3W = every 3 weeks.
a Includes events with causality ratings of 'Possible,' Probable,' or 'Definite'.
b MedDRA v12.1 & v15.1.

Additional details regarding margetuximab adverse events can be found in the Package Insert.

7.1.1.1 Adverse Event List for Trastuzumab (or biosimilar)

The following information is from the package insert for trastuzumab (Herceptin®; Genentech, Inc.). This is a list of those adverse events most likely to occur to this study; please refer to the trastuzumab package insert for the comprehensive list of adverse events that have occurred with trastuzumab administration. The side effect profile for trastuzumab biosimilars is the same as trastuzumab.

Cardiac Failure/Dysfunction: Signs and symptoms of cardiac dysfunction, such as dyspnea, increased cough, paroxysmal nocturnal dyspnea, peripheral edema, S3 gallop, or reduced ejection fraction, have been observed in participants treated with trastuzumab. Congestive heart failure associated with trastuzumab therapy may be severe and has been associated with disabling cardiac failure, death, and mural thrombosis leading to stroke. The probability of cardiac dysfunction was highest in participants who received trastuzumab concurrently with anthracyclines. The data suggest that advanced age may increase the probability of cardiac dysfunction. Subjects entering this study will undergo a baseline cardiac assessment including history, physical exam and an echocardiogram or MUGA scan.

Anemia and Leukopenia: An increased incidence of anemia and leukopenia was observed in the treatment group receiving trastuzumab and chemotherapy, especially in the trastuzumab and adriamycin/cytoxan subgroup, compared with the treatment group receiving chemotherapy alone. The majority of these cytopenic events were mild or moderate in intensity, reversible, and none resulted in discontinuation of therapy with trastuzumab. Hematologic toxicity is infrequent following the administration of trastuzumab as a single agent, with an incidence of Grade III toxicities for WBC, platelets, hemoglobin all <1%. No Grade IV toxicities were observed.

Diarrhea: Of participants treated with trastuzumab as a single agent, 25% experienced diarrhea. An increased incidence of diarrhea, primarily mild to moderate in severity, was observed in participants receiving trastuzumab in combination with chemotherapy.

Infection: An increased incidence of infections, primarily mild upper respiratory infections of minor clinical significance or catheter infections was observed in participants receiving trastuzumab in combination with chemotherapy.

Infusion Reactions: During the first infusion with trastuzumab, a symptom complex most commonly consisting of chills and/or fever was observed in about 40% of participants in clinical trials. The symptoms were usually mild to moderate in severity and were treated with acetaminophen, diphenhydramine, and meperidine (with or without reduction in the rate of trastuzumab infusion). Trastuzumab discontinuation was infrequent. Other signs and/or symptoms may include nausea, vomiting, pain (in some cases at tumor sites), rigors, headache, dizziness, dyspnea, hypotension, rash and asthenia. The symptoms occurred infrequently with subsequent trastuzumab infusions.

7.1.1.2 Adverse Event List for Pertuzumab

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

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

7.1.1.3 Adverse Event List for Paclitaxel

The following information is from the package insert for paclitaxel. This is a list of those adverse events most likely to occur to this study; please refer to the paclitaxel package insert for the comprehensive list of adverse events that have occurred with paclitaxel administration.

<u>Myelosuppression</u>
Myelosuppression occurs in the majority of patients (neutropenia, leukopenia, thrombocytopenia, and anemia). Myelosuppression is dose related, schedule related, and infusion-rate dependent and, in general, rapidly reversible upon discontinuation.

<u>Anaphylactic-Like Reactions</u>
Hypersensitivity is thought to be caused by the Cremophor vehicle. Minor symptoms include hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, and tachycardia. More severe reactions include hypotension requiring treatment, dyspnea with bronchospasm, generalized urticaria, and angioedema. The majority (53%) of the reported reactions occurred within 2-3 minutes of initiation of treatment and 78% occurred within the first 10 minutes. Reactions usually occurred with the first and second doses.

Cardiovascular toxicity
Atrial arrhythmia (sinus bradycardia [usually transient and asymptomatic], sinus tachycardia, and premature beats); significant events include syncope, hypotension, other rhythm abnormalities (including ventricular tachycardia, bigeminy, and complete heart block requiring pacemaker placement), and myocardial infarction. Hypertension (possibly related to concomitant medication - Dexamethasone) may also occur.

Neurotoxicity
Sensory (taste changes); peripheral neuropathy; arthralgia and myalgia (dose-related, more common when colony-stimulating factors are also administered); seizures; mood alterations; neuroencephalopathy; hepatic encephalopathy; motor neuropathy; and autonomic neuropathy (paralytic ileus and symptomatic hypotension)

Dermatologic toxicity
Alopecia (universal, complete and often sudden, between days 14- 21); injection site reactions (erythema, induration, tenderness, skin discoloration); infiltration (phlebitis, cellulitis, ulceration, and necrosis, rare); radiation recall; and rash.

Gastrointestinal toxicity
Nausea, vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis (neutropenic enterocolitis), ischemic colitis, and pancreatitis.
Hepatic: Increased AST, ALT, bilirubin, alkaline phosphatase; hepatic failure, and hepatic necrosis.

Other
Fatigue, headache, light-headedness, myopathy, elevated serum creatinine, elevated serum triglycerides, and visual abnormalities (sensation of flashing lights, blurred vision).

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Expectedness:**
  - **Expected** adverse events are those adverse events that are listed or characterized in the current adverse event list, the Package Insert, or is included in the informed consent document as a potential risk.

  - **Unexpected** adverse events are those not listed in the Package Insert (P.I.) 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

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Serious Adverse Event Reporting

7.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the DF/HCC Sponsor-Investigator.

7.3.2 Investigators **must** report to the DF/HCC Sponsor-Investigator any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.3 For Multi-Center Trials where a DF/HCC investigator is serving as the Sponsor-Investigator, each participating institution **must** abide by the reporting requirements set by the DF/HCC (listed in section 7.4). The Sponsor-Investigator and Coordinating Center should be notified of SAEs within 1 working day of learning of the event.

7.4 DF/HCC Adverse Event Expedited Reporting Guidelines

The following adverse events must be reported to the DFCI IRB according to the expedited reporting guidelines:

- All locally occurring Adverse Events that are *Serious*, *Unexpected*, and there is a *Reasonable Possibility* the Adverse Event is related to the study intervention should be reported to the IRB.

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

1. **All** life threatening and fatal Serious Adverse Events (grade 4 and 5) considered serious, unexpected, and related, must be submitted via a written adverse event report to OHRS **within 5 working days** from notification of the event.
2. For all other Serious Adverse Events considered serious, unexpected, and related, a full written adverse event report must be submitted to OHRS **within 10 working days** from notification of the event.
3. Any unrelated adverse event does not require reporting to OHRS except grade 5 events which must be reported at the time of continuing review.

Other investigative sites will report SAEs to their respective IRB according to their local IRB's policies and procedures for reporting adverse events. A copy of the submitted institutional AE

form will be forwarded to the Coordinating Center.

The Coordinating Center will submit AE reports from outside institutions to the DFCI IRB according to DFCI IRB policies and procedures in reporting adverse events.

7.5 Reporting to the Food and Drug Administration (FDA) and TerSera Therapeutics LLC

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

As required by 21 C.F.R. 312, Institution shall be responsible for reporting all necessary adverse events associated with the Study to the FDA. Institution shall notify FDA and TerSera of any unexpected fatal or life-threatening experience associated with the use of the Study Drug as soon as possible but in no event later than twenty-four (24) hours after the Institution's or Investigator's initial receipt of the information. Additionally, Institution shall promptly report all other adverse events, regardless of expectedness or causality, to TerSera [redacted]) within one (1) business day after the Institution's or Investigator's initial receipt of the information, and, when necessary, to FDA and the applicable IRB. Institution will promptly make available such records as TerSera may deem necessary to investigate and/or report on an adverse event associated with the use of the Study Drug.

7.6 Reporting to Hospital Risk Management

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

7.7 Routine Adverse Event Reporting

All Grade 2 or higher adverse events **must** be reported in routine study data submissions to the PI on the toxicity case report forms. Abnormal laboratory results are only reported as adverse events in the case report forms if deemed to be clinically significant by a treating investigator. **ALL AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

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

8.1 Margetuximab

8.1.1 Description and Form

Margetuximab (MGAH22) is a sterile, clear-to-slightly-opalescent, colorless-to-pale-yellow or pale brown, preservative-free solution for IV administration. Some visible, translucent,
proteinaceous, margetuximab particles may be present. Margetuximab is supplied in single use, 10-mL clear glass vials with FluroTec® coated gray butyl rubber serum stoppers and aluminum seals with plastic overseals. Each vial contains 250 mg margetuximab (25 mg/mL) in 10 mL solution. The product is formulated in a buffer containing 1.1 mg/mL sodium phosphate monobasic, monohydrate, 0.58 mg/mL sodium phosphate dibasic, heptahydrate, 2.9 mg/mL sodium chloride, 11 mg/mL L-arginine hydrochloride, 30 mg/mL sucrose, and 0.1 mg/mL Polysorbate 80, in Sterile Water for Injection, pH 6.1.

8.1.2 Storage, Stability, and Handling

Vials containing margetuximab should be stored under refrigeration at 2°–8°C (36°–46°F) in an appropriate location. Margetuximab must not be frozen. Protect from light during storage. DO NOT SHAKE. Vials are single-use containers and contain no preservatives; therefore, once margetuximab is diluted in normal saline, administration should begin immediately after preparation. Any partially used vials or diluted dosing solutions should be discarded using appropriate drug disposal procedures.

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

8.1.3 Compatibility

There are no known incompatibles with margetuximab. Margetuximab should not be mixed or infused with any other agents.

8.1.4 Availability

Margetuximab will be supplied free of charge by TerSera in vials for intravenous administration.

8.1.5 Preparation

<u>Preparation for Intravenous Infusion</u>

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

- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. The solution is clear to slightly opalescent, colorless to pale yellow or pale brown. Some visible, translucent, inherent proteinaceous particles may be present.
- **Swirl the vial(s) gently.** Do not shake the vial(s).

- Calculate the required volume of MARGENZA needed to obtain the appropriate dose according to patient's body weight. The calculated total dose volume should be rounded to the nearest 0.1 mL.
- Withdraw appropriate volume of MARGENZA solution from the vial(s) using a syringe.
- Transfer MARGENZA into an intravenous bag containing 100 mL or 250 mL 0.9% Sodium Chloride Injection, USP. Polyvinyl chloride (PVC) intravenous bags or intravenous bags made with polyolefins (polyethylene and polypropylene) and polyamide or polyolefins only or copolymer of olefins may be used. Do not use 5% Dextrose Injection, USP solution.
- The final concentration of the diluted solution should be between 0.5 mg/mL to 7.2 mg/mL.
- **Gently invert the intravenous bag to mix the diluted solution.** Do not shake the intravenous bag.
- Discard any unused portion left in the vial(s).

Do not administer as an intravenous push or bolus. Do not mix MARGENZA with other drugs.

Storage of Diluted Solution

- The product does not contain a preservative. If diluted infusion solution is not used immediately, it can be stored at room temperature up to 4 hours or stored refrigerated at 2°C to 8°C (36°F to 46°F) up to 24 hours. If refrigerated, allow the diluted solution to come to room temperature prior to administration. **Do not freeze.**

8.1.6 Administration

Margetuximab should not be administered as an IV push or bolus and should not be mixed or diluted with other drugs.

Administer diluted infusion solution intravenously over 120 minutes for the initial dose, then over a minimum of 30 minutes every 3 weeks for all subsequent doses. Administer through an intravenous line containing a sterile, non-pyrogenic, low-protein binding polyethersulfone (PES) 0.2 micron in-line or add-on filter.

