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IIT2018-04-McArthur-neoHP

Neoadjuvant HER2-targeted Therapy and Immunotherapy with Pembrolizumab (neoHIP)

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The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Signature	**Date**

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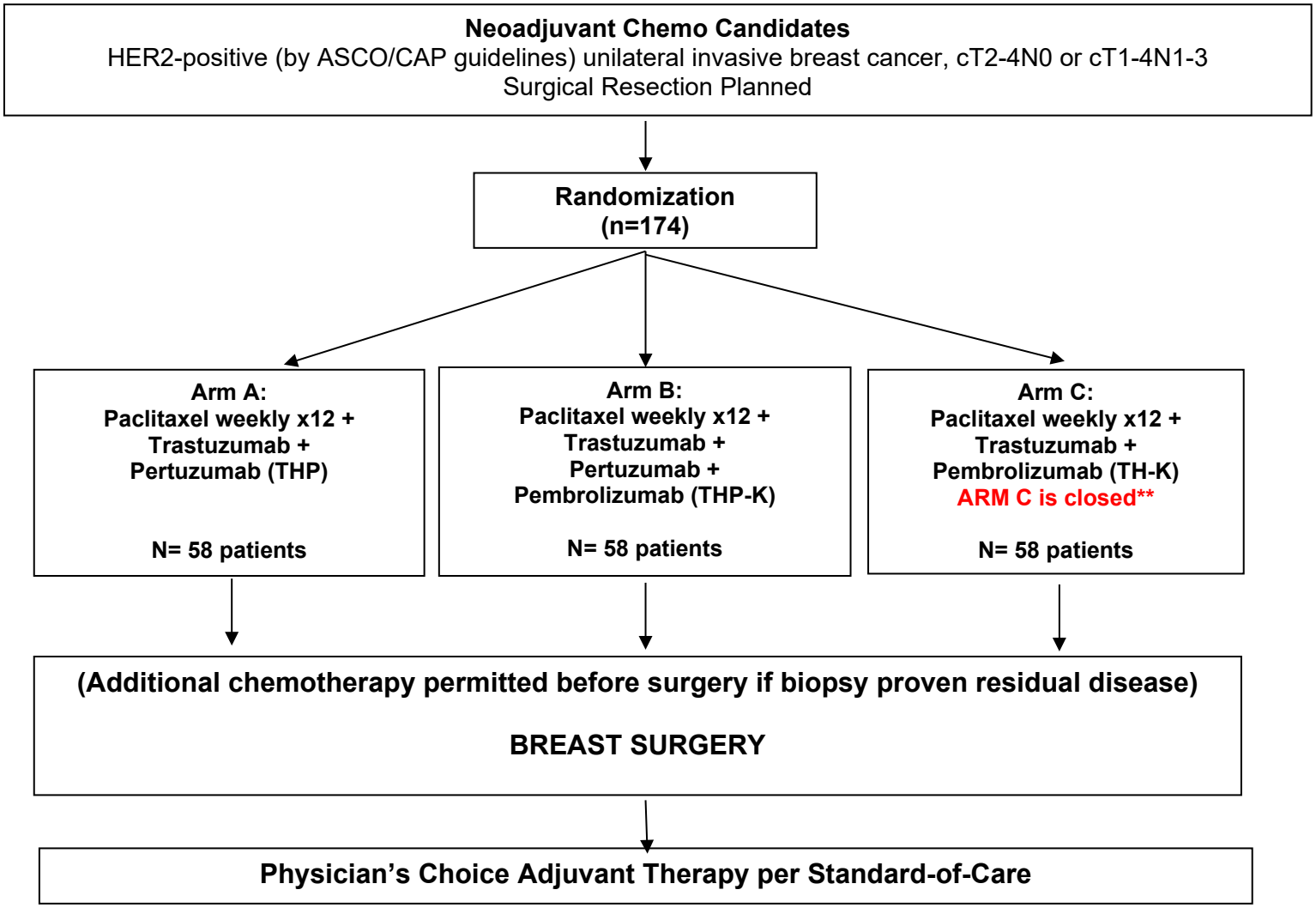
LIST OF ABBREVIATIONS

ADCC	Antibody Dependent Cell-Mediated Cytotoxicity
ACTH	Adrenocorticotropic Hormone
AE	Adverse Event
AJCC	American Joint Committee on Cancer
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
aPTT	activated Partial Thromboplastin Time
ASCO-CAP	American Society of Clinical Oncology-College of American Pathologists
AST	Aspartate Aminotransferase
AUC	Area Under Curve
BCG	Bacillus Calmette–Guérin
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CBE	Clinical Breast Exam
CBR	Clinical Benefit Rate
CD	Cluster of differentiation
chemo	chemotherapy
CHF	Congestive Heart Failure
CISH	Chromogenic in Situ Hybridization
CMP	Complete Metabolic Panel
Conmed	Concomitant medication
CPT	Cell Preparation tube
CR	Complete Response
CRA	Clinical Research Associate
CrCl	Creatinine Clearance
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
CT CAP	Computed Tomographic Chest Abdomen Pelvis
CTFG	Clinical Trial Facilitation Group
CTL	Cytotoxic T-lymphocyte
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DC	Dendritic Cell
ddAC	Dose-dense Adriamycin + Cytoxan
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ECI	Event of Clinical Interest
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture

EF	Ejection Fraction
EFS	Event Free Survival
ER	Estrogen Receptor
FDA	Food and Drug Administration
FFPE	Fresh Frozen Paraffin Embedded
FISH	Fluorescence in-situ hybridization
FOXP3	Forkhead box P3
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GFR	Glomerular Filtration Rate
H&P	History & Physical Exam
Hr	Hour
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HER2	Human Epidermal Growth Receptor 2
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HRT	Hormone replacement therapy
IB	Investigator's brochure
IDFS	Invasive Disease-Free Survival
IFN	Interferon
IHC	Immunohistochemistry
IL-2	Interleukin-2
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
irAE	Immune-related adverse event
ITT	Intent-To-Treat
IUD	Intrauterine device
IV	Intravenously
Kg	Kilogram
LH	Luteinizing Hormone
LVEF	Left Ventricular Ejection Fraction
Mg	Milligram
MHC	Major Histocompatibility Complex
mIHC	Multispectral immunohistochemistry
mRNA	Messenger Ribonucleic acid
MDSCs	Myeloid Derived Suppressor Cell
MK	Merck
Mg	Milligram
MHC	Major Histocompatibility Complex
mITT	Modified Intent-To-Treat
mL	Milliliters
MRI	Magnetic Resonance Imaging

MTD	Maximum Tolerated Dose
MUGA scan	Multigated Acquisition Scan
N	Nodal status
NCI	National Cancer Institute
NSAIDs	Non-Steroidal Anti Inflammatory Drugs
NYHA	New York Heart Association
ORR	Objective Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
pCR	Pathologic Complete Response
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Cell Death Ligand 1
PET/CT	Positron Emission Tomography/Computed Tomography
PFS	Progression Free Survival
PHI	Personal Health Information
PI	Principal Investigator
PO	Per oral
PR	Progesterone Receptor
PRs	Partial Response
PT	Prothrombin Time
RCB	Residual Cancer Burden
Reg	Regulatory
RNA	Ribonucleic acid
RSD	Reference Safety Dataset
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SOC	Standard of care
SGPT	Serum Glutamic Pyruvic Transaminase
T	Tumor size
T3/T4	Triiodothyronine / Thyroxine
TAA	Tumor Associated Antigen
TB	Tuberculosis
TCR	T-Cell Receptor
TIL	Tumor infiltrating lymphocyte
TNBC	Triple-Negative Breast Cancer
TNF-α	Tumor necrosis factor-alpha
Treg	T regulatory cell
TSH	Thyroid Stimulating Hormone
ULN	Upper limit of normal
WOCBP	Women of child-bearing potential

STUDY SCHEMA:



Primary endpoint: pathologic complete response (pCR) in the breast and axilla (ypT0/is ypN0).

Patients will be stratified by clinical nodal status (positive vs. negative) and hormone receptor status (positive vs. negative).

Trastuzumab loading dose of 8 mg/kg Cycle 1, and subsequent maintenance dose of 6 mg/kg Q3 weeks IV
Pertuzumab loading dose of 840 mg and subsequent maintenance dose of 420 mg Q3 weeks IV
Pembrolizumab is at fixed dose of 200mg Q3 weeks IV
Paclitaxel will be administered weekly at a dose of 80mg/m^2 for 12 weeks

STUDY SUMMARY

Title	**Neo**adjuvant **H**ER2-targeted Therapy and **I**mmunotherapy with **P**embrolizumab (**neoHIP**).
Protocol Number	IIT2018-04-MCARTHUR-NEOHP
Phase	2
Methodology	Prospective, open-label, multi-center therapeutic trial
Study Duration	36-month projected enrollment period and additional 84 months median follow-up
Study Center(s)	Cedars-Sinai Medical Center, Providence (Portland, OR), Massachusetts General Hospital (Boston, MA), University of Texas at Southwestern (Dallas, TX), New Mexico Cancer Center
Objectives	Primary Objectives: To explore rates of pathologic complete response (pCR) in the breast and axilla (ypT0/Tis ypN0) following 12 weeks of neoadjuvant HER2-directed systemic therapy with or without pembrolizumab Secondary Objective: To define the impact of the interventions on pathological invasive and in situ complete response in breast and axilla (ypT0 ypN0), pathologic invasive complete response in the breast only (ypT0/is), residual cancer burden (RCB), breast conserving surgery rate, event free survival (EFS), invasive disease free survival (IDFS), overall survival (OS), and safety. Exploratory Objective: 1) To characterize the immunologic effect of pembrolizumab, pertuzumab and trastuzumab administration in peripheral blood, intratumorally, and within the microbiome. 2) To correlate clinical outcomes with exploratory correlates wherever feasible, in order to explore potential predictive biomarkers of clinical efficacy or toxicity
Number of Subjects	174 patients
Diagnosis and Main Inclusion Criteria	-HER2-positive (by ASCO/CAP guidelines) invasive unilateral breast cancer -cT2-4N0 or cT1-4N1-3 -Patients will be stratified by clinical nodal status (positive vs. negative) and hormone receptor status (positive vs. negative)
Study Product(s), Dose, Route, Regimen	-Paclitaxel 80mg/m^2 weekly for 12 weeks -Trastuzumab 8mg/kg (loading dose) and then 6mg/kg IV every 3 weeks x 3 doses. Per local standard practice, an FDA-approved biosimilar administered intravenously may be substituted for trastuzumab at the same dose. Trastuzumab and hyaluronidase-oysk for subcutaneous administration may be substituted for trastuzumab, at 600 mg/hyaluronidase 10,000 units once every 3 weeks for total of 4 doses. No loading dose is required for trastuzumab and hyaluronidase-oysk. -Pertuzumab 840mg (1 loading dose) and then 420mg/kg IV every 3 weeks x 3 doses -Pembrolizumab 200mg IV every 3 weeks x 4 doses
Duration of administration	12 weeks

Reference therapy	Trastuzumab, pertuzumab and paclitaxel
Statistical Methodology	The rate of pCR (ypT0/Tis ypN0) in the control group (SOC THP Arm A) is expected to be 50%. We assume the treatment group will achieve a pCR rate of 73%. With a trial design 1:1:1 of THP vs. THP+pembrolizumab (THP-K) vs. TH+pembrolizumab (TH-K), for alpha=0.05 and power=80%, 55 patients are required for each arm. To account for a 5% loss to follow up during the trial, 3 additional patients will be enrolled to each arm (58 patients/arm). Due to the binary nature of the primary endpoint, patients who discontinue study therapy without undergoing surgery will be considered as a non-responder (non-pCR). A total of 174 patients will be enrolled in this trial. A safety interim analysis will take place after every 15th patient receives at least one dose of study treatment in each of the experimental arms. An efficacy analysis will take place after 20 patients have enrolled to Arm C.