8.1.7 Ordering

Margetuximab will be provided to participating institutions by TerSera. Sites will order directly from TerSera.

8.1.8 Accountability

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

8.1.9 Destruction and Return

Unused and expired supplies of margetuximab should be destroyed according to institutional policies.

8.2 Trastuzumab and Biosimilars

8.2.1 Description and Form

This study will use trastuzumab from commercial supply. Trastuzumab is a sterile, white to pale yellow, preservative free lyophilized powder for intravenous (IV) administration.

150 mg single-dose vial:
Each single-dose vial of Herceptin delivers 150 mg trastuzumab, 136.2 mg α,α-trehalose dihydrate, 3.4 mg L-histidine HCl monohydrate, 2.2 mg L-histidine, and 0.6 mg polysorbate 20.
Use appropriate aseptic technique. Reconstitute each 150 mg vial of single-dose Trastuzumab with 7.4 mL of sterile water for injection (SWFI) to yield a solution containing 21mg/mL trastuzumab that delivers 7.15 mL (150 mg trastuzumab), at a pH of approximately 6.
Use the trastuzumab solution immediately following reconstitution with SWFI, as it contains no preservative and is intended for single-dose only. If not used immediately, store the reconstituted trastuzumab solution for up to 24 hours at 2○C to 8○C (36○F to 46○F); discard any unused trastuzumab after 24 hours.

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

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

Refer to the FDA-approved package insert for more information.
If a trastuzumab biosimilar is used, please refer to the manufacturer's prescribing information/ package insert for information about dosage and administration.

8.2.2 Storage and Stability

Vials of trastuzumab are stable at 2ºC–8ºC (36ºF–46ºF) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of 440 mg trastuzumab reconstituted with BWFI is stable for 28 days after reconstitution when stored refrigerated at 2ºC–8ºC (36ºF–46ºF), and the solution is preserved for multiple use. Discard any remaining multi dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the

reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. A vial of 150 mg trastuzumab once reconstituted with SWFI should be used immediately, as it contains no preservative and is intended for single-dose only. If not used immediately, store the reconstituted trastuzumab solution for up to 24 hours at 2°C–8°C; discard any unused trastuzumab after 24 hours.

DO NOT FREEZE TRASTUZUMAB THAT HAS BEEN RECONSTITUTED.

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

If a trastuzumab biosimilar is used, please refer to the manufacturer's prescribing information/ package insert for information about storage conditions.

8.2.3 Compatibility

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

If a trastuzumab biosimilar is used, please refer to the manufacturer's prescribing information/package insert for information about drug compatibility.

8.2.4 Handling

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

If a trastuzumab biosimilar is used, please refer to the manufacturer's prescribing information/package insert for information about handling of the agent.

8.2.5 Availability

Trastuzumab is a commercially available agent. The cost of trastuzumab will be charged to the patient and/or his/her insurance company since its use is considered standard of care for neoadjuvant treatment of HER2-positive breast cancer.

A biosimilar for trastuzumab may be used on this study according to the standard of care at the participating institution and/or the insurance company of the participant.

8.2.6 Administration

Trastuzumab (or biosimilar) will be administered per local institutional guidelines. Patients should be observed for fever and chills or other infusion-associated symptoms per local institutional guidelines. Documentation of observation is not required. Refer to Section 5.1 and Section 5.3 for more details.

If a biosimilar for trastuzumab is used, the administration of the agent should be according to the participating site's institutional policy/standard of care.

8.2.7 Ordering

As trastuzumab will be used as a commercially-supplied agent, ordering will be at the discretion of each local institution pharmacy's standard practices.

If a biosimilar for trastuzumab is used, the agent should be ordered according to the participating site's institutional policy/standard of care.

8.2.8 Accountability

As commercial supply of trastuzumab will be used, inventory will be kept according to local institutional pharmacy practices.

If a biosimilar for trastuzumab is used, accountability of the agent should be according to the participating site's institutional policy/standard of care.

8.2.9 Destruction and Return

Unused supplies of trastuzumab should be destroyed according to institutional policies.

8.3 Paclitaxel

8.3.1 Description and Form

Other Names
Taxol (NSC 125973)

Classification
Antimicrotubule agent.

Mode of Action
Promotes microtubule assembly and stabilizes tubulin polymers by preventing their

depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell functions.

Refer to the FDA-approved package insert for more information.

8.3.2 Storage and Stability

Intact vials should be stored between 20° - 25° C (68° - 77° F) in the original package to protect from light and remain stable until the expiration date on the label. Neither freezing nor refrigeration adversely affects stability. Upon refrigeration components in the paclitaxel vial may precipitate but will re-dissolve upon reaching room temperature with little or no agitation. There is no impact on product quality under these circumstances. If the solution remains cloudy or if an insoluble precipitate is noted, the vial should be discarded. Solutions for infusion prepared as recommended are stable at ambient temperature (approximately 25° C) and lighting conditions for up to 27 hours.

8.3.3 Compatibility

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

8.3.4 Handling

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

8.3.5 Availability

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

8.3.6 Preparation

Preparation of paclitaxel should follow institutional guidelines and may slightly deviate from the suggested preparations guidelines in the remainder of this paragraph to accommodate different institutional guidelines. Paclitaxel may be diluted in 0.9% sodium chloride injection, USP or 5% dextrose injection, USP. Paclitaxel must be prepared in glass, plypropylene or plyolefin containers and non-PVC containing (nitroglycerin) infusion sets. In-line filtration with a 0.22-micron filter is required.

8.3.7 Administration

Paclitaxel will be administered over 30-180 minutes, or as per local institutional guidelines. Refer to Section 5.1 and Section 5.3 for more details.

8.3.8 Ordering

As paclitaxel will be used as a commercially supplied agent, ordering will be at the discretion of each local institution pharmacy's standard practices.

8.3.9 Accountability

As commercial supply of paclitaxel will be used, inventory will be kept according to local institutional pharmacy practices.

8.3.10 Destruction and Return

Unused supplies of paclitaxel should be destroyed according to institutional policies.

8.4 Pertuzumab

8.4.1 Description, Form, Preparation

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). **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** The preparation of pertuzumab solution for infusion, using aseptic technique, should be as follows:

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

Refer to the FDA-approved package insert for more information.

8.4.2 Storage and Stability

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

refrigerated (2°C–8°C).

8.4.3 Compatibility

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

8.4.4 Handling

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

8.4.5 Availability

Pertuzumab is a commercially available agent. The cost of pertuzumab will be charged to the patient and/or his/her insurance company since its use is considered standard of care for neoadjuvant treatment of HER2-positive breast cancer.

8.4.6 Administration

Pertuzumab will be administered over 30-60 minutes, or as per local institutional guidelines. Patients should be observed for fever and chills or other infusion-associated symptoms for at least 60 minutes after the initial dose and for 30 minutes after subsequent doses, or as per local institutional guidelines. Refer to Section 5.1 and Section 5.3 for more details.

8.4.7 Ordering

As pertuzumab will be used as a commercially-supplied agent, ordering will be at the discretion of each local institution pharmacy's standard practices.

8.4.8 Accountability

As commercial supply of pertuzumab will be used, it will be supplied, and the inventory kept according to local institutional pharmacy practices.

8.4.9 Destruction and Return

Unused supplies of pertuzumab should be destroyed according to institutional

policies.

8.5 Trastuzumab + Pertuzumab SC FDC (PHESGO)

8.5.1 Description, Form, and Preparation

FDC of pertuzumab and trastuzumab is available in two different forms:

- 1,200 mg pertuzumab, 600 mg trastuzumab, and 30,000 units hyaluronidase/15 mL (80 mg, 40 mg, and 2,000 units/mL) of solution in a single-dose vial. (3)
- 600 mg pertuzumab, 600 mg trastuzumab, and 20,000 units hyaluronidase/10 mL (60 mg, 60 mg, and 2,000 units/mL) of solution in a single-dose vial

Refer to the FDA-approved package insert for more information.

8.5.2 Storage and Stability

The recommended storage conditions for the drug product are between 2°C - 8°C, protected from light. The drug product must not be frozen. For batch-specific instructions and information on shelf-life, see the packaging.

Trastuzumab + pertuzumab SC FDC loading and maintenance doses are ready-to-use solutions and must be prepared for dosing under appropriate aseptic conditions, as they do not contain antimicrobial preservatives. The dose solution should be used immediately. If not used immediately, the total storage time of the dose solution prior to administration should not exceed 24 hours to limit the risk of microbial growth in case of accidental contamination. The recommended storage condition for the dose solution is 2°C - 8°C, but dose solutions may be stored at room temperature for up to a maximum of 4 hours.

8.5.3 Handling

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

8.5.4 Availability

Trastuzumab + pertuzumab SC FDC is commercially available agent. Each institutional pharmacy should assure availability for the study.

8.5.5 Administration

Administration of trastuzumab + pertuzumab SC FDC will be according to the FDA approved package insert.

8.5.6 Ordering

As trastuzumab + pertuzumab SC FDC will be used as a commercially supplied agent, ordering will be at the discretion of each local institution pharmacy's standard practices.

8.5.7 Accountability

As trastuzumab + pertuzumab SC FDC will be used as a commercially supplied agent on this study, accountability will be at the discretion of each local institution pharmacy's standard practices.

8.5.8 Destruction and Return

As trastuzumab + pertuzumab SC FDC will be used as a commercially supplied agent on this study, destruction will be at the discretion of each local institution pharmacy's standard practices.

8.6 Nab-Paclitaxel

Please refer to the FDA-approved package insert for nab-paclitaxel for product information, extensive preparation instructions, and a comprehensive list of adverse events.

8.6.1 Description

ABRAXANE® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound) is an albumin- bound form of paclitaxel with a mean particle size of approximately 130 nanometers. Paclitaxel exists in the particles in a non crystalline, amorphous state. ABRAXANE® is free of solvents.

The active agent in ABRAXANE® is paclitaxel, a microtubule inhibitor. The chemical name for paclitaxel is 5β,20-Epoxy 1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine.

8.6.2 Storage and Stability

Store vials in original cartons at room temperature (20°C-25°C; 68°F-77°F). Retain the original package to protect from bright light. Unopened vials of albumin-bound paclitaxel are stable until the date indicated on the package when stored at the above temperature in the original package.

Reconstituted vials of nab-paclitaxel may be refrigerated at (2°C-8°C; 38°F-46°F) for a maximum of 8 hours and should be protected from bright light.

8.6.3 Handling

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

8.6.4 Availability

Nab-paclitaxel is commercially available and will be obtained according to the local institution pharmacy's standard practices.

8.6.5 Preparation

Nab-paclitaxel will be prepared according to the local institution pharmacy's standard practices.

8.6.6 Administration
Nab-paclitaxel will be administered according to the local institution pharmacy's standard practices.

8.6.7 Ordering
Nab-paclitaxel will be ordered per the local institution pharmacy's standard practices.

8.6.8 Accountability
Accountability will be per the local institution pharmacy's standard practices.

8.6.9 Destruction and Return
Destruction and return will be per the local institution pharmacy's standard practices.

9. CORRELATIVE AND SPECIAL STUDIES

9.1 Correlative Science Background

The following outline provides a brief overview of the correlative studies. Additional exploratory studies not listed here may be pursued in the future if deemed relevant to the study. A comprehensive collection of biological materials is requested in order to be able to understand the immune microenvironment and assess immune responses to THP vs. TMP. The goals of the translational research will be to determine mechanisms and biomarkers for efficacy and resistance. Determining the variation in patients who respond well and those who respond poorly to treatment may further aid in defining the most appropriate group of patients to benefit from the given anti-HER2 therapy.

Breast tumor tissue sampling is required at baseline, on-treatment, at time of surgery, and (if applicable) at time of recurrence for all patients enrolled on this trial. For patients with obvious residual disease following completion of neoadjuvant THP or TMP, additional systemic therapy may be given in the neoadjuvant setting (see Section 5.5). In this case, a biopsy of the presumed residual disease is required both for clinical purposes (to confirm presence of malignant cells) **and** for research purposes (see Section 9).

Blood collections will be performed at 5 timepoints on trial (one baseline timepoint plus 4 on-treatment timepoints) as detailed below (and at a 6^{th} timepoint for patients who experience recurrence). Blood will be used for profiling of peripheral blood mononuclear cells (PBMCs) and banked for future sequencing of circulating tumor DNA (ctDNA), assessment of anti-HER2 antibody responses, and/or other future assays of interest.

Stool will be collected at baseline, during cycle 2 and pre-surgery to explore the structure and function of the gut microbiome. Samples will be processed and then stored at BTIL at Dana-Farber Cancer Institute.

9.2 Specimen Collection Requirements

The following specimens are to be submitted to the indicated lab. Refer to the separate Operations Manual for additional processing and shipping instructions.

Specimen Type	Collection Time Point							Shipping Condition	Ship to
	(Pre-Treatment)[1]	Day 1 of Cycle 2 and 4	Cycle 2 (Within 7 days prior to C2D1)	End of THP or TMP	Surgery/ After Surgery	End of Adjuvant Therapy	Recurrence		
Paraffin block (or 15 unstained slides)	X				X		X	Ambient temperature	DFCI study team
1 H&E stained slide	X				X		X	Ambient temperature	DFCI study team
PBMCs + plasma (three or four 10mL CPT tubes)[2]	X	X			X	X[7]	X[6]	-80 degrees Celsius (batch shipping after local processing)	DFCI Translational Hub
Research Biopsy/ Fresh tumor sample	X		X	X[3]	X			OCT & FFPE	DFCI Translational Hub
Stool Collection	X		X[4]	X[5]				Ambient temperature	DFCI Translational Hub

1. Baseline/Pre-Treatment samples to be collected after registration/randomization but prior to initiation of therapy for participating sites outside of DF/HCC (may be collected at C1D1 pre-treatment)
2. Four blood collection tubes for PBMCs + plasma will be drawn pre-treatment. Three tubes will be drawn at all other timepoints.
3. For patients with residual disease at the completion of neoadjuvant THP or TMP a biopsy is required prior to initiation of additional neoadjuvant systemic therapy
4. Cycle 2 stool sample to be collected (any time from C2D1- C3D1)
5. Pre-surgery stool sample to be collected any time after completion of THP/TMP but prior to surgery or initiation of additional non-protocol neoadjuvant systemic therapy
6. Blood draw at recurrence is strongly recommended but not required
7. Missed blood draw at end of adjuvant therapy will not be considered a protocol violation

Additional details regarding samples to be collected at each timepoint are further summarized in table below.

Research Sampling	**Timepoint** *(more details follow)*	**Contents**
Fresh tissue	Baseline (≤14 days before cycle 1 day 1)	5-7 cores
	C2D1 (prior to C2D1 drug administration; details below)	5-7 cores
	Prior to neoadjuvant therapy switch*	5-7 cores

	Surgery	4 core-sized pieces of tissue from residual tumor or tumor bed
Archival Tissue	Baseline and Surgical Tissue	FFPE Block and 1 H&E **OR** 15 unstained slides cut at 4-5um and 1 H&E
	Breast Cancer Recurrence	FFPE Block and 1 H&E **OR** 15 unstained slides cut at 4-5um and 1 H&E
Blood	Baseline (≤14 days prior to C1D1)	4 – 10 mL CPT tubes (plasma + PBMCs)
	Day 1, cycle 2 and 4	3 – 10 mL CPT tubes (plasma + PBMCs)
	Postoperative (≤6 weeks post-surgery)	3 – 10 mL CPT tubes (plasma + PBMCs)
	Completion of HER2-directed adjuvant therapy +/- 3 months for ALL patients, regardless of adjuvant therapy regimen (MP, HP, T-DM1, etc.)	3 – 10 mL CPT tubes (plasma + PBMCs)
	Breast Cancer Recurrence	3 – 10 mL CPT tubes (plasma + PBMCs)
Stool	Baseline	Home Collection Kit and questionnaires
	Cycle 2 Pre-Surgery	

* ONLY for patients with obvious residual disease at the completion of neoadjuvant THP or TMP, for whom additional neoadjuvant systemic therapy is planned.

9.3 Research Tumor Biopsies

Tumor biopsy will be performed at the following time-points:

- **Mandatory Pre-Treatment**: should be performed within 14 days prior to treatment initiation
- **Mandatory Pre-Cycle 2**: To be performed at or up to 7 days before C2D1, after drug administration on C1D15 and prior to drug administration on C2D1. Ideally as close to C2D1 as possible.
- **Mandatory at time of treatment change**: For patients with residual disease at the completion of neoadjuvant THP or TMP a biopsy is required (unless waived by the PI) prior to initiation of additional neoadjuvant systemic therapy both for clinical purposes (to confirm presence of malignant cells) **and** for research purposes

Ideally five, and at most seven, core biopsies will be collected at each timepoint.

Collection supplies for research biopsy samples will be in the research sample collection kit. Please see the Operations Manual for detailed instructions for collection, processing, labeling and shipping of research biopsy samples.

It is mandatory that core biopsies be image-guided. A clip should be placed in the biopsy site at the time of the research biopsy if a clip was not placed during diagnostic biopsy. Pre- and post-procedure 90-degree lateral and craniocaudal mammogram is recommended to ensure that the correct lesion has been biopsied and to determine the relationship of the clip to the lesion that was visualized prebiopsy. Clip migration following biopsy has been reported and the distance from the original biopsy cavity can be measured.[44] If sufficiently far away from the biopsy cavity, then an addendum should be made to the report documenting that the clip should NOT be used to guide post-treatment tumor sampling. Experience with NeoALTTO and I-SPY1 suggest that taking four core biopsy samples from one area is feasible and acceptable to participants and ethics committees.[45]

Collection of fresh tissue from surgical specimens

At the time of definitive surgery, the pathologist or pathology assistant will take core-sized pieces of tissue from the tumor or residual tumor bed in the breast (goal is at least four core-sized pieces of tissue; more or less are allowable per protocol, though a minimum of four is strongly preferred). Every effort should be made to obtain the sample as soon as possible after the time of resection. Processing of the fresh surgical specimens after they are obtained is described in the Study Operations Manual accompanying this protocol.

If surgery is taking place at a location where there is insufficient research infrastructure for the described tissue collection at the surgical timepoint, surgical specimens should not be collected. Instead, the FFPE surgical tissue may substitute, as described in Section 9.5. Surgical specimens will be collected on all participants regardless of receipt of additional non-protocol neoadjuvant systemic therapy.

9.4 Overview of blood collection protocols

Research blood will be collected at the following timepoints:

Baseline (≤14 days prior to C1D1)

- 4-10mL CPT tubes for whole blood

Day 1 of cycles 2 and 4

- 3-10mL CPT tubes for whole blood

Postoperative timepoint (≤6 weeks post-surgery):

- 3-10mL CPT tubes for whole blood

At final cycle of adjuvant HP/MP/T-DM1 *(note: blood draw timeframe should be kept constant, even if patient does not complete HP/MP/T-DM1 therapy)*

- 3-10mL CPT tubes for whole blood

Breast Cancer Recurrence

- 3-10mL CPT tubes for whole blood at time of biopsy-proven recurrence event

Collection supplies for research blood samples will be in the research sample collection kit.

Please see the Operations Manual for detailed instructions for collection, processing, labeling and shipping of research blood samples.

Of note, every effort will be made to coordinate research blood draws with clinically-indicated blood tests to minimize additional venipuncture. Research coordinators will track participants closely and make every effort to collect blood at required timepoints. However, if research blood draws are missed or fall outside window due unanticipated changes in treatment/scheduling issues, the sample will be collected at the subject's next clinic appointment with a blood draw and this will not be considered a violation of the protocol. These cases should be discussed with the coordinating center.

9.5 Archival Tissue Collection

Archival tumor tissue specimens will be submitted from following surgical procedures:

- Diagnostic biopsy (from time of first diagnosis)
- Surgery (after completion of neoadjuvant chemotherapy)
- Disease Progression/Recurrence (if applicable)

Either 1 paraffin block or 15 unstained slides and an H&E slide 4-5μm thick will be submitted.

Archival tissue is shipped ambient in conventional paraffin block or slide shipper with the Specimen Requisition form found in the Operations Manual. The Operations Manual contains additional information on labeling and shipment of archival tissue to DFCI. Paraffin blocks will be returned after completion of correlative research. Unstained slides will not be returned.

9.6 Stool Sample Collection

All stool samples will be collected by each patient at home using a home-based, self-collection and mail method for stool that has been proven to provide nearly equivalent metagenomic and meta-transcriptomic data as the state-of-the-art, fresh-frozen sample collection protocol.

Samples will be collected at the below timepoints:

- Baseline
- Cycle 2 (any time from C2D1-C3D1 is allowed)
- Pre-Surgery (any time after completion of THP/TMP but prior to surgery or receipt of additional non-protocol neoadjuvant systemic therapy)

A patient questionnaire is included in the kits (Appendix E). The questionnaire should be completed at the time of stool collection and mailed back along with the sample. Stool samples and questionnaires that are not collected at the protocol-specified collection time points will not be protocol violations.

Handling and shipping of stool specimens

All kits will be provided to the patients at a clinic visit. Patients will also be provided with a mailer in which to return the sample. All samples will be shipped within 24 hours of collection at ambient

temperature to the Breast Tumor Immunology Lab (BTIL), where they will be processed and stored (see Lab Manual). Samples will be stored until shipped out to an external lab vendor, such as Microbiome Dx, who will perform the analysis of the samples.

9.7 Hypotheses

We hypothesize that immune activation will be detected in both tissue and blood following treatment with THP and TMP, and features of this immune activation may differ between the two regimens. In particular, we hypothesize that ADCC activity will increase on treatment with both THP and TMP. We hypothesize that there will be evidence of greater ADCC activity in patients treated with TMP versus THP.

9.8 Translational Research: Planned Assays

The below-mentioned analyses may be altered or added based on updated best practices and major questions of interest in immuno-oncology and breast cancer biology in general at the time that correlative analyses are performed. Alternative technologies (based on evolving scientific knowledge or techniques) may be used. Some of the assays also may be eliminated if they are no longer deemed relevant at the time that correlative science is performed.

9.8.1 Tissue-based assays: characterization of the immune microenvironment

Assays will be performed to characterize immunologic aspects of the tumor microenvironment and immunologic responses to THP versus TMP. Potential assays include: (1) single and/or multiplex immunohistochemistry (IHC) or immunofluorescence (IF) performed on FFPE tissue to identify various lymphocyte and myeloid populations (e.g. NK cells, T cells, B cells, macrophages, dendritic cells) and their spatial relationships; (2) gene expression analysis or other genomic tests; (3) antigen-specific T-cell receptor (TCR) spectrotyping; (4) flow cytometry analyses performed on single cell suspensions evaluating immune markers characterizing of various immune cell populations (e.g. CD56+Ki67+ to identify proliferating NK cells).

9.8.2 Blood-based assays: Fc-gamma receptor genotyping, and characterization of the circulating immune response

CD16A (Fc-gamma RIII) receptor genotype will be determined in all patients via germline sequencing from PBMCs.

Assays will be performed to assess the presence and functional activity of various circulating immune cell populations. Potential assays include: (1) flow cytometry to characterize PBMCs; (2) cytokine analysis, to assess PBMC effector functions; (3) ELISA and/or ELISPOT to assess HER2-specific antibody responses.