1.0 BACKGROUND AND RATIONALE

1.1 Breast Cancer

Breast cancer is the most frequent women cancer worldwide, with an incidence of more than 1.7 million new cases[1]. The American Cancer Society estimates for 2017 over 255.000 new breast cancer cases within the United States[2]. Screening programs and effective local and systemic adjuvant therapies have been responsible for increasing survival rates, but still, more than 500.000 and 41000 breast cancer deaths are expected in 2017, globally and in the U.S. respectively.

One of the major advances in cancer research throughout the years was an in depth understanding of disease intrinsic biology, enabling a better clinical pathological and biological classification of breast cancer. There are three major clinically relevant subtypes that are managed differently: luminal (expressing the estrogen receptor (ER) and/or progesterone receptor (PR), human epidermal growth factor receptor-2+ (HER2+), and triple-negative, lacking expression of ER, PR, and HER2 (TNBC)[3]. The 8th edition of the American Joint Committee on Cancer (AJCC) Manual has incorporated biologic factors including tumor grade, ER, PR and HER2 status and the OncotypeDx Recurrence Score, helping clinicians with prognostic and predictive factors related to breast cancer[4]. Of these subtypes, HER2+ breast cancers and TNBCs are more likely than luminal breast cancers to harbor stromal tumor infiltrating lymphocytes (TILs) at diagnosis, with a linear relationship between stromal TIL content and clinical outcomes[5]. HER2+ breast cancers and TNBCs are also more likely to express the programmed cell death ligand 1 (PD-L1) in the tumor microenvironment than luminal breast cancers[6,7].

The classical approach to early stage breast cancer treatment is surgery, systemic adjuvant therapy and radiation therapy[8]. But some types of breast cancer are diagnosed at a locally advanced stage of disease, with increased rates of recurrence and metastasis after traditional treatment approaches[9]. The incorporation of neoadjuvant systemic therapy at this scenario has brought tremendous improvements, with some patients achieving a pathological complete response and consequently better survival[10]. Some subtypes of breast cancer derive better outcomes with this strategy (like HER2 positive and triple negative breast cancer)[11].
About 20% of breast cancer overexpresses HER2 protein[12], conferring those patients with a worse prognosis. Since the discovery of trastuzumab and drug incorporation on the metastatic setting, increasing response and overall survival, a tremendous evolution of and understanding of this disease has taken place. In the adjuvant setting, several trials have demonstrated increased survival[13]. Since then, newer HER2 directed agents

have been developed[14]. The dual HER2 blockade therapeutic strategy demonstrated increased PFS and OS in the metastatic setting[15], and increased pCR in the neoadjuvant setting[16,17]. Although, with greater pCR, comes more toxicity. In the NeoSphere adjuvant trial, one of the arms had a non-chemotherapy strategy using trastuzumab and pertuzumab for four cycles, achieving a 17% pCR and no chemotherapy related toxicity[18]. Definitely the therapeutic strategy needs to be challenged, with better treatment and less toxicity.

Immunotherapy trials have focused mainly on triple negative breast cancer and at the metastatic scenario, with overall response rate of 18.5% in patients heavily pretreated that received pembrolizumab[19]. Even though responses are not of a great magnitude, some of those patients are long term survivors, suggesting a possible long term protective immune response. Also, some retrospective analysis suggests that higher levels of TILs at diagnosis predict benefit from adjuvant and neoadjuvant chemotherapy, with longer progression-free survival (PFS) and overall survival (OS)[5]. A higher CD8+ T cell/Treg ratio may also be associated with a greater likelihood of pCR to neoadjuvant chemotherapy[20].

Since HER2 directed therapies, such as trastuzumab and pertuzumab, have an immune mediated mechanism of action[21], and most of the toxicity on the neoadjuvant trials is related to the chemotherapy backbone, this trial intends to explore the synergistic role of neoadjuvant HER2 directed therapy (trastuzumab and pertuzumab) associated with an anti-PD1 therapy (pembrolizumab), on patients with HER2 positive non-metastatic breast cancer. Besides the classical clinical factors associated with response to treatment, our study design will allow for examination on immune correlatives.

1.2 Rationale for Immune Therapy in Breast Cancer

The evolving understanding of breast cancer heterogeneity and biology and the tremendous efforts on advances in clinical trials through recent years has made possible for researchers to shift immune therapy from bench to bedside. Some pre-clinical studies have elucidated the relation between inflammation and innate immunity in mammary tumorigenesis. It all starts with acute inflammation, activation of innate immunity with tumor cell death and maturation of dendritic cells, priming tumor-specific T-cell response. If tumor cells are able to evade host immune response, there is a shift to chronic inflammation and a complex tumor microenvironment takes place, with lots of suppressive immune cells (Tregs, MDSCs and B-cells) and stromal cells (fibroblasts and endothelial cells), allowing immune escape and tumor progression[22]. At this point, immune checkpoint molecules are upregulated on tumor and immune cells[23]. This is the pre-clinical rationale for development of checkpoint blockade antibodies.

The two most common target pathways are cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and the programmed cell death protein 1 / programmed cell death ligand 1 (PD-1/PD-L1). Blocking these pathways would remove the inhibitory signals and allow the host immune system robust responses to tumor antigens[24]. Several phase 1 and 2 clinical trials have tested this hypothesis in metastatic breast cancer, with modest but encouraging initial results. Tremelimumab and ipilimumab, two anti-CTLA-4 antibodies, have been tested in Phase 1 or Pilot studies concurrent with other cancer therapies[25] or cryoablation, with modest clinical results but very interesting correlative biological analysis that has been moved forward to the expansion phase[26]. Studies with the PD-1/PD-L1 monoclonal antibodies avelumab[27], atezolizumab[28] and pembrolizumab[29] alone in metastatic breast cancer have demonstrated overall response rates from 4.8% to 44%, depending on the tumor subtype (ER positive, PR positive, HER2 positive or triple negative breast cancer), expression of PD-L1 or line of treatment for metastatic breast

cancer. Phase 3 trials with pembrolizumab and atezolizumab in metastatic breast cancer are ongoing (NCT03199885, NCT02555657, NCT02954874, NCT02819518, NCT03125902, NCT02425891, NCT03371017).

The utility of PD-1/PD-L1 blockade for TNBC in the adjuvant and neoadjuvant settings is under intensive investigation. Early data from the I-SPY trial revealed that adding pembrolizumab to neoadjuvant paclitaxel results in an estimated pCR rate of 46% versus 16% in HER2 negative patients, 60% versus 20% in TNBC patients, and 34% versus 13% in ER+/PR+/HER2- patients[30]. A trial investigating the combination of Pembrolizumab with chemotherapy in the neoadjuvant setting had preliminary results presented at ASCO 2017, suggesting that PD-L1 positive TNBC are more likely to respond to treatment[31].

Chemotherapy has a varied impact on immune responses, depending on the drug, dose and timing. By killing cancer cells and releasing tumor antigens, it would enhance immune priming and DC modulation. This is one rationale for combining chemotherapy to PD-1/PD-L1 blockade[32]. A phase 1b trial that enrolled patients with metastatic TNBC regardless of the PD-L1 status tested atezolizumab with nab-paclitaxel[33]. Thirty-two patients were evaluable for safety and response. Grade 3-4 hematologic toxicity occurred in over half the patients, but was manageable. At a median follow up of >5 months, the ORR was 38%, with 1 CR, 11 PRs, and 2 additional patients demonstrating a non-classical response. Responses occurred in patients with both PD-L1+ and PD-L1- disease, and there was a trend for higher response rate in patients treated first line relative to later line. A phase 3 registrational trial in first line is under way (NCT02425891). Pembrolizumab has been tested with eribulin in an ongoing trial designed to enroll 95 patients with metastatic TNBC of any PD-L1 status treated with < 2 prior lines of chemotherapy[34]. An interim analysis of 39 patients demonstrated the safety of the combination, with the most common side effects of fatigue, alopecia, nausea, neutropenia, and peripheral neuropathy. The ORR for 17 patients treated first line was 41.2%, and for those treated second or third line was 27.3%; this included 1 CR and 12 PRs. The CBR was 41%. PD-L1 status did not appear to impact the likelihood of response; the ORR and CBR for PD-L1+ patients were 29.4% and 35.3%, and for PD-L1- (negative) patients were 33.3% and 44.4%. The KEYNOTE-355 Phase 3 trial is evaluating pembrolizumab with chemotherapy relative to various chemotherapy regimens alone as first-line therapy for incurable TNBC (NCT02819518).

1.3 Rationale for Immune Therapy and HER2 Directed Therapy in Breast Cancer

Trastuzumab (H), a humanized monoclonal antibody that specifically binds to HER2 homodimers, is the cornerstone of therapy for both early and late stage HER2-overexpressing breast cancer. Trastuzumab itself has intrinsic immune modulating activity mediating ADCC[35] and promoting a HER2-specific T-cell response[36]. A group from Johns Hopkins University, Baltimore, MD, has demonstrated that the pre-clinical equivalent of trastuzumab or trastuzumab itself can augment the activity of a cell based vaccine in preclinical models[37] and in patients[38]. Also, pre-clinical models using HER2 transgenic mice demonstrated enhanced Fc-mediated immune priming by DCs, augmented effector T-cell activity, and a durable memory T-cell response[39]. Stagg J. et al. have shown that combined therapy with a trastuzumab-like antibody plus a PD-1 antibody markedly augments the clearance of HER2+ tumors in mice[40].
Multiple clinical trials are underway testing the addition of PD-1/PD-L1 blockade to HER2-based therapies for both locally advanced and metastatic HER2+ breast cancer. A Phase 2 global, randomized, double-blind, placebo controlled study is currently underway evaluating whether the addition of atezolizumab to TDM1 can further improve clinical

outcomes in patients with metastatic HER2+ breast cancer previously treated with trastuzumab and a taxane (NCT02924883).

1.4 Rationale for Combining Trastuzumab, Pertuzumab, Paclitaxel

Trastuzumab is a recombinant humanized anti-p185HER2 monoclonal antibody that binds with high affinity to the HER2 protein[41]. It was the first monoclonal antibody directed against the HER2 family receptors, showing clinical efficacy on HER2 overexpressing tumors[42]. Later trials on the metastatic[43] and adjuvant scenario[13] confirmed that when added to chemotherapy, trastuzumab based treatment provided better symptom control and prolonged survival on breast cancer patients whose tumors overexpressed HER2. Unfortunately, some patients develop disease recurrence after adjuvant treatment and, of those, near all of them develops metastatic disease[44]. The main toxicity signal related to trastuzumab is transient myocardial dysfunction, presented by decline on left ventricular ejection fraction on MUGA scans[45]. Strategies to overcome resistance to trastuzumab based therapy initially included: maintaining trastuzumab and changing the chemotherapy backbone[46]; changing trastuzumab for a HER2 directed tyrosine kinase inhibitor and Capecitabine[47]; dual HER2 directed therapy[48] and the use of an antibody-drug conjugate trastuzumab entamsine (TDM-1)[49].