Circulating tumor DNA (ctDNA) may undergo sequencing analysis at the timepoints outlined, and may be used to explore (1) the correlation between ctDNA at the preoperative timepoint and

presence of pCR versus residual disease; (2) the correlation between ctDNA at a postoperative timepoint and long-term breast cancer outcomes; (3) mechanisms of response and resistance to HER2-directed therapy.

9.8.3 Analysis of intestinal microbiome

We will quantify microbiome features from amplicon, metagenome, and metatranscriptome using established pipelines to identify strain-level taxonomic, functional, transcriptional, and microbially-mediated metabolite profiles.

Microbial DNA is extracted using the Mag-Bind Universal Pathogen DNA Kit (Omega Bio-Tek). Briefly, 250 mg of the specimen is transferred to a deep-well plate for bead beating followed by DNA precipitation and purification following the manufacturer's instructions. Finally, DNA is eluted in 100 uls of Elution Buffer and stored at -80°C until further use. 16S sequencing libraries are generated by amplifying the v3-v4 hypervariable regions of the 16S gene in a polymerase chain reaction using primers F341and R785. Resulting amplicons are tagged with unique molecular barcodes that are later used to demultiplex sequencing reads into individual sample buckets. Libraries are loaded on a MiSeq flow cell and sequenced following Illumina's loading instructions. Sequence data are retrieved from the instrument by converting base call format files into fastq files for data processing purposes.

9.8.4 Sites Performing Correlative Analysis

De-identified biospecimens and data may be shared with the following external entities for the purposes described above:

Broad Institute
Memorial Sloan Kettering Cancer Center

9.9 Diet and Physical Activity Assessments

Dietary composition will be assessed through the Block Fat/Sugar/Fruit/Vegetable Screener, supplemented with 3 additional questions about fiber intake from the Block Vegetable/Fruit/Fiber Screener. The dietary assessment contains 58 total questions and takes about 10-12 minutes to complete. Analysis produces estimates of saturated fat, trans fat, total sugars, "added sugars" (in sweetened cereals, soft drinks, and sweets), fruit and fruit juice, vegetable intake, glycemic load, glycemic index and fiber intake.

Physical activity patterns will be assessed with the Leisure Score Index of Godin Leisure-Time Exercise Questionnaire (LSI). The LSI is a short instrument which asks participants to quantify the number of minutes spent in the last week engaging in vigorous, moderate, and light/mild physical activity. The LSI has undergone extensive reliability and validity testing in a number of cancer and general populations. We will be using a modified form of the instrument which includes not only frequency of activity but also exercise duration. Weekly minutes of moderate and vigorous physical activity will be calculated at each time point using standard scoring algorithms.

Please refer to Appendices C and D for the Diet and Physical Activity Assessments.

Assessments will be conducted at the below timepoints (in conjunction with collection of stool samples):

- Baseline
- Cycle 2 (any time from C2D1-C3D1 is allowed)
- Pre-Surgery (any time after completion of THP/TMP but prior to surgery or receipt of additional non-protocol neoadjuvant therapy)

The assessments should be completed at the time of stool collection and mailed back along with the sample and stool questionnaire. Diet and physical activity assessments that are not collected at the protocol-specified collection time points will not be protocol violations.

9.10 Genetic Testing

Participants will be given information as part of the informed consent process that samples will be used for research tests that will include genetic studies and testing. The intent is not to give participants (or his/her medical providers) the results of any testing done for research purposes; however, incidental germline (heritable) mutations may be identified of which a participant may or may not already be aware. In the case that an incidental genetic finding is identified, the Protocol Chair of this project will be notified. The possible decisions for handling incidental findings may include notification of the participant (and provider); recommendation for genetic counseling, which may or may not include genetic testing (e.g., if the finding was not done in a CLIA certified laboratory); or, neither. In general, a member of the participant's treating team will be given the information to help with notification. In all cases, the current policy of the Dana Farber Cancer Institute and local/participating site IRB, as applicable, will be followed and any additional approvals that may be required prior to participant notification will be secured in advance.

9.11 Additional Information

Submission of data for Genome Wide Association Studies (GWAS) is not currently planned; however, subjects will be asked for permission in the informed consent process. A revision to the protocol and/or any regulatory approvals will be secured prior to any GWAS submission or inclusions in the future, if applicable.

9.12 Specimen Banking

The Protocol Chair and collaborators have approval by the TBCRC to use all research bio-specimens 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.

Secondary use of biospecimens for new endpoints must be submitted to the TBCRC Central Office for possible review by the TBCRC Correlative Science Review Committee.

9.13 Assessment of Adjuvant Therapy Decision-Making

9.13.1 Designation of adjuvant systemic therapy status

Based on pCR status (yes/no) plus systemic adjuvant therapy planned, a, patients will be

designated as belonging to one of three categories: (1) non-de-escalator; (2) unplanned-de-escalator; (3) protocol-consistent adjuvant therapy. Of note, in all patients with HR+ disease, any regimen of adjuvant hormonal therapy may be given at the investigator's discretion (and will not be considered in the assessment of escalation/de-escalation). Adjuvant bone-modifying agent use will also not be considered in the assessment of escalation/de-escalation.

	pCR: Yes	**pCR: No**
Plan adjuvant HP/MP* or T-DM1 alone (+/- hormonal therapy)	Protocol-consistent adjuvant therapy	***Unplanned-de-escalator***
Plan adjuvant HP/MP* or T-DM1 + additional adjuvant chemotherapy (+/- hormonal therapy)	***Non-de-escalator***	Protocol-consistent adjuvant therapy

***** In the adjuvant setting, patients should receive either HP or MP according to maintaining the regimen they received in the neoadjuvant setting, if it led to pCR. I.e. patients who achieved pCR after neoadjuvant HP-containing regimen should go on to receive adjuvant HP, patients who achieved pCR after neoadjuvant MP-containing regimen should go on to receive adjuvant MP.

This designation will be established at the time of the post-operative medical oncology visit and recorded in the CRF. The planned adjuvant chemotherapy questionnaire (Questionnaire Packet; to be completed by a study team member) will also be completed at that time. If for any reason the discussion of planned adjuvant chemotherapy is not completed at the post-operative visit, CRF recording of planned adjuvant chemotherapy may be delayed until a final decision is made regarding planned adjuvant chemotherapy, though it is strongly encouraged that this decision be made within 28 days (and as soon as possible) after the post-operative visit.

9.13.2 Patient questionnaire completion for non-de-escalator patients and unplanned-de-escalator patients

For patients designated to fall in the non-de-escalator and unplanned-de-escalator groups, a questionnaire should be administered at the timepoint at which this designation is made. The goal of these questionnaires is to assess the rationale behind planning for non-protocol-consistent adjuvant therapy.

Questionnaire for non-de-escalator patients, see Appendix A of the Questionnaire Packet.

Questionnaire for unplanned-de-escalator patients, see Appendix B of the Questionnaire Packet.

Of note, while it is strongly preferred that patients complete the questionnaire in person, phone, email, or mail completion/return of the questionnaire is allowed as a back-up.

9.13.3 Patient questionnaire completion for protocol-consistent adjuvant therapy patients

For patients designated to fall in the protocol-consistent adjuvant therapy group, a questionnaire should be administered at the timepoint at which this designation is made.

This questionnaire can be found in Appendix E of the Questionnaire Packet.

Of note, while it is strongly preferred that patients complete the questionnaire in person, phone, email, or mail completion/return of the questionnaire is allowed as a back-up.

9.13.4 Structured physician medical record review for non-de-escalator patients and unplanned-de-escalator patients

Following patient designation into the non-de-escalator or unplanned-de-escalator groups, medical record review of all notes relating to adjuvant chemotherapy decision-making (meaning notes filed prior to the final, recorded decision being made) will be performed by at least one physician member of the study team. Every effort will be made for the same physician study team member to review all charts, for consistency. External sites must submit a printed copy of a patient's post-operative visit note to [redacted]. The physician study team member will record, using the same structure of responses as provided on patient questionnaires, their interpretation of the reasons for planning a non-protocol-consistent adjuvant therapy regimen. For each case, this will be recorded on a structured physician review form. Every effort will be made for each record to be independently reviewed and questionnaire completed by two physicians, in order to ensure validity of results as much as possible.

Structured physician review form for non-de-escalator patients, see Appendix C of the Questionnaire Packet. This form will be completed by DFCI principal investigator during central review at the end of the study.

Structured physician review form for unplanned-de-escalator patients, see Appendix D of the Questionnaire Packet. This form will be completed by DFCI principal investigator during central review at the end of the study.

9.14 Patient-Reported Outcomes

9.14.1 Overview and rationale

Patient-reported outcomes (PROs) will be assessed via questionnaires in order to capture a comprehensive picture of the patient experience and quality of life on TMP versus THP in the early-stage breast cancer setting. No data yet exist regarding PROs in patients receiving margetuximab in the early-stage breast cancer setting, or for patients receiving margetuximab in combination with paclitaxel and pertuzumab. Quality of life (QOL) data from the phase III SOPHIA trial of margetuximab plus chemotherapy in the setting of metastatic HER2+ breast cancer is not yet reported. Based on toxicity reporting by standard CTCAE (non-PRO) in the SOPHIA trial,[25] any grade infusion reactions and fatigue were both numerically slightly worse with margetuximab plus chemotherapy versus trastuzumab plus chemotherapy, however, any grade nausea, anemia, diarrhea and

neutropenic fever were numerically less frequent in the margetuximab arm. Notably, toxicities were not statistically compared between the two arms in the SOPHIA trial and overall safety profiles for both arms were thought to be similar. GI symptoms were reported in both arms of the trial. PROs were also not included in earlier phase studies of margetuximab in the advanced breast cancer setting. Notable toxicities observed in these studies included fatigue, infusion reaction, nausea, vomiting, diarrhea, anorexia, upper respiratory infection, laboratory abnormalities and headache.[22]

9.14.2 Objectives

Objectives related to PROs are detailed in Section 1.4

9.14.3 Assessment items and timepoints

PRO measures, number of questions, recall period and time points are listed below. PROs will be completed by patients on paper questionnaires or tablet.

Quality of life assessments that are not collected at the protocol-specified collection time points will not be protocol violations.

PRO Questionnaire is Appendix G of the Questionnaire Packet.

Domain	Measure	# of questions	Recall period	Time points
Fatigue	PROMIS v 1.0 short form 7a	7	7 days	- Day 1 of neoadjuvant cycle 1 through 4 (all patients) - Pre-operative visit (all patients) - Day 1 of adjuvant cycle 1, 4, 7, and 13 (only patients who continue on adjuvant HP or MP on study)
Fatigue	PROCTCAE questions for severity and interference	2	7 days	
Nausea	PROCTCAE questions for frequency and severity	2	7 days	
Vomiting	PROCTCAE questions for frequency and severity	2	7 days	
Diarrhea	PROCTCAE question for frequency	1	7 days	
Decreased appetite	PROCTCAE questions for severity and interference	2	7 days	
Abdominal pain	PROCTCAE questions for frequency, severity, interference	3	7 days	
Rash	PROCTCAE question for rash (yes/no)	1	7 days	
Cough	PROCTCAE questions for severity and interference	2	7 days	
Shortness of breath	PROCTCAE questions for severity and interference	2	7 days	

Headache	PROCTCAE questions for frequency, severity, interference	3	7 days	
QOL	PROMIS Global Health Scale v1.2 *2 scores (physical health and mental health)	10	7 days	
Infusion Reaction	Self-report of severity of infusion reaction (0-10)	1	0 days	Day 1 of neoadjuvant cycle 1 through 4 immediately after infusion

9.14.4 PRO eligibility

Patients must be English-speaking in order to complete PRO questionnaires. Otherwise, eligibility will be identical to overall trial eligibility. Inability or unwillingness to complete PRO questionnaires will not exclude a patient from participating in the trial.

10. STUDY CALENDAR

All screening tests must be completed within 28 days of registration, with the exception of breast and axillary imaging, which may be completed up to 42 days prior to registration, pregnancy test (only required in specified patients), which should be completed within 14 days of treatment start, and LVEF assessment which should be completed within 3 months prior to registration. Axillary ultrasound and/or biopsy do not need to be repeated if performed prior to the screening period.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted.

Assessment	Pre-Study		Cycle 1-4 (+/- 3 days)			Pre-Op Visit	Post-Op Visit[q]	Adjuvant therapy[m, s]		Follow-up
	≤ 28 days of registration[a]	≤ 14 days of tx start	D1	D8[l]	D15[l]			Q3Wks	Q9Wks (+/- 3 weeks)	
Medical history	X									
Physical exam[a]	X		X			X	X		X	
ECOG PS	X									
Vital signs, weight, (& height at screening only)	X		X					X		
Breast imaging[b]	X					X				
Axillary assessment[c]	X									
Whole-body staging[d]	X									
Chemistry and hematology[e]	X		X	X	X			X		
Serum B-HCG[f]		X								
LVEF Assessment (Echo or MUGA)[g]	X						X[g, r]	X (Q12wks) [g]		
Concomitant medication review	X		X			X	X		X	X
Adverse Event evaluation			X			X	X		X	
Visit with breast surgeon	X					X[h]				
planned adjuvant therapy questionnaire[i]							X			
Research Blood		X	X (D1 of C2& C4)				X	X (C13D1 only)		X[o]
Research Biopsy[k]		X	X (C2D1)			X[k]				
Tissue collection		X					X[n]			X[o]
Stool sample collection and questionnaire[p]		X	X (anytime During Cycle 2)			X				
PROs			X			X			X[j]	

a: All physical examinations should include examination of breast and loco-regional lymphatics, with breast tumor measurements recorded in the medical record if tumor is still in place (i.e. preoperatively). Additional components of the examination should be focused on organ system(s) related to adverse events. See Physical exam should be collected at either post-op visit or C1D1 of adjuvant therapy and then q9wks (+/- 3 weeks) during adjuvant tx. Section 5.6 for further details if necessary.

b: See Section 5.7 for further details regarding breast imaging requirements.
c: See Section 5.9 for further details regarding axillary assessment.
d: Whole-body staging recommended only in patients with stage III disease according to AJCC staging manual edition 8, anatomic staging table. CT chest/abdomen/pelvis with or without bone scan is preferred, but PET CT scan is allowed per investigator discretion.
e: See patient eligibility for lab values that must be verified at the time of screening. Thereafter, comprehensive metabolic panel and complete blood count with differential should be drawn at day 1,8, and 15 of each neoadjuvant cycle, and at day 1 of each adjuvant cycle.
f: Pregnancy test only required in women of childbearing potential (within 14 days of treatment start).
g: For patients who receive adjuvant HP or MP on study, another echo or MUGA should be performed in the post-operative period prior to receipt of additional anti-HER2 therapy, and every 12 weeks (+/- 3 weeks) thereafter from the start of adjuvant therapy. Q12 week Echo/MUGA assessments in the adjuvant setting do not need to be performed prior to treatment administration.
h: Pre-op visit with surgeon is recommended to occur any time on or after C4D8, but can be performed earlier at the discretion of the surgeon.
i: See Section 9.11 for further details regarding administration of adjuvant systemic therapy decision-making questionnaire
j: In the adjuvant setting, PROs will be administered on day 1 of cycles 1, 4, 7, and 13 (only in patients who continue on adjuvant HP or MP on study).
k: Pre-Treatment and Pre- Cycle 2 biopsies are mandatory for all participants. For patients with residual disease at the completion of neoadjuvant THP or TMP a biopsy is required prior to initiation of additional neoadjuvant systemic therapy. (See Section 9)
l: Day 8 and Day 15 may occur at a local facility on either arm
m: Adjuvant therapy with 13 cycles of HP or MP (matched to neoadjuvant regimen) will only be administered on trial in patients who achieve pCR following 4 cycles of THP or TMP.
n: see section 9 for guidance on collection of surgical tissue (fresh or FFPE for sites unable to collect fresh tissue)
o: Blood and tissue only collected during follow-up at time of recurrence
p: Stool samples to be collect pre-treatment, during cycle 2 (any time from C2D1-C3D1) and pre-surgery (any time after completion of THP/TMP treatment but prior to surgery (or prior to any additional non-protocol neoadjuvant chemotherapy)).
q: Post-operative visits are recommended to occur within 6 weeks (42 days) from breast surgery.
r: If clinically indicated, the post-operative LVEF assessment can occur pre-operatively.
s: Adjuvant treatment and assessments outside of these designated windows will not result in a violation.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

A baseline and presurgical radiographic study of the breast is required; MRI is recommended. The same radiographic modality should be used consistently if at all possible. The baseline scan must be obtained within 42 days prior to registration. The presurgical scan should occur a maximum of 4 weeks after the last neoadjuvant therapy. If the participant clinically progresses, repeat imaging is required. If there is discordance (clinical progression, but radiographic stable disease or response), and further input is desired, contact the overall study PI.

11.2 Radiographic assessment

Each participant will have pre- and post-therapy radiographic tumor measurements, preferably by MRI, however if logistic or practical issues preclude MRI use, mammogram or ultrasound may be substituted. The longest diameter (LD) of the target lesion at the time of study initiation will be reported as the baseline LD. The baseline LD of the target lesion may be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

Response criteria are based on the RECIST 1.1 criteria:

Radiographic Complete Response (CR): Complete disappearance of the target lesion

Radiographic Partial Response (PR): Greater than or equal to 30% decrease in the longest diameter (LD) of the target lesion taking as reference the baseline LD.

Radiographic Progressive Disease (PD): Greater than or equal to 20% increase in the LD of target lesion taking as reference the baseline LD or the appearance of one or more new lesions
.
Radiographic Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the baseline LD

11.3 Clinical assessments

Both target and, in the event of multifocal or multicentric invasive cancer, nontarget lesions should be followed clinically and their clinical size recorded at baseline. Measurements thereafter are required; these lesions should be categorized at subsequent visits regarding whether there is evidence of progression. If "yes", the study chair may be notified in order to determine whether the participant should come off protocol treatment.

11.4 Pathologic Response

For the purpose of this study pCR will be defined as RCB 0.

Pathologic response will also be reported using the Residual Cancer Burden calculator [1] from M.D

Anderson: http://www.mdanderson.org/breastcancer_RCB.

The following parameters are required from pathologic examination in order to calculate Residual Cancer Burden (RCB) after neoadjuvant treatment:

- The largest two dimensions (mms) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)
- Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following: 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%
- To assess cellularity it is helpful to scan across the sections of tumor bed and then estimate the average cellularity from the different microscopic fields.
- When estimating percentage cancer cellularity in any microscopic field, compare the involved area with obvious standards, e.g. more or less than half, one quarter, one fifth, one tenth, one twentieth, etc.
- Expect there to be variable cellularity within the cross section of any tumor bed, but estimate the overall cellularity from the average of the estimates in different microscopic fields of the tumor bed. E.g., if cellularity in different fields of the tumor bed were estimated as 20%, 10%, 20%, 0%, 20%, 30%, then an average estimate of overall cellularity would be 20%.
- Histologic estimate of the percentage of the carcinoma in the tumor bed that is in situ, select one of the following: 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%
- The number of positive (metastatic) lymph nodes
- The largest diameter (mm) of the largest nodal metastasis

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity,

safety and other data from this study. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed by the Overall PI, statistician and study team. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the study.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual; treatment regimen information; adverse events and serious adverse events reported by category; summary of any deaths on study; audit results; and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multi-Center Guidelines

This protocol will adhere to DF/HCC Policy MULTI-100 and the requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the O PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix B.

- The PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.
- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

12.4 Collaborative Research and Future Use of Data and Samples

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

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples or data are sent to collaborators and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, date of birth, or MRNs.

These samples may be used for future research studies and may be provided to collaborating investigators both within and outside of DF/HCC. Samples and data may be shared with outside non-profit academic investigators as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples are sent to investigators, and when any research is performed on samples, the samples will be identified with a code, but will not contain patient identification information such as name, birthdate, or medical record numbers.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one of more publicly-accessible scientific databases, such as the National Institutes of Health's Database for Genotypes and Phenotypes (dbGaP). The

results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data.

13. REGULATORY CONSIDERATIONS AND MULTICENTER GUIDELINES

13.1 Protocol Review and Amendments

Unless otherwise specified, each participating institution must obtain its own IRB approval. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

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

Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB of each institution prior to implementation. IRB approval at each participating site is required within 90 days of receipt of amended documents.

The Protocol Chair (or designee) is responsible for the coordination and development of all protocol amendments and will disseminate this information to the participating centers.

13.2 Informed Consent

The physician- investigator will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary, that s/he may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment or relationship with the treating physician(s) or institution. The informed consent will be given by means of a standard written statement, written in non-technical language, which will be IRB approved. The subject should read and consider the statement before signing and dating it and will be given a copy of the document. No subject will enter the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information 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.

13.3 Ethics and GCP

This study will 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.

13.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. The Sponsor-Investigator has delegated responsibility to the Coordinating Center for registering the trial and posting the results on clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information.

13.5 Study 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.

13.6 Records 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.

14. STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

This is a 2:1 randomized open-label phase II trial of paclitaxel/margetuximab/pertuzumab (TMP) compared to paclitaxel/trastuzumab/pertuzumab (THP) in patients with anatomic stage II-III HER2+ breast cancer. We hypothesize that patients with stage II-III HER2+ breast cancer will have improved pCR rates to neoadjuvant TMP compared to THP. Eligible patients will have anatomic stage II-III (according to AJCC 8th edition anatomic staging table) HER2+ breast cancer. Patients may have any hormone receptor status, may be either pre- or post-menopausal, and must be treatment-naïve for this cancer. Following registration, all patients will be randomized 2:1 to neoadjuvant TMP or THP, respectively. All patients will receive 4 cycles of neoadjuvant therapy (1 cycle=21 days), with paclitaxel delivered days 1, 8, and 15 of each 21-day cycle for all patients, and MP or HP delivered on day 1 of each 21-day cycle according to randomization.

Pathologic response will be assessed at surgery per standard clinical practice. Patients with pCR (defined as RCB 0) will complete one year of their assigned MP or HP therapy in the adjuvant setting, without additional chemotherapy. Patients who do not achieve pCR will receive additional/alternative adjuvant therapy of the investigator's choice (four cycles of adjuvant adriamycin/cyclophosphamide (AC) chemotherapy followed by 14 cycles of adjuvant trastuzumab-emtansine (T-DM1) or 14 cycles of adjuvant T-DM1 alone, depending on extent of residual disease are recommended, but not mandated). In all patients with hormone receptor-positive (HR+) disease, any adjuvant hormonal therapy may be given at the investigator's discretion.

Of note, all patients who receive additional pre-surgery therapy will be considered as non-pCR for purposes of the primary endpoint, regardless of whether they had a biopsy to confirm the presence of residual disease

All patients will be followed for recurrence and survival events for up to 10 years from definitive surgery.

14.2 Endpoints

Primary endpoint: Rate of pCR (defined as RCB 0).