Pertuzumab is a humanized monoclonal antibody and consists of two heavy chains (449 amino acid residues) and two light chains (214 residues). Like trastuzumab, pertuzumab is directed against the extracellular domain of HER2. However, it differs from trastuzumab in the epitope-binding regions of the light chain (12 amino acid differences) and heavy chain (29 amino acid differences). As a result, pertuzumab binds to the dimerization epitope of the HER2 receptor, thereby inhibiting dimerization of HER2 with other HER family receptors[50]. Trials that evaluated pertuzumab with chemotherapy for breast and other types of cancer showed modest clinical activity, with no extra cardiac signal toxicity, but with diarrhea, rash and mucosal inflammation[51]. Since trastuzumab and pertuzumab are HER2 directed monoclonal antibody with complementary modes of action, clinical trials were designed to confirm the possible synergism. Initial phase 2 trials confirmed this hypothesis[52]. The registrational phase 3 CLEOPATRA Trial[53] was a large randomized trial that tested the hypothesis of dual HER2 blockade using trastuzumab, pertuzumab and docetaxel. The results were impressive, with more than 80% of the patients experiencing a clinical response, a 50% reduction in the risk of progression from breast cancer and an overall survival gain never observed on such a population, with median overall survival of almost 60 months. No extra cardiac toxicity signals were observed with rash, diarrhea and mucosal inflammation as the major toxicity signals with major improvements after chemotherapy cessation. Based on that, FDA approved the use of trastuzumab, pertuzumab and a taxane for the first line treatment of patients with HER2 positive metastatic breast cancer. Other trials have tested paclitaxel with this dual HER2 blockade, showing similar efficacy as the use of docetaxel[54], with a better toxicity profile, regarding diarrhea and neutropenia.

The synergistic effect of dual HER2 blockade with trastuzumab and pertuzumab have also been exemplified at the neoadjuvant setting: when systemic chemotherapy and/or biological therapy are administered before surgery. The initial goal of trials with neoadjuvant chemotherapy was to convert an inoperable breast tumor into an operable one, and to convert mastectomies into breast conserving surgery[55]. The CTNeoBC pooled analysis confirmed later on that patients with HER2 positive disease that achieve a pCR have significantly better event-free and overall survivor[11], although there was not a direct correlation between the magnitude of the pCR and the survival benefit. Some

trials have tested the hypothesis that the addition of pertuzumab to a trastuzumab/chemotherapy neoadjuvant schema would increase the pCR rates. Trials like TRYPHAENA[16] and NeoSphere[18] have demonstrated levels of pCR never seen before, up to 66% in the hormone receptor negative population. Even though not technically powered to detect a survival difference, in the NeoSphere trial, patients that received trastuzumab, pertuzumab and docetaxel had a significantly better disease-free survival compared to those that received just trastuzumab and docetaxel. Based on that, the FDA granted approval for dual HER2 blockade with pertuzumab and trastuzumab in patients with HER2 positive breast cancer with tumors greater than 2 cm or with clinically axillary lymph node involvement[56]. Recently published, the APHINITY trial tested the hypothesis that adding pertuzumab to a trastuzumab based treatment for the adjuvant setting would increase invasive disease-free survival in breast cancer patients clinical stage I-III[57]. The trial demonstrated a statistically significant benefit on adding the dual HER2 direct therapy compared to trastuzumab based therapy, but with a modest 0.9% magnitude of a benefit. Thus, optimization of HER2 directed therapy in the neoadjuvant and adjuvant setting is needed.

1.5 Rationale for Combining Trastuzumab, Pertuzumab, Paclitaxel and Pembrolizumab

Some pre-clinical studies show that monoclonal antibodies against PD-1 or CTLA-4 could substantially boost the therapeutic activity of anti-HER2 in immunocompetent mice[40]. Their findings suggest that optimum activity of anti-HER2 treatment needs type I and type II interferons, and interferon γ can also enhance MHC class I and PD-L1 expression on tumor cells. Scientific evidence from translational data obtained from the neoadjuvant trials with dual HER2 blockade links tumor immune profile and pathological complete response. Using the NeoSphere trial tumor and blood samples, a group from Milan performed correlative analyses between immune gene mRNA expression (IFNγ, PD-1, PD-L1, PD-L2 and CTLA-4), lymphocyte infiltration and PD-L1 expression by immunohistochemistry and pCR in the breast[58]. What the authors found was an interesting contribution of the immune system with regards to HER2 direct therapy. In particular, expression of the immune check point PD-L1 was related to resistance to all regimens used in the trial (trastuzumab, pertuzumab, and docetaxel; trastuzumab and docetaxel; trastuzumab and pertuzumab; pertuzumab and docetaxel). Also, the expression of MHC I-related genes, which inhibit antibody-dependent cell cytotoxicity (ADCC), was also related to resistance.

The only clinical data available to date testing the hypothesis of immune therapy reverting resistance to HER2 directed treatment comes from the Phase 1b/2 PANACEA Trial, presented at the 2017 San Antonio Breast Cancer Symposium[59]. In this trial, patients with HER2 positive metastatic breast cancer that had progressed previously on trastuzumab-based treatment received a combination of trastuzumab and pembrolizumab. Tumors were assessed centrally for HER2 positivity and PD-L1 status, and for quantity of TILs. In the phase 1 portion, a dose escalation of pembrolizumab with trastuzumab showed no dose limiting toxicities. In the phase II part of the trial, 58 patients received the combination of trastuzumab at full dose and pembrolizumab 200mg IV every 3 weeks. In the PD-L1 positive patients (N=40), they observed a 15% objective response rate and a 25% disease control rate. In patients with 5% or more TILs present in the metastatic lesion, the objective response rate was 39% and the disease control rate was 47%. These findings confirm the safety of pembrolizumab with HER2-directed therapy and confirms activity in HER2-positive subsets. This strategy could be further improved if co-administered earlier in the course of disease in trastuzumab-naïve populations (i.e. in the curative intent setting) and may synergize when given with chemotherapy. The role of dual HER2-blockade when pembrolizumab and trastuzumab are co-administered is

uncertain. The goal is to improve responses with biologically rational strategies and ultimately improve cure rates, in part, by potentiating adaptive immunity, rendering long term cancer-specific immunity.[60]

In terms of safety, pembrolizumab has been tested concomitantly with chemotherapy in the metastatic and neoadjuvant setting. The Keynote 173 trial had patients with triple negative breast cancer suited to neoadjuvant therapy used pembrolizumab at a dose of 200mg every 3 weeks, together with nab-paclitaxel 125 mg/m2 for 4 cycles; then pembrolizumab 200mg every 3 weeks together with doxorubicin 60mg/2 and cyclophosphamide 600mg/m2 for another 4 cycles (Arm A).[31] The Arm B was the same regimen, but added Carboplatin AUC 6 with the pembrolizumab and nab-paclitaxel part. In this small trial, all patients presented with treatment adverse events, but all were non-fatal with 3 patients discontinuing due to adverse events. We hypothesize that checkpoint inhibitor therapy in combination with HER2-directed and cytotoxic chemotherapy in the preoperative setting has the potential to engage the immune system and lead to higher rates of pCR in women with HER2+ stage II-III breast cancer.

1.6 Rationale for Correlative Studies

Tumor Tissue:

Infiltration of immune cells has predicted improved prognosis and response to therapy in many different tumor types including breast cancer[61,62]. The greatest clinical benefit is seen when TILs comprise at least 50-60% of the tumor[60]. While a baseline TIL score indicates the immunogenicity of a tumor, the influx of TILs with therapy indicates the engagement of the immune system as a result of therapy.

In addition to the amount of lymphocytic infiltrate, the phenotype of that infiltrate has also been shown to be prognostic. Type 1 T-cells are associated with favorable prognosis. Type 1 $CD4^+$ T-helper cells facilitate antigen presentation and activation of $CD8^+$ cytotoxic T-cells (CTL), which are essential for tumor destruction[63]. On the other hand, type 2 $CD4^+$ T-helper cells, including Forkhead box P3 (FOXP3) $CD4^+$ regulatory T-cells, contribute to a tumor environment that inhibits CTL function and promotes tumor growth[64].

Multiplex IHC (mIHC) allows for multiple fluorescently tagged antibodies to be applied to paraffin-embedded tissue quantification of cell types including macrophages (CD163+), effector T-cells (CD3+CD8+), and regulatory T-cells (CD3+CD4+FOXP3+). Assays can also be used to assess the activation and proliferation of TILs using markers such as Ki-67, PD-1, CTLA-4. Flow cytometry can similarly be used on fresh or frozen tissue to quantify cell types including effector T-cells (CD3+CD8+), and regulatory T-cells (CD3+CD4+FOXP3+).

Activation of the immune system can be further assessed using sequencing technology. Using TCR sequencing analysis on extracted DNA from paraffin-embedded tissue, increase in shared clones suggests that therapy is enhancing proliferation of antigen experienced cells within the tumor.

Single cell gene expression profiling can be used to assess the transcriptome of individual cells. Innovative technology allows for the evaluation of the transcriptome in single cells within the tumor and the tumor microenvironment[65]. Serial assessment of the transcriptome through the course of therapy can identify genes that are indicators and/or effectors of safety and response.

Whole Blood:

An increase in circulating cytokines is an indicator of systemic immune activation. Further, flow cytometry can also be performed on fresh or frozen blood to assess the composition of systemic immune cells. Similarly, TCR sequencing can be conducted on blood to assess the clonality of systemic immune cells.

Optional Fecal Samples for Analysis

Many factors have been shown to regulate the anti-tumor immune response. The interaction of the immune system and the individual gastrointestinal microbiome can impact responses to immune therapy [66]. The microbiome can also influence the toxicity from immune therapy[67]. Thus, we will collect optional fecal samples for microbiome analysis as a potential biological correlate for efficacy and/or toxicity.

Optional Breast Tumor Biopsies for Analysis

We hypothesize that given the high expression of Fc receptors on macrophages (Shiao *Cancer Immunol Res* 2015) and the importance of PD-1 in regulating macrophage phagocytosis and antigen-presentation (Gordon *Nature* 2017) that adding pembrolizumab to HER2-directed therapy and chemotherapy will lead to increased numbers of phagocytic and antigen-presenting macrophages that promote the formation and activation of cytotoxic T cells.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

To explore the impact of checkpoint blockade administration with (mono- vs. dual) HER2-directed therapy on post-surgery pathologic complete response (pCR) rate in the breast and axilla (ypT0/Tis ypN0) following 4 cycles (12 weeks) of treatment and surgery. A pCR is defined at the time of surgery and the rate will be reported as the proportion of the intent-to-treat population that achieve a pCR.

The purpose of this phase II study is to identify whether Arm B (THP-K) or Arm C (TH-K) or both demonstrate a clinically significant pCR rate improvement over Arm A (THP).

2.2 Secondary Objectives

To define the impact of the interventions on pathological invasive and in situ complete response in breast and axilla (ypT0 ypN0), pathologic invasive complete response in the breast only (ypT0/is), breast conserving surgery rate, event free survival (EFS), invasive disease free survival (IDFS) overall survival, and safety.

2.3 Exploratory Objectives

2.3.1 To characterize the immunologic effect of pembrolizumab, pertuzumab and trastuzumab administration, both in peripheral blood, intratumorally and on the microbiome, for example, by:

- Describing changes in peripheral blood CD3+, CD4+, CD8+, or regulatory (CD4+FOXP3+) T-cells by flow cytometry over time;
- Characterizing the effect of the intervention on peripheral levels of Th1 and Th2-type cytokines over time;
- Characterizing tumor infiltrating lymphocytes (TILs) isolated from tumor biopsies and resection specimens using flow cytometry;
- Assessing changes in the microbiome during the study period.

2.3.2 To correlate clinical outcomes with exploratory correlates wherever feasible, in order to explore potential predictive biomarkers of clinical efficacy or toxicity

2.4 Endpoints

2.4.1 Primary Efficacy Endpoint

The primary endpoint is pathological complete response rate (pCR) in the breast and axilla (ypT0/Tis ypN0). It is defined as the proportion of subjects without residual invasive cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy by AJCC staging criteria (8^{th} edition) assessed by the local pathologist at the time of definitive surgery.

Subjects who are discontinued from the study treatment and continue with another neoadjuvant treatment not specified by the study prior to definitive surgery will be

classified as not having a pCR (non-responders). Subjects who are discontinued from study treatment due to the reasons that preclude surgery are considered non-responders.

2.4.2 Secondary Efficacy Endpoints

The secondary efficacy variables are pathological invasive and in situ complete response in breast and axilla (ypT0 ypN0), pathologic invasive complete response in the breast only (ypT0/is), residual cancer burden (RCB), the proportion of patients achieving breast conserving surgery, invasive disease-free survival, event-free survival, overall survival, and safety .

Pathological complete invasive and in situ response rate (pCR) in breast and axilla (ypT0 ypN0): It is defined as the proportion of subjects without residual invasive cancer and in situ disease on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy by AJCC staging criteria (8^{th} edition) assessed by the local pathologist at the time of definitive surgery.

Pathological complete response rate (pCR) in the breast only (ypT0/is): It is defined as the proportion of subjects without residual invasive cancer on hematoxylin and eosin evaluation of the complete resected breast specimen following completion of neoadjuvant systemic therapy by AJCC staging criteria (8th edition) assessed by the local pathologist at the time of definitive surgery.

Residual cancer burden (RCB) index: it is defined as a component of four distinct pathological parameters: bi-dimensional diameter of primary tumor bed, percent of cellularity in the tumor bed, number of involved lymph nodes and size of the largest nodal metastasis. This information is inputted in a web based mathematical model and four different classes are possible depending on the scores: RCB 0 (pCR), RCB I, RCB II, RCB III
(http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3).
The method has been described and validated before[68], pathologists involved in this protocol have been trained to use the RCB index.

Breast conserving surgery rate: This is defined as the proportion of patients who achieved breast conserving surgery out of the intent-to-treat population without inflammatory breast cancer, as these patients will receive mastectomy irrespective of their response to neoadjuvant treatment.

Event Free Survival: this is defined as time from randomization to any of the following events: progression of disease that precludes surgery, local or distant recurrence, or death due to any cause.

Invasive Disease-free survival (IDFS): This is defined as the time from the first date of no disease (i.e. date of surgery) to the first documentation of invasive progressive disease or death. All in situ cancer events (ipsilateral or contralateral DCIS, ipsilateral or contralateral LCIS and all in situ cancers of non-breast sites) are excluded as an event in the endpoint. IDFS will be described separately in patients who achieve a pCR from those who do not and overall for all patients that had surgery. Patients who are withdrawn from the study without documented progression and for whom there exists

eCRF evidence that evaluations have been made, will be censored at the date of the last assessment when the patient was known to be disease-free.

Overall survival (OS): This is defined as the time from the date of randomization to the first documentation of death from any cause, measured in the intent-to-treat population.

Safety: Safety of the pre-operative (neoadjuvant) treatment regimen will be evaluated as follows:

1. Incidence of symptomatic cardiac events and asymptomatic LVEF events (LVEF < 50% or a decrease ≥ 10 ejection fraction points from baseline);
2. LVEF measures over the course of the study;
3. Incidence and severity of adverse events and serious adverse events;
4. Laboratory test abnormalities.

All patients who received at least one dose of treatment and received at least one post-baseline safety assessment will be included in the safety evaluation.

2.4.3 Exploratory Endpoints:

Exploratory correlative studies will be performed to characterize the immunologic response of the intervention, and to correlate immunologic laboratory parameters with clinical efficacy and toxicity. Breast cancer tissue specimens, axillary lymph node specimens when available, peripheral blood samples and optional fecal samples will be studied. The correlatives will be conducted by the Cedars Sinai collaborating scientists.

The exploratory aims of this study are:

1. To characterize the immunologic effect of pre-operative pembrolizumab and HER2-directed therapy administration, both in peripheral blood, intratumorally and on the microbiome;
2. To evaluate for the induction of an antigen-specific immune response;
3. To evaluate the effect of subsequent systemic cytotoxic therapy on the immune response;
4. To evaluate molecular tumor characteristics, such as mutational burden, RNA expression and protein expression, and correlate with markers of immune response;
5. To evaluate these laboratory correlates as potential predictive biomarkers of clinical efficacy or toxicity.

To this end, specimens to be collected for evaluation include:

1. Core needle biopsy of primary tumor with 2 cores (1 cm each) obtained for immune response studies and confirmation of diagnosis. If the diagnostic core needle biopsy specimen has enough material, another procedure will not be mandated;
2. Lumpectomy/mastectomy specimens for all study participants;
3. Sentinel or non-sentinel lymph node specimens where available (collected on the core biopsy and/or surgical date);
4. Serial peripheral blood samples – collected from all study participants, as per the schedule in Table 3;
5. Optional fecal samples collected as per the schedule in Table 3;

6. Optional breast biopsies collected as per the schedule in Table 3.

3.0 STUDY DESIGN

3.1 Overview of Study Design:

This is a Phase 2 open-label, randomized, multi-center trial to evaluate the efficacy and safety of trastuzumab, pertuzumab and weekly paclitaxel as compared to trastuzumab, pertuzumab, pembrolizumab and weekly paclitaxel, or trastuzumab, pembrolizumab and weekly paclitaxel in chemo naive patients with invasive HER2 positive (by ASCO/CAP guidelines) unilateral breast cancer whose primary tumors are > 2 cm or clinically lymph node positive (cT2-4N0 or cT1-4N1-3).

To participate in the trial, a patient must fulfill all inclusion/exclusion criteria and will need to consent to allow the storage of blood and tumor tissue samples for biomarker research as specified in Section 4.1.

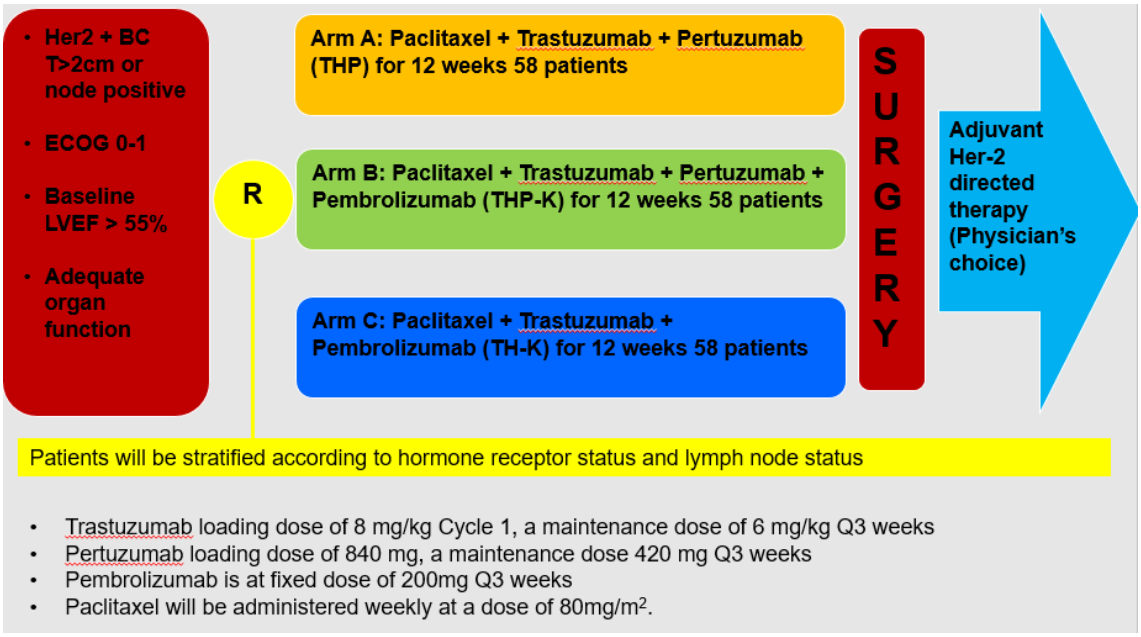
Patients will be randomized (see Figure 1) to either Arm A (trastuzumab, pertuzumab and weekly paclitaxel), Arm B (trastuzumab, pertuzumab, pembrolizumab and weekly paclitaxel) or Arm C (trastuzumab, pembrolizumab and weekly paclitaxel). Patients will be stratified according to hormone receptor status (positive versus negative) and lymph node status (clinically positive versus negative). All patients will be treated every three weeks for four cycles (only paclitaxel will be administered weekly) and then undergo breast surgery. Arm A patients will be regarded as the reference group.

After surgery, patients in all arms will be treated at their physician's discretion, with standard of care HER2 positive breast cancer adjuvant therapy after receiving neoadjuvant taxane and dual HER2 blockade. After completion of post-operative chemotherapy, patients will receive radiotherapy as per local clinical standard and those patients whose tumors were estrogen-receptor positive will receive hormone manipulation as per local clinical standard. Further details of the study treatment and administration can be found in Section 5.1.

Patients whose neoadjuvant study treatment is discontinued prior to surgery will be managed as per local practice. Patients, whose adjuvant (post-operative) chemotherapy is discontinued due to standard chemotherapy related intolerable toxicity, will continue with trastuzumab until they have received a total of 17 cycles of treatment. Patients with biopsy-proven residual disease after completing the study treatment may receive additional chemotherapy (i.e. four cycles of adriamycin + – cyclophosphamide administered every two weeks) prior to surgery at the treating physician discretion and should be seen in follow-up at each additional treatment prior to surgery.

Subjects will be followed on protocol after surgery every 3 months until 36 (+/-1) months from the date of surgical resection and every 6 months during year 4 and 5, then annually until year 10. Thereafter, every effort will be made to continue monitoring these subjects via standard-of-care visits, so that late recurrences can be identified and overall survival can be estimated.

Figure 1: Study treatment plan



3.1.1 Rationale of Dosage Selection of Paclitaxel

For further information see Sections 1.4 and 5.0.

3.1.2 Rationale of Dosage Selection of Trastuzumab and Pertuzumab

For further information see Sections 1.4 and 5.0.

3.1.3 Rationale of Dosage Selection of Pembrolizumab

For further information see Sections 1.5 and 5.0.

4.0 PATIENT ELIGIBILITY

4.1 Participant Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Male/female patients with histologically confirmed invasive HER2-positive (by ASCO/CAP guidelines) unilateral breast cancer.
2. Patient has untreated non-metastatic (M0), cT2-4N0 or cT1-4N1-3 (biopsies of clinically suspicious lymph nodes to confirm nodal status is encouraged).
3. Multifocal/centric disease is permitted if all suspicious foci have been biopsied and are consistent with HER2-positive (by ASCO/CAP guidelines) invasive breast cancer.
4. Be a male or female subject ≥18 years of age on day of signing informed consent.
5. Male participants:

   A male participant must agree to use a contraception as detailed in Appendix C of this protocol during the treatment period and for at least 6 months after the last dose of study treatment and refrain from donating sperm during this period.
6. Female participants:

   A female participant is eligible to participate if she is not pregnant (see Appendix C), not breastfeeding, and at least one of the following conditions applies:
   a.) Not a woman of childbearing potential (WOCBP) as defined in Appendix C OR
   b.) A WOCBP who agrees to follow the contraceptive guidance in Appendix C during the treatment period and for at least 6 months after the last dose of study treatment.
7. The participant (or legally acceptable representative if applicable) provides written informed consent for the trial.
8. Provides adequate archival tumor tissue sample or newly obtained core or excisional biopsy of a tumor lesion not previously irradiated. Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. Newly obtained biopsies are preferred to archived tissue. Note: If submitting unstained cut slides, newly cut slides should be submitted to the testing laboratory within 14 days from the date slides are cut (see section 8.1).
9. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1.
10. Have adequate organ function as defined in the following table (Table 1).

Table 1: Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/μL
Platelets	≥100 000/μL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Creatinine OR Measured or calculated[b] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants
Cardiac	
Echocardiogram or MUGA	Baseline LVEF ≥ 55%

ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.

[a] Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks.