Secondary endpoints:

- Rate of pCR (RCB) in patients treated with TMP or THP, by HR status (positive/negative).
- RCB scores in in patients treated with TMP or THP, overall and by HR status (positive/negative).
- Radiographic response to neoadjuvant therapy in patients treated with TMP or THP, overall and by HR status (positive/negative)
- Safety and tolerability profile
    - Assessment of DLTs on Arm A during the first 21 days of treatment
    - Maximum grade of all treatment-related adverse events according to CTCAE v5.0
    - Patient-reported outcomes
- All adjuvant therapies administered in all patients.
- Feasibility of sentinel lymph node mapping and biopsy (defined by ability to identify at least three sentinel lymph nodes, or the clipped node plus two sentinel lymph nodes).
- Pathologic outcomes of axillary lymph node dissection after sentinel lymph node biopsy among patients with positive sentinel lymph node(s).
- EFS, RFI, and OS.

Correlative, patient-related outcomes, and exploratory endpoints:

- Patient/physician considerations in choice of adjuvant therapy regimen.
- Humoral and cellular immune response measured serially in both tissue and blood.
- Symptoms and quality of life (both physical and mental health) during neoadjuvant TMP and THP.

14.3 Sample Size and Analysis of Primary Endpoint

Target accrual is 171 patients. The primary endpoint is pCR. The expected pCR rate for the THP control arm is 45% based on the previously published NeoSphere and CALGB 40601 trials of neoadjuvant taxane plus dual HER2-directed therapy.[20,46] We assume that an improvement in pCR rate from 45% to 65% would make neoadjuvant TMP (experimental regimen) worthy of further investigation. The planned sample size of

171 patients, with improvement in pCR rate from 45% to 65% and a 2:1 randomization (experimental:control) corresponds to 80% power (chi squared test, single side testing, type I error 0.05) accounting for interim analysis.

14.4 Stratification Factors

Patients will be randomized using block stratified randomization. Patients will be stratified according to ER status (≥10% or <10%) and clinical anatomic stage (II vs III).

14.5 Interim Monitoring Plan for pCR

The interim analysis of pCR will compare pCR rates of TMP and THP. The analysis will use the first 90 outcomes (pCR-status). Enrollment is terminated if (i) the TMP pCR estimate at interim analysis is inferior compared to THP and (ii) a final inferiority result—assuming study continuation—is predicted with high probability >80%. Prediction probability will be computed based on a standard Beta-Binomial model.

14.6 Interim Monitoring Plan for Post-Operative Outcomes

We anticipate a low rate of recurrence during the time patients are receiving de-escalated adjuvant HP or MP. Continuous monitoring of unacceptable levels of recurrence will use Pocock-style boundaries constructed following the method of Ivanova, et al (2005).[47] Simulation based evaluation of the sensitivity of design operating characteristics to the enrollment rate--which determine follow up times—have revealed power variations of
2% (range: 2 to 10 patients per month). An event rate of 5% or less would be accepted (where only a biopsy-proven recurrence of metastatic breast cancer is considered an event), and the desired probability of stopping in the anticipated pCR population (N=90 calculated from 45% of THP patients and 65% of TMP patients) is 0.1, leading to the following stopping rules.

The thresholds tabled below can determine early termination of the study, and the will be operationalized with quarterly interim analyses:

Number de-escalated	1-20	20 - 50	51 -80	81 - 120	121 - 160
Event (biopsy-proven recurrence of metastatic breast cancer)	4	6	8	11	14

The probabilities of stopping the trial early are presented below:

True rate	Probability of stopping early
---	---
0.05	0.097
0.10	0.8
0.20	0.99
0.30	>0.99

14.7 Supplemental Interim Monitoring of Safety

Several measures will be taken to ensure the safety of patients participating in this study. The phase III SOPHIA study[25] suggested that the safety profile of margetuximab is globally acceptable and similar to the safety profile of trastuzumab. However, as margetuximab has not been previously combined with paclitaxel or pertuzumab in a prospective trial, DLTs will be closely monitored. Safety monitoring reported to the DSMB will be supplemented by 1) a formal, prespecified safety monitoring guidance for pausing study accrual to more fully evaluate safety in case of unexpectedly high dose-limiting toxicities; 2) a pause of study accrual after the first 6 patients in the investigational treatment group to more fully review safety, regardless of the number of dose-limiting toxicities observed.

14.7.1 Monitoring of dose-limiting toxicity (DLT)

During enrollment of the first one third (N=40) of patients to the investigational TMP arm, DLTs will be summarized with pre-specified criteria based on sequential boundaries to pause enrollment to more fully review safety if excessive numbers of DLTs are observed. The criteria are such that the probability of pausing is at most 0.05 if the true DLT rate is equal to 15%, and the probabilities of pausing will be 0.66 or 0.95 if the true DLT rate is equal to 30% or 40% [48].

The cumulative number of patients (X) experiencing DLT will be compared with the number of safety-evaluable patients (N), i.e., in the safety population (defined in Section 14.9.1, at that time, and an associated cutoff of safety-evaluable patients (Nx). If the number of patients N is greater than Nx then enrollment will continue; if the number of patients N is less than or equal to Nx then enrollment will pause in order to more fully review the nature, frequency, severity and timing of the events. The Table gives the criteria for pausing enrollment to more fully evaluate safety.

Criteria for pausing enrollment because of DLTs to more fully review safety, for sample size up to 40 patients, with probability of pausing of 0.05 when the true toxicity rate is 15%.

Number of patients for whom DLT is reported	X	1	2	3	4	5	6	7	8	9	10	11	12
Pause enrollment if safety-evaluable patients N is ≤Nx	Nx	-	-	≤4	≤7	≤10	≤14	≤18	≤23	≤27	≤32	≤36	≤40
Continue enrollment if safety-evaluable patients N is ≥ Nx	Nx	≥1	≥2	≥5	≥8	≥11	≥15	≥19	≥24	≥28	≥33	≥37	-

14.7.2 Assessment of first 6 patients in the TMP (investigational) treatment group

Once 6 patients assigned to the experimental arm (TMP) have received at least one cycle of assigned treatment, a safety review will be conducted before additional patients are enrolled. Based on the monitoring criteria, if ≥4 of 6 patients (or before 6, if 3/3, ≥3/4, ≥4/5 patients) experience DLT within the first cycle of treatment, then the treatment regimen will be modified or discontinued. The review will include a per-patient listing of all reported AEs to date, including actions required for dosing, to more fully review the nature, frequency, severity and timing of the events. This information combined with fewer DLTs may also result in modification of the experimental treatment regimen. See Section 5.3 regarding DLTs for more

information.

14.8 Analysis of Secondary Endpoints

Descriptive statistics will be used to summarize patient and disease characteristics and for secondary endpoints related to adjuvant treatments received, adherence, and reported reasons for off-protocol treatment decisions.

The observed rates of pCR and RCB will be reported with exact binomial two-sided 90% confidence intervals. The distribution of the survival function and cumulative incidence function for EFS, RFI, and OS will be summarized using the Kaplan Meier product limit estimator and 90% confidence interval (CI) using Greenwood's formula for the standard error.

Clinical outcomes will be evaluated in the entire study population—including those patients who are evaluable for the primary endpoint and those who are not—and in the subgroups defined by HR+ and HR- breast cancer. Contrasts in the long-term outcomes (EFS, RFI, and OS) in patient subgroups according to endpoints at time of surgery will use landmark survival analyses to avoid guarantee time bias. Contrasts will be estimation-only and reported as ratios (odds ratios and hazard ratios) with two-sided 95% confidence intervals.

Analyses of correlative endpoints will be exploratory, and will use logistic regression and Cox proportional hazard models to evaluate the association between molecular phenotypes and clinical outcomes: binomial and time-to-event endpoints, respectively. For assessments at the time of surgery, landmark survival analyses will be performed. For post-baseline and serial assessments, including circulating tumor DNA, time-varying covariates will be used in survival analysis to infer the association.

Descriptive statistics will describe the PRO outcomes over time. With 114 participants receiving TMP and 57 participants receiving THP, we will have 80% power to find a standardized effect size of 0.45 (i.e. standard deviation units; considered a moderate effect size) in the change in physical health scores from baseline to pre-operative visit between the two intervention groups assuming a two-sided type I error of 5%. For PROMIS measures, we will describe mean and median T score, SD and range at each time point for each arm. We are primarily interested in the difference in change scores from baseline to the pre-operative visit between treatment groups and we will compare these differences between the TMP and THP groups using two sample t-tests. For PRO-CTCAE measures we will describe proportions who report each answer for each question at each time point for each arm. We will further model the trajectory over time for each PRO outcome using linear mixed effects models. Normalizing transformations will be used if necessary to ensure accurate model fit. Random effects include random intercept for participants and random slope for follow-up time. Treatment arm will be included as a covariate and interactions between treatment and time will be explored.

14.9 Reporting and Exclusions

14.9.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment.

14.9.2 Evaluation of the Primary Efficacy Endpoint

Analysis of the primary endpoint will be by a modified intention-to-treat of all patients who initiate therapy. Of note, all patients who receive additional pre-surgery therapy will be considered as non-pCR for purposes of the primary endpoint, regardless of whether they had a biopsy to confirm the presence of residual disease

15. PUBLICATION PLAN

It is understood that any manuscript or releases resulting from the collaborative research must be approved by the Protocol Chair and will be circulated to applicable participating sites/investigators prior to submission for publication or presentation.

Additionally, any publication of study data and results must conform to the publications policy as stated the Translational Breast Cancer Research Consortium's (TBCRC) "Policies and Procedures".

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B DF/HCC DATA-SAFETY MONITORING PLAN

1 INTRODUCTION

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

1.1 Purpose

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

2 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the following general responsibilities apply, in addition to those outlined in DF/HCC Policies for Sponsor-Investigators:

2.1 Coordinating Center

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

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

2.2 External Site

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

Each External Site is expected to comply with all applicable DF/HCC requirements stated within this Data and Safety Monitoring Plan and/or the protocol document.

The general responsibilities for each External Site may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their IRB of record. For studies under a single IRB, the Coordinating Center will facilitate any study-wide submissions..
- Maintain regulatory files as per ICH GCP and federal requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required.
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities when required by the sponsor.
- Submit Serious Adverse Event (SAE) reports to sponsor, Coordinating Center, and IRB of record as applicable, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to the Sponsor, Coordinating Center, and IRB of record as applicable.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.
- Notify the sponsor immediately of any regulatory authority inspection of this protocol at the External Site.

3 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

Certain DF/HCC Policy requirements apply to External Sites participating in DF/HCC research. The following section will clarify DF/HCC requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Revisions and Closures

The External Sites will receive notification of protocol revisions and closures from the Coordinating Center. When under a separate IRB, it is the individual External Site's responsibility to notify its IRB of these revisions.

- **Protocol revisions:** External Sites will receive written notification of protocol revisions from the Coordinating Center. All protocol revisions should be IRB approved and implemented within a timely manner from receipt of the notification.

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

3.2 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for External Sites. The External Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for Investigator-Sponsored Multi-Center Trials. This document will be provided separately to each External Site upon request.

External Sites must send their version of the informed consent document to the Coordinating Center for sponsor review and approval. If the HIPAA authorization is a separate document, please submit to the sponsor for the study record. Once sponsor approval is obtained, the External site may submit to their IRB of record, as applicable. In these cases, the approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each External Site will identify the appropriate members of the study team who will be obtaining consent and signing the consent form for protocols. External Sites must follow the DF/HCC requirement that for all interventional drug, biologic, or device research, only attending physicians may obtain initial informed consent and any re-consent that requires a full revised consent form.

3.3 IRB Re-Approval

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

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

3.4. DF/HCC Multi-Center Protocol Confidentiality

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

3.5. Participant Registration and Randomization

Refer to Protocol Section 4 for participant registration information. Treatment cannot begin until site has received confirmation that participant has been registered with DF/HCC CTMS.