[b] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

4.2 Participant Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. A WOCBP who has a positive urine pregnancy test within 72 hours prior to randomization (see Appendix C). If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

   Note: in the event that 72 hours have elapsed between the screening pregnancy test and the first dose of study treatment, another pregnancy test (urine or serum) must be performed and must be negative in order for subject to start receiving study medication.

2. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (e.g., CTLA-4, OX 40, CD137).

3. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks prior to randomization.

   Note: If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment.

4. Has received prior radiotherapy within 2 weeks of start of study treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis.

5. Has received a live vaccine or live-attenuated vaccine within 30 days prior to the first dose of study drug. Administration of killed vaccines is allowed.

6. Is currently participating in or has participated in a study of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of study treatment.

   Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

7. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drug.

8. Has a known additional malignancy (other than their current breast cancer diagnosis) that is progressing or has required active systemic treatment within the past 3 years.

   Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or carcinoma in situ (e.g. breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.

9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

10. Has a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or has current pneumonitis/interstitial lung disease.

11. Has an active infection requiring systemic therapy.

12. Has a known history of Human Immunodeficiency Virus (HIV).

13. Has known active Hepatitis B or Hepatitis C virus infection.

14. Has a known history of active TB (Bacillus Tuberculosis).
15. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the subject's participation for the full duration of the study, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
16. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
17. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the study, starting with the screening visit through 120 days after the last dose of trial treatment.
18. Has significant cardiovascular disease, such as:
    - History of myocardial infarction, acute coronary syndrome or coronary angioplasty/stenting/bypass grafting within the last 6 months
    - Congestive heart failure (CHF) New York Heart Association (NYHA) Class II-IV or history of CHF NYHA class III or IV
    - Angina pectoris requiring anti-anginal medication, uncontrolled arrhythmias, or uncontrolled hypertension (systolic blood pressure > 180mmHg and/or diastolic blood pressure > 100mmHg).

5.0 TREATMENT PLAN

5.1 Treatment Dosage and Administration

Arm A

Trastuzumab I.V. followed by pertuzumab I.V. every three weeks for four cycles. A loading dose of 8 mg/kg of trastuzumab and 840 mg of pertuzumab is required on Day 1 Cycle 1, thereafter a maintenance dose of 6 mg/kg and 420 mg is required from Cycle 2 onwards, respectively. Paclitaxel will be administered weekly at a dose of 80mg/m^2 for 12 weeks.

Arm B

Trastuzumab I.V. followed by pertuzumab I.V. followed by pembrolizumab I.V. every three weeks for four cycles. A loading dose of 8 mg/kg of trastuzumab and 840 mg of pertuzumab is required on Day 1 Cycle 1, thereafter a maintenance dose of 6 mg/kg and 420 mg is required from Cycle 2 onwards, respectively. Pembrolizumab is administered at fixed dose of 200mg. Paclitaxel will be administered weekly at a dose of 80mg/ m^2 for 12 weeks.

Arm C

Trastuzumab I.V. followed by pembrolizumab I.V. every three weeks for four cycles. A loading dose of 8 mg/kg of trastuzumab is required on Day 1 Cycle 1, thereafter a maintenance dose of 6 mg/kg is required from Cycle 2 onwards. Pembrolizumab is at fixed dose of 200mg. Paclitaxel will be administered weekly at a dose of 80mg/ m^2 for 12 weeks.

Study treatment will be administered every three weeks for four cycles then the patient will undergo breast surgery.

Trastuzumab

Trastuzumab will be administered on Day 1 of Cycle 1 at the required loading dose of 8 mg/kg, as an I.V. infusion. On Day 22 (three weeks after the first dose) and every three weeks thereafter, trastuzumab will be administered at a dose of 6 mg/kg as an I.V. infusion. The initial dose of trastuzumab will be administered over 90 (± 10) minutes. Interruption or slowing of the infusion may help control infusion-related symptoms and infusion may be resumed when symptoms. abate. If the infusion is well tolerated, subsequent infusions may be administered over 30 (± 10) minutes. All infusion-related symptoms must have resolved before study treatment is given or the patient is discharged (see Section 7.5.1). Patients who experience infusion-related symptoms may be pre-medicated with paracetamol and antihistamines for subsequent infusions. Dose reductions for toxicity are not permitted. Trastuzumab will be administered every three weeks for four cycles.

Per local standard practice or institutional guidelines, an FDA-approve biosimilar is an appropriate substitute for trastuzumab per the US FDA labeling information (e.g. trastuzumab-anns, trastuzumab-dkst, trastuzumab-pkrb or trastuzumab-qyyp). Dose is similar to trastuzumab administered as an IV infusion.

Trastuzumab and hyaluronidase administered subcutaneously may be substituted for trastuzumab at 600 mg of trastuzumab/10,000 units of hyaluronidase every 3 weeks. No loading dose is required for trastuzumab/hyaluronidase.

Pertuzumab

Pertuzumab will be administered on Day 1 of cycle 1 at the required loading dose of 840 mg as an I.V. infusion. On Day 22 (three weeks after the first dose), and every three weeks thereafter, pertuzumab will be administered at a dose of 420 mg as an I.V. infusion. The initial dose of pertuzumab will be administered over 60 (± 10) minutes. Following infusion, it is recommended, but not required that patients be observed for a further 30-60 minutes or per institutional standards of practice on Day 1 of Cycle 1 for symptoms like fever, chills and other infusion-related symptoms. The infusion should be slowed or interrupted if the patient experiencing fever, chills and other infusion-related symptoms. If the infusion is well tolerated, subsequent doses may be administered over 30 (± 10) minutes. All infusion-related symptoms must have resolved before chemotherapy (Arm B only) is given or the patient is discharged. Patients who experience infusion-related symptoms may be pre-medicated with paracetamol and antihistamines for subsequent infusions. Dose reductions for toxicity are not permitted. Pertuzumab will be administered every three weeks for four cycles.

Pembrolizumab

Pembrolizumab is a highly selective humanized monoclonal antibody designed to block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Subjects will receive pembrolizumab 200 mg as an IV infusion. Pembrolizumab will be administered as a 30-minute IV infusion (every effort should be made to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Pembrolizumab is manufactured by Merck. Please refer to investigator brochure[69] and MK-3475 Drug Preparation Instructions' manual.

Table 2 Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/4 mL	Solution for Injection

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational products in accordance with the protocol and any applicable laws and regulations. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

Paclitaxel

Paclitaxel will be administered weekly for 12 weeks at 80 mg/m^2 as an I.V. infusion over a minimum of 1 hour per institutional standards. Paclitaxel will be administered after the trastuzumab and pertuzumab infusion observation period in Arm A, after the trastuzumab, pertuzumab and pembrolizumab infusion observation period in Arm B, and after trastuzumab and pembrolizumab infusion observation period in Arm C. Additional premedication should be administered as per standard practice.

Nab-paclitaxel may be substituted for paclitaxel due to medical necessity (e.g. hypersensitivity reaction) per local standard practice. If substituted, administer nab-paclitaxel at 125 mg/m^2 IV over 30 minutes (±10 minutes) weekly for total of 12 weeks including paclitaxel doses that were previously administered before substitution). Premedication is not generally necessary prior to nab-paclitaxel, although may be administered per standard practice or in patients with prior mild to moderate hypersensitivity reactions.

5.2 Dose Modification and Delays

5.2.1 Modification of the Amount of Study Drug Administered due to Changes in Patient's Weight

The amount of trastuzumab administered is calculated according to the patient's actual body weight, with no upper limit. Weight should be recorded at baseline and at every scheduled visit for all patients. The amount to be administered must be recalculated if the patient's body weight has changed by > 10% (increased or decreased) from baseline.

The amount of paclitaxel or nab-paclitaxel is calculated according to the patient's body surface area (BSA). Weight and height should be recorded at baseline and the BSA calculated; thereafter, at every scheduled visit all patients should be re-weighed. The amount to be administered must be recalculated if the patient's body weight has changed by > 10% (increased or decreased) from baseline.

5.2.2 Dose Modifications for Pertuzumab, Trastuzumab, and Paclitaxel

Administration may be delayed to assess or treat adverse events, such as cardiac adverse events, myelosuppression and immune mediated events. Since trastuzumab, pertuzumab and paclitaxel are widely available drugs on daily practice and FDA approved for neoadjuvant breast cancer treatment, dose delays or adjustments according to toxicity should follow the current standard of care guidelines.

Please refer to Section 7.4 for drug specific Supportive Care Guidelines.

In the neo-adjuvant setting (Cycles 1-4), a dose delay of up to two weeks will be permitted to allow recovery to baseline. Thereafter, one further dose delay of up to two weeks will be permitted before the patient is discontinued from the study. Following a dose delay of two weeks or less, trastuzumab does not need to be re-loaded (the maintenance dose only needs to be given).

5.2.3 Dose Modifications and delays for pembrolizumab

AEs associated with pembrolizumab exposure, including coadministration with additional compounds, may represent an immunologic aetiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the

last dose of pembrolizumab/combination treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab/combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab/combination treatment are provided in Table 6.

Attribution of Toxicity:

When study interventions are administered in combination, attribution of an adverse event to a single component is likely to be difficult. Therefore, while the investigator may attribute a toxicity event to the combination, or to pembrolizumab alone, for adverse events listed in Table 6, both interventions must be held according to the criteria in Table 6 Dose Modification and Toxicity Management Guidelines for Immune-Related Adverse Events Associated with Pembrolizumab.

Holding Study Interventions:

When study interventions are administered in combination, if the AE is considered immune-related, both interventions should be held according to recommended dose modifications.

Restarting Study Interventions:

Participants may not have any dose modifications (no change in dose or schedule) of pembrolizumab in this study, as described in Table 6.

If the toxicity does not resolve or the criteria for resuming treatment are not met, the participant must be discontinued from all study interventions.

If the toxicities do resolve and conditions are aligned with what is defined in Table 3, the combination and pembrolizumab may be restarted at the discretion of the investigator. In these cases where the toxicity is attributed to the combination, re-initiation of pembrolizumab as a monotherapy may be considered at the principal investigator's discretion.

Dosing Interruptions:

Table 6 shows treatment guidelines for dose modification and toxicity management guidelines for immune-related AEs associated with administration of pembrolizumab. Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical/surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within

3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

5.3 Method of Assigning Subjects to Treatment Groups

Randomization will be conducted using a web-based randomization system within the EDC database Clinical Studio. Randomization will be accomplished by the method of random permuted block. Eligible patients will be randomized in a 1:1:1 ratio between the experimental arm and control arm.
Patients will be randomized and assigned unique randomization numbers. As confirmation, the investigator will be provided a written verification of each patient's registration. **Treatment should occur within 5 business days of the patient being randomized into the study.**
No patient may begin treatment prior to randomization and assignment of a medication number.
The patient randomization numbers are to be allocated dynamically in the order in which the patients are enrolled. The dynamic allocation will be stratified by hormone receptor status (positive or negative) and lymph node status (positive or negative).

5.4 Concomitant Medication and Treatment

Allowed Therapies

In general, all medications taken by the patient for concomitant diseases should continue during the study treatment period and should be recorded on the eCRF. The following list of allowed medications is provided as a guidance, treatment prescribed to the patients should be adapted according to local standard practice.

- H_1 and H_2 antagonist (e.g. diphenhydramine, cimetidine);
- Analgesics (e.g. paracetamol, meperidine, opioids);
- Corticosteroids to treat or prevent allergic or infusion reactions;
- Corticosteroids to treat immune related adverse events
- Thyroid medications
- Anti-emetic (approved prophylactic serotonin-antagonists, benzodiazepines, ondansetron, etc.);
- Medication to treat diarrhea (e.g. loperamide);
- Colony stimulating factors (e.g. G-CSF);

- Estrogen receptor antagonist (e.g. tamoxifen) or aromatase inhibitors(e.g. anastrozole, exemestane) in the adjuvant setting per local practice;

- Gonadotropin-Releasing Hormone Analog/Agonists for fertility preservation during chemotherapy

- Acceptable methods of contraception must be used when the female patient or male partner are not surgically sterilized or do not meet the study definition of post-menopausal (≥12 months of amenorrhea).

Excluded Therapies

The following therapies are excluded during the study:

- Anti-cancer therapies other than those administered in this study, including cytotoxic chemotherapy (except for ddAC in patients with biopsy proven residual disease before surgery), radiotherapy (except for adjuvant radiotherapy for breast cancer after completion of chemotherapy), immunotherapy, and biological/targeted anti-cancer therapy;

- Any investigational agent, except for those used for this study;

- Initiation of herbal remedies. Herbal remedies initiated prior to study entry and continuing during the study are permitted and must be reported on the appropriate eCRF;

- Any oral, injected or implanted hormonal methods of contraception;

- Proton pump inhibitors, while not excluded are discouraged while on protocol treatment. It is strongly encouraged that proton pump inhibitors (i.e. pantoprazole, etc.) be discontinued from the time of consent until the Post-Treatment Follow-up phase;

- Live vaccines or live-attenuated vaccines within 30 days prior to the first dose of study treatment and while participating in the study. Administration of killed vaccines is allowed.
  Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. All treatments that the Investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care.

  Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing study. If there is a clinical indication for any medication or vaccination specifically prohibited during the study, discontinuation from study therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the participant's primary physician decision. However, the decision to continue the participant on study treatment requires the mutual agreement of the investigator, the Sponsor and the participant.

  There are no prohibited therapies during the Post-Treatment Follow-up Phase.

Rescue Medications and Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 7.4.2 [Table 6]. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to Table 6 in Section 7.4.2 for guidelines regarding dose modification and supportive care.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

5.5 Other Modalities or Procedures/ Research vs. Standard of Care Procedures

After the completed neoadjuvant treatment, patients will continue to standard of care breast surgery per local SOC guidelines approximately 3-6 weeks following discontinuation or completion of study treatment in the neoadjuvant treatment phase. After surgery, patients will receive adjuvant chemotherapy and HER2-directed therapy, by their physician's choice. Standard of care is the use of adjuvant anthracycline-based chemotherapy followed by trastuzumab for a total of 12 months (considering the neoadjuvant treatment).

5.6 Duration of Study Participation

The study duration per subject will be up to 37 months, with up to 28 days of screening, up to 90 days on treatment, and 84 months of follow-up. The total enrollment period is 36 months.

5.7 Criteria for Premature Withdrawal

Subjects have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw subjects from the study in the event of intercurrent illness, adverse events, treatment failure after a prescribed procedure, protocol violation, cure, administrative reasons or for other reasons. An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided.

Should a subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

The investigator should contact the subject or a responsible relative by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the subject's withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an adverse event, the principal specific event will be recorded on the Electronic Case Report Form.

In the case that the subject decides to prematurely discontinue study treatment ["refuses treatment"], he/she should be asked if he/she can still be contacted for further information. The outcome of that discussion should be documented in both the medical records and in the eCRF.

5.8 Removal of Patients from Protocol

Subjects participating may withdraw from the study at any time and for any reason. Subjects may be removed from the study at any time due to severe unacceptable side effects, death, patient's non-compliance with the defined treatment plan, identification of recurrent/metastatic disease, pregnancy, or at the patient's request to withdraw consent.

5.9 Replacement Policy

5.9.1 For Subjects

Patients randomized into the study will not be replaced

5.9.2 For Centers

A center may be replaced for the following administrative reasons:

- Excessively slow recruitment.
- Poor protocol adherence.

6.0 STUDY PROCEDURES

6.1 Screening/Baseline Procedures

After the patient is identified as a potentially appropriate study candidate an initial history, physical exam, and baseline CTCAEv.5.0 assessment will be performed. If potentially eligible, the consent form will be reviewed, and consent will be signed by the study candidate.

Following consent, candidates will then undergo screening for eligibility for up to 28 days. Required components of the screening include: screening bloods, breast imaging (mammogram with ultrasound or MRI as indicated), cross-sectional imaging to rule out metastatic disease if AJCC stage III (either PET/CT or CT CAP + bone scan).

Leftover tumor tissue will be collected from standard of care biopsy prior to Cycle 1, Day 1. Collection requirements provided in section 8.4.

6.1.1 Informed Consent

6.1.2 Comprehensive metabolic panel (CMP) must include the following: aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), total bilirubin, calcium, creatinine, glucose, total protein, BUN, sodium, potassium, chloride, bicarbonate or carbon dioxide.

6.1.3 Complete blood cell count with differential and platelet count (CBC).

6.1.4 Echo or MUGA.

6.1.5 Coagulation PT/INR and aPTT

6.1.6 Thyroid stimulating hormone TSH with reflex to T3 and free T4 OR TSH with T3 and free T4 per institutional standards.

6.2 Procedures During Treatment

6.2.1 Subjects randomized to control arm

Subjects randomized to the control arm will receive standard-of-care neoadjuvant trastuzumab followed by pertuzumab and weekly paclitaxel, as stated on section 5.0 above. Before starting treatment, the tumor should be marked using the method which is standard locally (for example skin tattoo or surgical clip) so that the appropriate excision can be made should the patient experience complete clinical regression of the tumor during therapy. Also, patients will have a medical oncology visit and blood test collected before each treatment cycle. If residual disease is suspected after completion of Cycle 4, a biopsy can be performed at the treating physician's discretion. If residual disease is biopsy confirmed, additional chemotherapy (i.e. q2weekly doxorubicin and cyclophosphamide) can be administered prior to surgery with clinical assessments completed on treatment days.

Additional trastuzumab and pertuzumab can be administered if surgery is delayed greater than 6 weeks since the last paclitaxel administration.

In order to provide a control comparison for long-term toxicity assessment and immunologic laboratory correlatives, subjects will be monitored following definitive surgery (table 3). Following the date of surgery, subjects will be monitored with history, physical exam, toxicity assessment, and research bloods at 30 days and 60 days +/- 1 week after surgery. These visits will correspond with standard-of-care surgical oncology follow-up and/or medical oncology follow-up/chemotherapy treatment visits whenever feasible. Therefore, the research assessments may be conducted by a surgical oncologist or medical oncologist. Thus, study assessments will not require additional visits above and beyond the standard of care.

Research bloods will be drawn at Cycle 1 Day 1 and Cycle 3 Day 1 (prior to treatment) with standard-of-care routine bloods, at surgery (research items completed within +/- 3 days of surgery), 30 and 60 days (+/- 1 week) after surgery. Research bloods (2 CPT tubes, 1 Cyto tube and 1 red-top tube) for immune response studies will be sent to laboratory.

Optional fecal sample for microbiome analysis will be collected on Cycle 1 Day 1 and Cycle 3 Day 1 (prior to treatment), at surgery (+/- 3 days), and at 30 and 60 days (+/- 1 week) after surgery.

Optional fresh breast biopsies (Cedars site only) will be collected at baseline (after eligibility is confirmed but prior to start of treatment) and at 6 weeks (C3D1 +/- 1 week).

CBC and CMP will be drawn weekly on days 1, 8 and 15 of each cycle (+/-3 days) prior to treatment.

Echo or MUGA after last dose of paclitaxel and prior to surgery.

6.2.2 Subjects randomized to intervention arm

Subjects randomized to the intervention arm will receive either neoadjuvant trastuzumab, pertuzumab, pembrolizumab and weekly paclitaxel (Arm B) or trastuzumab, pembrolizumab and weekly paclitaxel (Arm C) as stated on section 5.0 above. Before starting treatment, the tumor should be marked using the method which is standard locally (for example skin tattoo or surgical clip) so that the appropriate excision can be made should the patient experience complete clinical regression of the tumor during therapy.

Patients will have a medical oncology visit and blood test collected before each treatment cycle. Additional trastuzumab and pertuzumab can be administered if surgery is delayed greater than 6 weeks since the last paclitaxel administration. Following the date of surgery, subjects will be monitored with history, physical exam, toxicity assessment, safety labs and research bloods 30 days +/- 1 week after surgery and again 60 days +/- 1 week. As in the control arm, long-term toxicity assessment and immunologic laboratory correlatives will be conducted during standard-of-care surgical/medical oncology follow-up appointments.

Research bloods will be drawn at Cycle 1 Day 1 and Cycle 3 Day 1 (prior to treatment) with standard-of-care routine bloods, at surgery (research items completed within +/- 3 days of surgery), 30 and 60 days (+/- 1 week) after surgery. Research Blood (2 CPT tubes, 1 Cyto tube and 1 red-top tube) for immune response studies will be sent to laboratory.

Optional fecal sample for microbiome analysis will be collected on Cycle 1 Day 1 and Cycle 3 Day 1 (prior to treatment), at surgery (+/- 3 days), and at 30 and 60 days post-surgery follow-up.

Optional fresh breast biopsies (Cedars site only) will be collected at baseline (after eligibility is confirmed but prior to start of treatment) and at 6 weeks (C3D1 +/- 1 week).

CBC and CMP will be drawn weekly on days 1, 8 and 15 of each cycle (+/-3 days) prior to each treatment.

Echo or MUGA after last dose of paclitaxel and prior to surgery.

Table 3: Time and Events Table

	Pre-study (28 days)	Cycle 1 Day 1 (+/- 3 days)	Cycle 2 Day 1 (+/- 3 days)	Cycle 3 Day 1 (+/- 3 days)	Cycle 4 Day 1 (+/- 3 days)	Surgery[8] (+/- 3 days)	Safety follow-up (30-days and 60-days post -surgery +/- 1 week)	Long term-follow-up and final study visit[14]
Informed Consent	X							
History and PE with Clinical Breast Exam	X	X	X	X	X		X	X
Vital signs[9]	X	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X		X	X
Toxicity evaluation[1]		X	X	X	X	X	X	X
Staging imaging[2]	X					X[10]		
Breast Imaging[10]	X					X		X
Safety bloodwork[3]	X	X	X	X	X	X	X	
Coagulation tests[13]	X							
Optional fecal samples[4]		X		X		X	X	
Research blood[5]		X		X		X	X	
Echo or MUGA[6]	X					X		X
Neoadjuvant treatment[7]		X	X	X	X			
Tissue collection[11]	X					X		
Optional fresh breast biopsies (Cedars site only)[15]		X		X				
Pregnancy testing[12] (urine or serum)	X	X						

1. Toxicity will be evaluated using CTCAE version 5.0 until 1-year post surgery.
2. Staging imaging will be with the discretion of the physician. For clinical stage IIB/III disease, staging with either a FDG PET/CT or CT chest, abdomen and pelvis and plus bone scan or PET/CT is recommended. Brain MRI is recommended for symptomatic patients only.
3. Safety bloodwork consists of CBC, CMP (including: AST, ALT, albumin, ALP, total bilirubin), and TSH with reflex to T3 and free T4 **OR** TSH with T3 and free T4 per institutional standards. C1D1 safety labs are not repeated if drawn for screening within 7 days of treatment initiation. CBC & CMP are drawn weekly during treatment on days 1, 8, and 15 of each cycle.
4. All fecal samples for microbiome analysis are optional.
5. 2 CPT tubes, 1 Cyto-Chex BC tube, and 1 red top tube of blood will be required at all timepoints. All research blood draws will be performed in conjunction with safety labs in order to minimize the number of venipunctures
6. Patients will undergo Echo or MUGA between last dose of paclitaxel and surgery date (+/- 3 days does not apply to these tests), then every 3 months (+/- 1 week) until completion of HER2-directed therapy. Cardiac monitoring after surgery will be performed per standard of care for patients receiving HER2 directed adjuvant therapy
7. Neoadjuvant treatment: consists of treatment Arm A, Arm B, and Arm C and will start within 5 business days of randomization. Each cycle is 3 weeks in duration and patients receive 3 doses of weekly paclitaxel within each cycle.