Registration can only occur during normal business hours, Monday through Friday from 8:00 AM to 5:00 PM Eastern Standard Time.

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

3.3.1 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the External Site receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and IRB of record must be notified of any violations to this policy.

3.3.2 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without IRB approval will be permitted. All External Sites are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.5. Data Management

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

3.5.1. Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

If study forms are not submitted on schedule, the External Sites will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

3.6. Protocol Reporting Requirements

3.6.1. Protocol Deviations, Exceptions and Violations

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

3.6.2. Reporting Procedures

Requests to deviate from the protocol require approval from the IRB of record and the sponsor.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

3.6.3. Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports per DF/HCC requirements, and ensure that all IND Safety Reports are distributed to the External Sites as required by DF/HCC Policy. External Sites will review/submit to the IRB according to their institutional policies and procedures.

4. MONITORING: QUALITY CONTROL

The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

4.4. Ongoing Monitoring of Protocol Compliance

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

The Coordinating Center will implement ongoing monitoring activities to ensure that External Sites are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

Additionally, a plan will be formulated to provide regular and ongoing communication to Participating Institutions about study related information which will include participation in regular Coordinating Center initiated teleconferences. Teleconferences will occur monthly and will continue regularly until completion of accrual. Frequency of teleconferences may be adjusted based

on rate of accrual. Upon completion of accrual, teleconferences will occur monthly until all patients complete protocol therapy. Upon completion of protocol therapy, teleconferences will occur on an as needed basis until study completion. Additional communication may be distributed via "Newsletter" or email as deemed appropriate by DF/HCC Sponsor.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. Participating Institutions will be required to provide access to participants' complete medical record and source documents for source documentation verification during the visit. In addition, Participating Institutions should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the Participating Site. On-site monitoring visits can be substituted with remote (virtual) monitoring visits at the discretion of the Principal Investigator.

Remote Monitoring: Remote monitoring will be performed on an as-needed basis by the Clinical Trial Monitor. Sites will be asked to provide source documentation via fax, email, or mail as specified by the Clinical Trial Monitor for virtual monitoring.

4.5. Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at External Sites that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

4.6. Accrual Monitoring

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

A minimum of 3 participants per site annually is recommended for Phase II trials. However, given the additional regulatory burden and cost of overseeing each site, a consideration of 5 participants per site should be a minimum target.

5. AUDITING: QUALITY ASSURANCE

5.4. DF/HCC Internal Audits

All External Sites are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2-day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

5.5. Audit Notifications

It is the External Site's responsibility to notify the Coordinating Center of all external audits or inspections (e.g., FDA, EMA, NCI) that involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

5.6. Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the IRB as applicable.

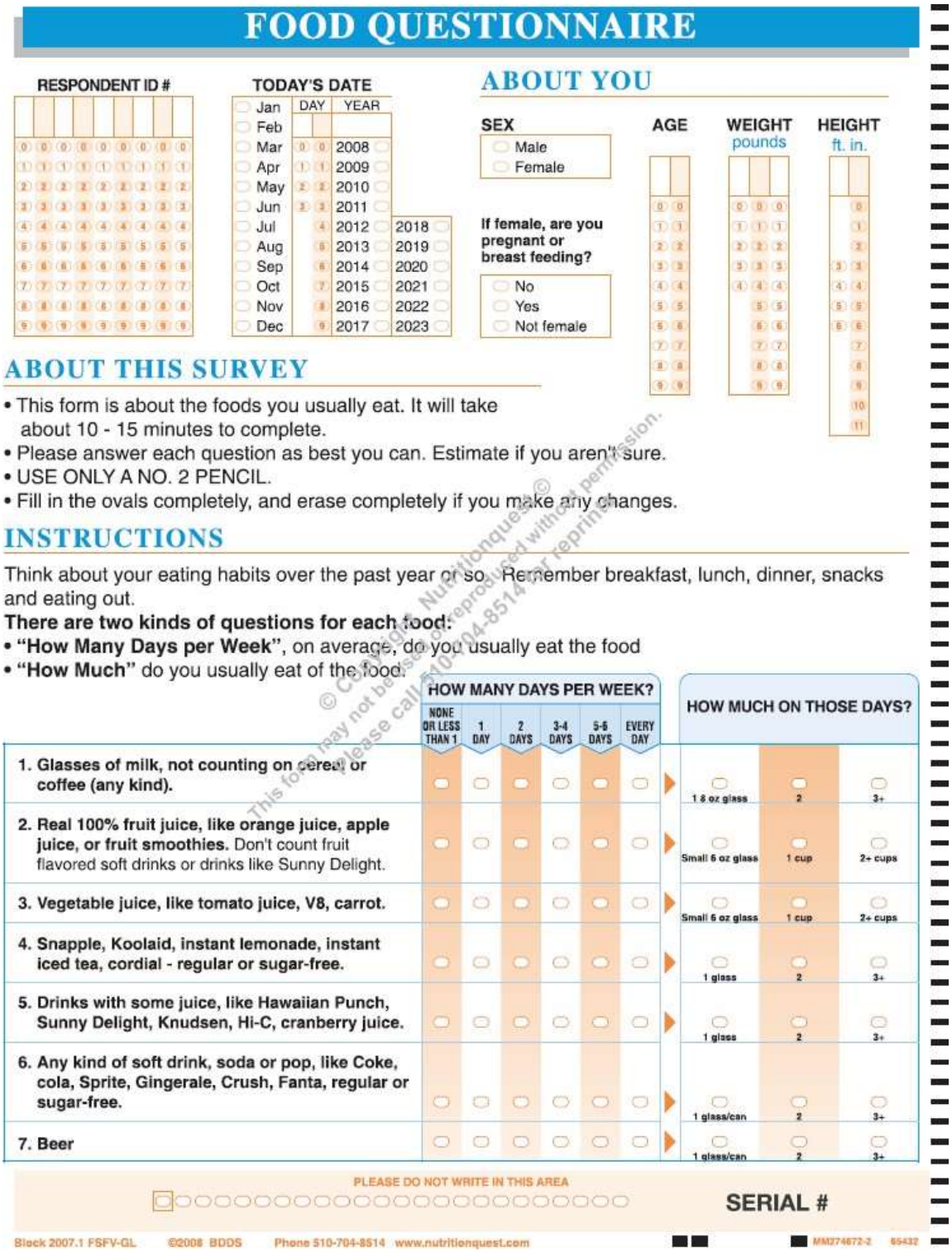
5.7. External Site Performance

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

External Sites that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be put on hold or closed.

APPENDIX C DIET AND PHYSICAL ACTIVITY ASSESSMENTS

Please note: the below is a copy of the **Block Fat/Sugar/Fruit/Vegetable Screener** for reference. *Please use the official Scantron version of the Screener rather than the appendix.*



FOOD QUESTIONNAIRE

RESPONDENT ID #

TODAY'S DATE

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

DAY

YEAR 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023

ABOUT YOU

SEX: Male, Female

If female, are you pregnant or breast feeding? No, Yes, Not female

AGE

WEIGHT pounds

HEIGHT ft. in.

ABOUT THIS SURVEY

- This form is about the foods you usually eat. It will take about 10 - 15 minutes to complete.
- Please answer each question as best you can. Estimate if you aren't sure.
- USE ONLY A NO. 2 PENCIL.
- Fill in the ovals completely, and erase completely if you make any changes.

INSTRUCTIONS

Think about your eating habits over the past year or so. Remember breakfast, lunch, dinner, snacks and eating out.

There are two kinds of questions for each food:

- **"How Many Days per Week"**, on average, do you usually eat the food
- **"How Much"** do you usually eat of the food.

	HOW MANY DAYS PER WEEK? None or less than 1	1 day	2 days	3-4 days	5-6 days	Every day	HOW MUCH ON THOSE DAYS?		
1. Glasses of milk, not counting on cereal or coffee (any kind).							1 8 oz glass	2	3+
2. Real 100% fruit juice, like orange juice, apple juice, or fruit smoothies. Don't count fruit flavored soft drinks or drinks like Sunny Delight.							Small 6 oz glass	1 cup	2+ cups
3. Vegetable juice, like tomato juice, V8, carrot.							Small 6 oz glass	1 cup	2+ cups
4. Snapple, Koolaid, instant lemonade, instant iced tea, cordial - regular or sugar-free.							1 glass	2	3+
5. Drinks with some juice, like Hawaiian Punch, Sunny Delight, Knudsen, Hi-C, cranberry juice.							1 glass	2	3+
6. Any kind of soft drink, soda or pop, like Coke, cola, Sprite, Gingerale, Crush, Fanta, regular or sugar-free.							1 glass/can	2	3+
7. Beer							1 glass/can	2	3+

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

Block 2007.1 FSFV-GL ©2008 BDDS Phone 510-704-8514 www.nutritionquest.com MM274672-2 65432

	HOW MANY DAYS PER WEEK?						HOW MUCH ON THOSE DAYS?		
	NONE OR LESS THAN 1	1 DAY	2 DAYS	3-4 DAYS	5-6 DAYS	EVERY DAY			
8. Eggs, or breakfast sandwiches with eggs, like Egg McMuffins (McDonalds).	○	○	○	○	○	○	○ 1 egg	○ 2	○ 3+
9. Cold cereal, any kind.	○	○	○	○	○	○	○ 1 small bowl	○ 1 medium bowl	○ 1 large bowl
10. Hot Cereal, cooked cereal like oatmeal or porridge, grits, or cream of wheat.	○	○	○	○	○	○	○ 1 small bowl	○ 1 medium bowl	○ 1 large bowl
11. Real sugar or honey in coffee or tea or on cereal.	○	○	○	○	○	○	○ 1 tsp	○ 2	○ 3+
12. Cheese, sliced cheese or cheese spread, including on sandwiches.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
13. Lunch meats like bologna, salami, sliced ham, turkey lunch meat, or any other cold meat cuts.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
14. Hamburgers, cheeseburgers, meat balls or meat loaf.	○	○	○	○	○	○	○ 1 small/3 oz	○ 1 large	○ 2 large
15. Hot dogs, or sausage like Polish, Italian or chorizo.	○	○	○	○	○	○	○ 1 hotdog	○ 2	○ 3+
16. Other beef or pork, such as steak, roast beef, ribs, or in sandwiches, tacos, burritos.	○	○	○	○	○	○	○ 3 oz small	○ 4-6 oz medium	○ 7+ oz large
17. Fried chicken, including chicken nuggets, wings, chicken patty.	○	○	○	○	○	○	○ 1 medium piece	○ 2 medium pieces or 6 nuggets	○ 3 medium pieces
18. Fish, any kind.	○	○	○	○	○	○	○ 2 oz	○ 4 oz	○ 6 oz
19. Pizza.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
20. Spaghetti, lasagna, other pasta, or noodles.	○	○	○	○	○	○	○ 1 cup	○ 2	○ 3+
21. Rice, or dishes made with rice.	○	○	○	○	○	○	○ 1 cup rice	○ 2	○ 3+
22. Green salad and vegetables you put in green salad.	○	○	○	○	○	○	○ 1 cup	○ 2	○ 3+
23. Any kind of fruit, fresh or canned (not counting juice).	○	○	○	○	○	○	○ 1 fruit or 1/2 cup	○ 2 fruits or 1 cup	○ 3 fruits or 2 cups
24. French fries, home fries, hash browns.	○	○	○	○	○	○	○ small (McD)	○ medium	○ large
25. Potatoes not fried, like baked, mashed.	○	○	○	○	○	○	○ 1/2 cup or 1/2 potato	○ 1 cup	○ 2+ cups
26. Vegetable soup, or stew with vegetables.	○	○	○	○	○	○	○ 1 cup	○ 1 1/2 cups	○ 2+ cups