Additional chemotherapy per local standard-of-care may be administered prior to surgery in patients with biopsy proven residual disease after study treatment.
8. Surgery will take place 3-6 weeks after last protocol treatment dose per SOC.
9. Vitals including height and weight to be obtained at each study visit prior to and during treatment. Once a subject completes study treatment, height is not required to be collected at long term follow up visits.
10. Baseline, pre-surgery and long-term follow up breast imaging (i.e. mammography, ultrasound, and/or MRI) is recommended per SOC.
11. Left-over tumor tissue will be collected from standard of care biopsy prior to C1D1 and from surgical specimen. Fresh tissue will be obtained if possible, see lab manual.
12. Must occur within 72 hours before first drug administration. If screening occurs within 72 hours of C1D1, then test will not be repeated.
13. Coagulation panel to include Prothrombin time (PT/INR) and aPTT (activated partial thromboplastin time).
14. Subjects will be followed as per standard-of-care after surgery every 3 months (+/- 1 month) for 3 years then every 6 months (+/- 1 month) in years 4 and 5 and then annually after 5 years (up to year 10) for assessment of recurrence or metastatic disease. During this follow-up phase, routine bloods, physical exam, and standard-of-care follow-up imaging will be performed to assess for disease recurrence. Toxicity will be assessed up to 1-year post surgery. If the subject does not return for protocol-specified or standard-of-care visits in the long-term follow-up period, all attempts should be made to contact the subject via telephone for survival status in accordance with the study schedule.
15. Optional fresh breast biopsies (Cedars patients only) will be collected at baseline (after eligibility is confirmed but before C1D1 treatment) and 6 weeks (+/- 1 week of cycle 3).

6.3 Follow-up Procedures

Long-term follow-up and final study visit:

Subjects will be followed on protocol after surgery every 3 months until 36 (+/-1) months from the date of surgical resection. Thereafter, every effort will be made to continue monitoring these subjects via standard-of-care visits, so that late recurrences can be identified and overall survival can be estimated. Specifically, all subjects will be followed as per standard-of-care after surgery every 3 months (+/- 1 month) for 3 years then every 6 months (+/- 1 month) in years 4 and 5 and then annually after 5 years (up to year 10) for assessment of recurrence or metastatic disease. During this follow-up phase, routine bloods, physical exam, and standard-of-care follow-up imaging will be performed to assess for disease recurrence. Toxicity will be assessed up to 1-year post surgery. If the subject does not return for protocol-specified or standard-of-care visits in the long-term follow-up period, all attempts should be made to contact the subject via telephone for survival status in accordance with the study schedule.

Cardiac (LVEF) monitoring while on adjuvant HER2-directed therapy should be obtained per standard-of-care (i.e. every 3 months while on therapy).

6.4 Removal of Subjects from Study

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

6.4.1 Patient voluntarily withdraws (follow-up permitted);

6.4.2 Patient withdraws consent (termination of treatment and follow-up);

6.4.3 Patient is unable to comply with protocol requirements;

6.4.4 Patient demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);

6.4.5 Patient experiences toxicity that makes continuation in the protocol unsafe;

6.4.6 Treating physician determines continuation on the study would not be in the patient's best interest;

6.4.7 Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);

6.4.8 Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;

6.4.9 Lost to follow-up. If a research subject cannot be located to document survival after a period of 20 weeks, the subject may be considered "lost to follow-up." All attempts to contact the subject during the follow up period must be documented. This will be reviewed during an interim data monitoring visit.

7.0 ADVERSE EVENTS (AE)

7.1 Definitions

7.1.1 Adverse Event

An adverse event is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention whether or not related to the intervention.

7.1.2 Serious Adverse Events (SAE)

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that:

7.1.2.1 Results in death. If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

7.1.2.2 Is life-threatening (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

7.1.2.3 Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.

7.1.2.4 Results in persistent or significant disability or incapacity.

7.1.2.5 Is a congenital anomaly / birth defect.

7.1.2.6 Is an important medical event.

Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event.” For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.1.3 Unanticipated Problem (UP)

Unanticipated problems include any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known to an individual or group of individuals (including research subjects, research staff, or others not directly involved in the research).

7.1.3.1 Evaluable for toxicity

All patients will be evaluable for toxicity from the time of their first treatment with study drug.

7.2 Severity of Adverse Events

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE current version is available at http://ctep.cancer.gov/reporting/ctc.html.

7.3 Adverse Event Monitoring

The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's medical records. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations result in a delay or dose modification of study treatment, or judged relevant by the investigator), should be reported as an adverse event.

The investigator or sub-investigator (treating physician if applicable) will provide the following for all adverse events (both serious and non-serious):

- Event term (as per CTCAE) - Descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting.
- Description of the event
- Date of onset and resolution
- Expectedness of the toxicity
- Grade of toxicity
- Attribution of relatedness to the investigational agent- (this must be assigned by an investigator, sub-investigator, or treating physician)
- Action taken as a result of the event, including but not limited to; no changes, dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received conmed or other intervention, etc.

- Outcome of event

All patients experiencing an adverse event, regardless of its relationship to study drug, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline
- any abnormal laboratory values have returned to baseline
- there is a satisfactory explanation other than the study drug for the changes observed, or death.

7.4 Toxicity profiles of investigational agents

7.4.1 Paclitaxel, Trastuzumab and Pertuzumab

This combination is FDA approved based on the results from the neoadjuvant trials, such as NeoSphere trial[18]. In this trial, the majority of the adverse events were related to the chemotherapy backbone, docetaxel. The association of paclitaxel, trastuzumab and pertuzumab has been tested in a phase II trial, also showing a good safety and efficacy profile[54].

Because of the risk to potentiate trastuzumab cardiac toxicity, these trials had a careful cardiac safety follow up and procedures. There were no new signals of cardiac toxicity with the combination of trastuzumab, pertuzumab and docetaxel or paclitaxel. However, we intend to carefully follow patients for the risk of cardiac toxicity signals in our trial, with the association of pembrolizumab. The algorithm for continuation and discontinuation of study medication based on cardiac safety will be the same used on the NeoSphere protocol, as outlined on Appendix A and B. It will be also graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE current version is available at http://ctep.cancer.gov/reporting/ctc.html.

Monoclonal antibodies may cause infusion-associated symptoms such as fever, chills, hypotension, shortness of breath, skin rash, headache, nausea, and/or vomiting. Such reactions typically occur during or very shortly after an infusion. Paclitaxel can also cause infusion-associated symptoms.

Since trastuzumab, pertuzumab and paclitaxel are widely available drugs on daily practice and FDA approved for neoadjuvant breast cancer treatment, toxicity management should follow the current standard of care guidelines. Tables 4 and 5 outline some of these guidelines.

Dose reductions are not recommended for trastuzumab and/or pertuzumab. Pertuzumab should be discontinued if trastuzumab is discontinued.

Delayed or missed trastuzumab and/or pertuzumab of ≥ 6 weeks, reloading dose should be administered per discretion of the investigator.

Table 4 Dose Modification Guideline for Paclitaxel

Toxicities	Grade or actual value	Paclitaxel
Hematological		
Neutropenia	ANC≥1000/mm^3 G2/G1	No change to paclitaxel • For ANC ≤1500/mm^3, prophylactic myeloid growth factors (filgrastim) use at the discretion of the investigator, o Should not be given on the same day as chemotherapy. o Pegfilgrastim may not be used with paclitaxel due to it's weekly dosing schedule
	ANC<1000/mm^3 G3/G4	Hold paclitaxel until ANC ≥1000/mm^3. G-CSF may be used between days 2–6 at discretion of the investigator. Pegfilgrastim may not be used with paclitaxel due to its weekly dosing schedule. Resume paclitaxel based on timing of recovery: • ≤1 week: No change to paclitaxel • >1 but <3 weeks: Dose-reduce paclitaxel to 70 mg/m^2 for all subsequent cycles. • ≥3 weeks: Stop paclitaxel
Febrile neutropenia	ANC ≤1000/mm^3, fever ≥38.5°C G3 and G4	Hold paclitaxel until resolved (ANC >1000/mm^3, fever <38.5°C, and resolution of any signs of infection). G-CSF may be used between days 2–6 at discretion of the investigator. Pegfilgrastim may not be used with paclitaxel due to its weekly dosing schedule. Resume paclitaxel according to number of episodes: • First episode: no change to paclitaxel. •Second episode: Reduce paclitaxel to 70 mg/m^2 for all subsequent doses. • Third episode: Discontinue paclitaxel
Thrombocytopenia	≥75,000-<100,000/mm^3 G1	Hold paclitaxel until ≥100,000/mm^3, resume treatment based on timing of recovery: • ≤1 week—no change to paclitaxel. • >1 but <3 weeks— Reduce paclitaxel to 70 mg/m^2 for all subsequent doses. • ≥3 weeks: Discontinue paclitaxel
	<75,000/mm^3 ≥G2	Hold paclitaxel until ≥100,000/mm^3. • Reduce paclitaxel to 70 mg/m^2 for all subsequent doses. • Stop paclitaxel if held for ≥ 3 weeks in a row.

Anemia	All grades	No change to paclitaxel • Iron studies should be done and iron should be replaced as indicated. Red blood cell transfusions can be given at the investigator's discretion.

Toxicities	Grade or actual value	Paclitaxel
Non-Hematological		
Nausea/Vomiting	Grade 1 or 2	No change to paclitaxel
	≥ Grade 3	Hold paclitaxel until resolved to≤ Grade 1. • Resume paclitaxel with modification of premedication • Second episode ≥ Grade 3 despite maximum supportive care, reduce paclitaxel to 70 mg/m^2 for all subsequent cycles
Mucositis/Stomatitis	Grade 1 or 2	No change to paclitaxel
	≥ Grade 3	Hold paclitaxel until resolved to ≤ Grade 1. • Resume paclitaxel at previous dose with modification of premedication • Second episode ≥ Grade 3 despite maximum supportive care, reduce paclitaxel to 70 mg/m^2 for all subsequent cycles
Neurotoxicity	Grade 1–2	No change to paclitaxel
	Grade 3	Hold paclitaxel until neuropathy improves to ≤ Grade 2. • Resume paclitaxel dose reduced to 70 mg/m^2 for all subsequent doses. • Discontinue paclitaxel if held for ≥ 3 weeks in a row.
	Grade 4	Discontinue paclitaxel if held for ≥ 3 weeks in a row (see general instruction below).
Hepatic	Grade 1	• No change to paclitaxel
	Grade 2 or 3	• Bilirubin fractionation should be performed if total bilirubin > 1.5xULN. Dose may continue if isolated bilirubinemia is mostly indirect such as in subject with Gilbert • Hold paclitaxel until resolved to Grade 1 and resume the dose at previous level. • Discontinue paclitaxel if held for ≥ 3 weeks in a row.

	Grade 4	• Discontinue paclitaxel. Note all concurrent ALT/AST >3xULN and Total bilirubin > 2xULN should be discontinued and evaluated (by the investigator) for potential drug induced liver injury.
Anaphylaxis /hypersensitivity	Mild	• Complete paclitaxel infusion w/medication.
	Moderate	• Stop infusion and treat per standard practice. • Resume infusion at half of the infusion speed if symptoms resolve. • Stop if symptoms recur.
	Severe	• Stop infusion immediately and discontinue treatment.
Other significant toxicities excluding fatigue, alopecia and leukopenia at discretion of the investigators	Grade 2	• Hold paclitaxel until resolved to ≤ Grade 1. • Resume at the previous dose and increase supportive care measure, if available.
	≥ Grade 3	• Hold paclitaxel and discuss with sponsor medical monitor for further instructions. • If ≥ Grade 3 toxicity recurs upon re-challenge, discontinue treatment permanently.