	HOW MANY DAYS PER WEEK?						HOW MUCH ON THOSE DAYS?		
	NONE OR LESS THAN 1	1 DAY	2 DAYS	3-4 DAYS	5-6 DAYS	EVERY DAY			
27. ALL other vegetables you eat, as a side dish or in any kind of dish, not counting salad or potatoes.	○	○	○	○	○	○	○ 1/2 cup altogether	○ 1 cup	○ 2 cups
28. Bread, rolls, bagels.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
29. Biscuits, muffins, croissants.	○	○	○	○	○	○	○ 1	○ 2	○ 3+
30. Snack chips like potato chips, tortilla, corn chips, Fritos, Doritos, popcorn (not pretzels).	○	○	○	○	○	○	○ 1 small handful	○ 1 oz bag 1 cup	○ Big bag 2 cups
31. Crackers, like Ritz, soda-crackers, Cheez-Its, or any other snack cracker.	○	○	○	○	○	○	○ 3-4 small crackers	○ 5-10 crackers	○ a lot
32. Ice cream, ice cream bars.	○	○	○	○	○	○	○ 1/2 cup	○ 1 cup	○ 2+ cups
33. Doughnuts.	○	○	○	○	○	○	○ 1	○ 2	○ 3+
34. Cake, cookies, or snack cakes like cupcakes, Twinkies or any other pastry.	○	○	○	○	○	○	○ 1 small piece	○ 1 medium	○ 2+
35. Pie including fast food pies or snack pies.	○	○	○	○	○	○	○ 1 small piece	○ 1 medium	○ 2+
36. Chocolate candy like chocolate bars, M&Ms, Mars Bars, Reeses.	○	○	○	○	○	○	○ 1 mini	○ 1 medium	○ 1 large
37. Any other candy (not chocolate) like hard candy, Lifesavers, Skittles, Starburst.	○	○	○	○	○	○	○ 1-2 pieces	○ 1/2 package	○ 1 package
38. Margarine (not butter) on bread or on vegetables.	○	○	○	○	○	○	○ 1 teaspoon	○ 2 teaspoons	○ 3 teaspoons
39. Butter (not margarine) on bread or on vegetables.	○	○	○	○	○	○	○ 1 teaspoon	○ 2 teaspoons	○ 3 teaspoons
40. Fat or oil in cooking.	○	○	○	○	○	○			

For each of the questions below, please fill in the oval that best describes your usual eating habits.

41. What kind of milk do you usually drink?	○ Whole milk ○ Reduced-fat 2% milk ○ Low-fat 1% milk	○ Skim milk ○ Soy milk ○ Rice milk	○ I don't drink milk or soy milk
42. If you drink soft drinks or pop, is it usually:	○ Diet or sugar free soft drinks	○ Regular ○ I don't drink soft drinks	
43. If you drink Snapple, KoolAid, instant iced tea, or instant lemonade, is it usually:	○ Sugar-free ○ Regular	○ I don't drink these	
44. If you eat hot dogs, are they usually:	○ Low Fat or turkey hot dogs	○ Regular hot dogs ○ I don't eat hot dogs	
45. If you eat lunch meats, are they usually:	○ Low Fat or turkey	○ Regular	○ I don't eat lunch meats
46. If you eat snacks like chips, are they usually:	○ Trans-fat free	○ Regular	○ I don't know ○ I don't eat them

For each of the questions below, please fill in the oval that best describes your usual eating habits.

47. If you eat crackers, are they usually:
- ◯ Trans-fat free
- ◯ Triscuits, Graham crackers, Ry-Vita
- ◯ Saltines or other snack crackers
- ◯ I don't eat them

48. If you eat ice cream, is it usually:
- ◯ Low carb, low sugar
- ◯ Low fat or ice milk
- ◯ Regular
- ◯ Premium
- ◯ I don't eat it

49. If you eat cake, snack cakes, cookies and other pastries, are they usually:
- ◯ Low carb, low sugar
- ◯ Low fat
- ◯ Regular
- ◯ I don't eat it

50. If you eat chocolate candy, is it usually:
- ◯ Low carb, low sugar
- ◯ Low fat
- ◯ Regular
- ◯ I don't eat it

51. If you eat other candy, not chocolate, is it usually:
- ◯ Sugar-free
- ◯ Regular
- ◯ I don't eat it

52. When you use margarine, is it usually:
- ◯ Stick margarine
- ◯ Soft tub margarine
- ◯ Low-fat margarine
- ◯ Butter-margarine blend
- ◯ Non-hydrogenated and trans-fat free
- ◯ I don't eat it

53. What kind of fat or oil do you usually use in cooking? MARK ONLY 1 or 2.
- ◯ Spray oil (i.e. Pam), or no oil
- ◯ Butter
- ◯ Butter/margarine blend
- ◯ Stick margarine
- ◯ Soft tub margarine
- ◯ Low-fat margarine
- ◯ Corn oil, vegetable oil
- ◯ Olive oil, canola oil
- ◯ Lard, fatback, bacon fat
- ◯ Crisco
- ◯ Trans-fat free brand
- ◯ I don't know, or don't cook

54. What kind of cold cereal do you usually eat? Choose 1 or 2 that you eat most often. (If you usually eat just one kind, mark one.)
- ◯ Low-carb cereals like Atkins, Low-Carb Special K
- ◯ Cheerios (plain), Shredded Wheat, Wheat Chex, Wheaties
- ◯ Sweetened cereals like Frosted Flakes, Honey Nut Cheerios, Fruit Loops, Cap'n Crunch, granola, instant sweetened oatmeal
- ◯ Other cold cereals, like Corn Flakes, Rice Krispies, Bran Flakes
- ◯ I don't eat cereal.

55. What kind of bread do you usually eat?
- ◯ Italian, French or local bakery
- ◯ Regular sliced white bread
- ◯ Dark bread like rye, cracked wheat
- ◯ 100% whole wheat
- ◯ I don't know or I don't eat bread

SOME LAST QUESTIONS ABOUT YOU

Are you ◯ Hispanic or Latino ◯ Not Hispanic or Latino

What race do you consider yourself to be? (MARK ALL THAT APPLY)
- ◯ White
- ◯ Black or African American
- ◯ Asian
- ◯ American Indian or Alaska Native
- ◯ Native Hawaiian or Other Pacific Islander
- ◯ Do not wish to provide this information

Thank you very much for filling out this questionnaire.
Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

PAGE 4

APPENDIX D DIET AND PHYSICAL ACTIVITY ASSESSMENTS

Below is a copy of the **Gordin Leisure-Time Exercise Questionnaire (LSI)** with three additional questions about fiber intake from the **Block Vegetable/Fruit/Fiber Screener**.

Godin Leisure-Time Questionnaire

When answering these questions please:

- only count exercise sessions that lasted 10 minutes or longer in duration.
- only count exercise that was done during free time (i.e., not occupation or housework).
- note that the main difference between the first three categories is the intensity of the endurance (aerobic) exercise.
- please write the average frequency on the first line and the average duration on the second.
- if you did not do any exercise in one of the categories, please write in "0".

Considering a typical week (7 days) how many times on the average did you do the following kinds of exercise in the last month?

	Times Per Week	Average Duration (min.)
a. VIGOROUS/STRENUOUS EXERCISE (HEART BEATS RAPIDLY, SWEATING) (e.g., running, aerobics classes, cross country skiing, vigorous swimming, vigorous bicycling).	_____	_____
b. MODERATE EXERCISE (NOT EXHAUSTING, LIGHT PERSPIRATION) (e.g., fast walking, tennis, easy bicycling, pilates, easy swimming, popular and folk dancing).	_____	_____
c. LIGHT/MILD EXERCISE (MINIMAL EFFORT, NO PERSPIRATION) (e.g., easy walking, yoga, golfing with a cart, and bowling).	_____	_____
d. STRENGTH TRAINING (eg. Dumbbells or Nautilus machines)	_____	_____

In addition to the above, please complete the below questions from the Dietary Fruit-Vegetable-Fiber Screener ©

Think about your eating habits over the past month or so. About how often do you eat each of the following foods? Remember breakfast, lunch, dinner, snacks and eating out. Mark one box for each food.

Fiber	Less than 1/Week	Once a Week	2-3 times a Week	4-6 times a Week	Once a Day	2+ a Day
Fiber cereals like Raisin Bran, Shredded Wheat or Fruit-n-Fiber	☐	☐	☐	☐	☐	☐
Beans such as baked beans, pinto, kidney, or lentils (not green beans)	☐	☐	☐	☐	☐	☐
Dark bread such as whole wheat or rye	☐	☐	☐	☐	☐	☐

BLOCK DIETARY DATA SYSTEMS
www.nutritionquest.com
(510) 704-8514

APPENDIX E STOOL QUESTIONNAIRE

Breast Cancer Stool Collection Questionnaire

Subject ID:	Date collected _____/_____/_____ Month Day Year	Hour collected _____ ○ am ○ pm

1. Based on the small chart included in the postcard, what did the stool you put into the tube look like? (Choose one or two answers).

2. Prior to this collection, when was your last bowel movement?

Earlier today, in the last 6 hours	Earlier today, more than 6 hours ago	Yesterday	Two days ago	More than two days ago
☐	☐	☐	☐	☐

3. In the past 2 months, how often have you had a bowel movement and what was the frequency of stool with the following textures?

	More than twice per day	Twice per day	Once per day	Every other day	Every 3-6 days	Once a week or less	Never
Any bowel movement	☐	☐	☐	☐	☐	☐	
Stool texture (The answers can be different from the above)							
Hard / lumpy	☐	☐	☐	☐	☐	☐	☐
Soft / smooth	☐	☐	☐	☐	☐	☐	☐
Watery liquid	☐	☐	☐	☐	☐	☐	☐

4. In the past 2 months, have you used any of the following medications?

	No	Yes, occasionally	Yes, regularly
Oral antibiotics	☐	☐	☐
If yes, what is the name (list of used)?			
Injected antibiotics	☐	☐	☐
If yes, what is the name (list of used)?			
Prilosec, Nexium, Prevacid (lansoprazole), Protonix, Aciphex	☐	☐	☐
H2 blocker: Pepcid, Tagamet, Zantac, Axid	☐	☐	☐

5. In the past 2 months, have you used any medications modifying bile production, including (but not limited to): Cholesytramine (e.g. Questran, Prevalite, Locholest), colestipol (e.g, colestid), colesevelam (e.g. Welchol), chenodeoxycholic acids (e.g. CDCA), or ursodeoxycholic acid (e.g. UDCA, Ursodiol, Actigall)?

☐No ☐Yes, occasionally ☐Yes, regularly

6. In the past 2 months, have you undergone a colonoscopy or other procedure requiring bowel preparation? ☐No ☐Yes

7. In the past week, have you taken (or eaten) any of the following? If so, please specify the frequency.

	Not used	Once or twice	Three to six times	Daily	More than once per day
Fiber substitute, such as Metamucil, Konsyl or Citracel	☐	☐	☐	☐	☐
Laxatives, such as Ex-lax, Dulcolax, MiraLax, Senna, or enema	☐	☐	☐	☐	☐
Stool softener, such as Colace	☐	☐	☐	☐	☐
Probiotic supplements	☐	☐	☐	☐	☐
Yogurt or kefir	☐	☐	☐	☐	☐
Other fermented foods, such as sauerkraut or kombucha	☐	☐	☐	☐	☐

8. In the past 6 months, did you gain or lose weight?

No	Gained ≤5 lbs.	Gained >5 lbs.	Lost ≤5 lbs.	Lost >5 lbs.
☐	☐	☐	☐	☐

9. Did you have any problems or concerns with the stool sample collection, for example the solution spilled out of the tube or you had problems with catching stool in the toilet accessory? (Please describe)

_____

_____

_____