If nab-paclitaxel is substituted per protocol section 5.0, do not administer nab-paclitaxel if baseline ANC less than 1,500/mcL. Refer to local standard practice and prescribing information for detailed information about management of nab-paclitaxel related toxicity and dose adjustment.

Table 5 Actions to be taken in case of Pertuzumab and Trastuzumab related toxicity

Toxicity related to study treatment	Action
1. Non-hematological, Grade 1 or 2 (NCI-CTCAE; excluding cardiac*) toxicity	Continue with study treatment
2. Non-hematological, Grade 3 or 4 (NCI-CTCAE; excluding cardiac*) toxicity	Hold study treatment (all medication in the cycle) until recovery to Grade ≤ 2. Toxicity resolved to Grade ≤ 2 within a maximum of 2 weeks calculated from last_administration: Resume study treatment. Toxicity did NOT resolve to Grade ≤ 2 within a maximum of 2 weeks calculated from last administration: Discontinue the related study medication (pertuzumab or trastuzumab) permanently. Continue treatment as deemed suitable by local investigator.
3. Recurrence of non-hematological, Grade 3 or 4 (NCI-CTCAE; excluding cardiac*) toxicity upon re-challenge	Discontinue the related study medication (pertuzumab or trastuzumab) permanently. Continue treatment as deemed suitable by local investigator.
4. Cardiac toxicity (asymptomatic drop in LVEF or symptomatic congestive heart failure)	Study treatment (all medication in the cycle) to be held, continued or resumed according to the algorithm depicted in C. Related study medication (pertuzumab or trastuzumab) to be discontinued permanently in case of symptomatic CHF.
5. Cardiac toxicity (NCI-CTCAE; other cardiac toxicities not covered by treatment algorithm in C)	Actions must follow rules 1. to 3. for non-hematological toxicities.
6. Hematological toxicity – Neutropenia	Hold study treatment (all medication in the cycle) until neutrophils ≥ 1.5 x 10^9/L.

7.4.2 Pembrolizumab

Pembrolizumab is an FDA-approved agent on formulary for the treatment of metastatic melanoma, metastatic non-small cell lung carcinoma, head and neck squamous cell carcinoma, Hodgkin Lymphoma, metastatic urothelial carcinoma, metastatic gastric cancer and microsatellite instability-high metastatic cancer. It is investigational for use in breast cancer. The following list of toxicities and supportive care guidelines were taken from the investigator's brochure 2017[69], based upon Sponsor's Reference Safety Dataset (RSD), a pooled data from monotherapy clinical trials (n=2799).

7.4.3 Pembrolizumab and chemotherapy

In the Keynote 173 Trial, 20 patients with triple negative breast cancer suited to neoadjuvant therapy used pembrolizumab at a dose of 200mg every 3 weeks, together with nab-paclitaxel 125mg/m^2 for 4 cycles; then pembrolizumab 200mg every 3 weeks together with doxorubicin 60mg/m^2 and cyclophosphamide 600mg/m2 for another 4 cycles (Cohort A, 10 patients). The Cohort B was the same regimen, but added carboplatin AUC 6 with the pembrolizumab and nab-paclitaxel part (10 patients). In terms of grade 3 and 4 toxicity, 50-80% of the patients experienced neutropenia, 10% anemia, 30% nausea, 10% vomiting, 10% fatigue, 10% decreased appetite, 10-20% febrile neutropenia and 10% pyrexia, 20% Grade 3 liver enzymes increase, 30% immune-mediated adverse events (hyperthyroidism, hypothyroidism and thyroiditis). 2 patients discontinued treatment because of toxicity. No reports of death due to treatment related adverse events. No reports of cardiac toxicity. Dose modifications (reductions, interruptions or withdrawals) were reported in 90% of patients. Most of the adverse events were related to the chemotherapy backbone, considered very toxic, as shown on other neoadjuvant chemotherapy trials.

Table 6 Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab monotherapy and IO combinations

General instructions:

1. **Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.**
2. **Study intervention must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last study intervention treatment.**
3. **The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.**
4. **If study intervention has been withheld, study intervention may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.**

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
T1DM or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis:	Grade 2	Withhold	• Administer corticosteroids	• Monitor changes of renal

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
grading according to increased creatinine or acute kidney injury	Grade 3 or 4	Permanently discontinue	(prednisone 1 to 2 mg/kg or equivalent) followed by taper	function
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
[a] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal **[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal** **[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal** **[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.** **[e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).**				

Table 7: Pembrolizumab Infusion reaction dose modification and treatment guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g. antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	Stop Infusion. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment	Participant may be premedicated with diphenhydramine 50 mg po (or equivalent dose of antihistamine) and acetaminophen 500-1000 mg po (or equivalent dose of analgesic) per institutional standards.

Grades 3 or 4 Grade 3: Prolonged (i.e. not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g. renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Stop Infusion. Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v 5.0 (CTCAE) at http://ctep.cancer.gov		

7.5 Reporting Requirements for Adverse Events

7.5.1 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE VERSION 5.0).

Step 2: Grade the adverse event using the NCI CTCAE VERSION 5.0

Step 3: Determine whether the adverse event is related to the protocol therapy

Attribution categories are as follows:

- 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.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is

attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.

Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in the Agent Information Section of this protocol;
- the drug package insert;
- the current Investigator's Brochure

7.5.2 Expedited Reporting

7.5.2.1 The Medical Monitor must be notified by study staff or co-investigators within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 1-year post surgery.

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Reporting SAEs and UPIRSOs to the Simmons Comprehensive Cancer Center (SCCC) Data Safety Monitoring Committee (DSMC):

Serious Adverse Events and UPIRSOs at all sites, which occur in research subjects on protocols for which the SCCC is the DSMC of record require reporting to the DSMC regardless of whether IRB reporting is required. All SAEs occurring during the protocol-specified monitoring period and all UPIRSOs should be submitted to the SCCC DSMC within 5 business days of the study team members awareness of the event(s). In addition, for participating centers other than UTSW, local IRB guidance should be followed for local reporting of serious adverse events or unanticipated problems.

The UTSW study PI is responsible for ensuring SAEs/UPIRSOs are submitted to the SCCC DSMC Coordinator. This may be facilitated by the IIT project manager, study team, sub-site or other designee. Hardcopies or electronic versions of the eIRB Reportable Event report; FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be submitted to the DSMC Coordinator. The DSMC Coordinator forwards the information onto the DSMC Chairman who determines if immediate action is required. Follow-up eIRB reports, and all subsequent SAE or UPIRSO documentation that is available are also submitted to the DSMC Chair who determines if further action is required.

If the event occurs on a multi-institutional clinical trial coordinated by the UTSW Simmons Comprehensive Cancer Center, the UTSW study PI or designee ensures that all

participating sites are notified of the event and resulting action, according to FDA guidance for expedited reporting. DSMC Chairperson reviews all SAEs and UPIRSOs upon receipt from the DSMC Coordinator. The DSMC Chairperson determines whether action is required and either takes action immediately, convenes a special DSMC session (physical or electronic), or defers the action until a regularly scheduled DSMC meeting.

Should you need to discuss any SAE (initial/follow up reports) contact: Heather McArthur (Telephone): 214-645-9380 Heather.McArthur@utsouthwestern.edu		
Lead Site (UTSW): Send all SAE initial and follow up reporting to the following contacts below: • Heather McArthur, MD Heather.McArthur@utsouthwestern.edu • Meredith Carter Meredith.Carter@utsouthwestern.edu • Stephanie Rice Stephanie.Rice@utsouthwestern.edu • UTSW SCCC Data Safety Monitoring Committee: Report through InfoReady SAE form (link below) [http://utsouthwestern.infoready4.com] SCCDSMC@utsouthwestern.edu	**Within 24 hours of learning of the event**	SAE report form
Sub Sites Send all SAE initial and follow up reporting to the following contacts below: • Heather McArthur, MD Heather.McArthur@utsouthwestern.edu • Meredith Carter Meredith.Carter@utsouthwestern.edu • Stephanie Rice Stephanie.Rice@utsouthwestern.edu	**Within 24 hours of learning of the event**	SAE report form; MedWatch 3400A if applicable

Lead Site: UTSW Institutional Review Board Submit a Reportable event within eIRB with a copy of the final sponsor report determination as attached supporting documentation Medical Monitor reporting Refer to section 7.5.2.1 Merck reporting Refer to section 7.6.6-7.6.8	Submit within time specified per policy	Submit required documentation per policy
Subsite: Local IRB submission per policy Medical Monitor reporting Refer to section 7.5.2.1 Merck reporting Refer to section 7.6.6-7.6.8	Submit within time specified per policy	Submit documentation per policy

Reporting Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) to the UTSW HRPP

UTSW reportable event guidance applies to all research conducted by or on behalf of UT Southwestern, its affiliates, and investigators, sites, or institutions relying on the UT Southwestern IRB. Additional reporting requirements apply for research relying on a non-UT Southwestern IRB.
According to UTSW HRPP policy, UPIRSOs are incidents, experiences, outcomes, etc. that meet **ALL three (3)** of the following criteria:

1. Unexpected in nature, frequency, or severity (i.e., generally not expected in a subject's underlying condition or not expected as a risk of the study; therefore, not included in the investigator's brochure, protocol, or informed consent document),AND
2. Probably or definitely related to participation in the research, AND
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. Note: According to OHRP, if the adverse event is serious, it would always suggest a greater risk of harm.

For purposes of this policy, UPIRSOs include unanticipated adverse device effects (UADEs) and death or serious injury related to a humanitarian use device (HUD).

UPIRSOs must be promptly reported to the UTSW HRPP within 5 working days of study team awareness.

For research relying on a non-UT Southwestern IRB (external, central, or single IRB):

Investigators relying on an external IRB who are conducting research on behalf of UT Southwestern or its affiliates are responsible for submitting LOCAL UPIRSOs to the UT Southwestern IRB within 5 working days of study team awareness. Investigators must report to their relying IRB according to the relying IRB's policy. In addition, the external IRB's responses or determinations on these local events must be submitted to the UT Southwestern IRB within 10 working days of receipt.

Events NOT meeting UPIRSO criteria:

Events that do NOT meet UPIRSO criteria should be tracked, evaluated, summarized, and submitted to the UTSW HRPP/IRB at continuing review.

For more information on UTSW HRPP/IRB reportable event policy, see https://www.utsouthwestern.edu/research/hrpp/quality-assurance/

7.5.2.2 Reporting to the Institutional Review Board (IRB)

The IRB must be notified per institutional standard of "any unanticipated problems involving risk to subjects or others."

1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
4. Any new information (e.g. publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
5. Any breach in confidentiality that may involve risk to the subject or others.

Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

7.6 Sponsor Reporting Requirements

7.6.1 Collection of Safety Information

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

All AEs, SAEs and other reportable safety events that occur after the consent form is signed, but before treatment allocation/randomization, must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event cause the participant to be excluded from the study, or is the result of a protocol-specified intervention, including, but not limited to, washout or discontinuation of usual therapy, diet, or a procedure.

● All AEs from the time of treatment allocation/randomization through 1-year post surgery must be reported by the investigator.

● All AEs meeting serious criteria from the time of treatment allocation/randomization through 90 days following cessation of study treatment or 30 days following cessation of study treatment, if the participant initiates new anticancer therapy (whichever is earlier), must be reported by the investigator.

● All pregnancies and exposure during breastfeeding, from the time of treatment allocation/randomization through 120 days following cessation of study treatment or 30 days following cessation of study treatment, if the participant initiates new anticancer therapy, must be reported by the investigator.

● Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately by the investigator, if the event is considered to be drug-related.

Investigators are not obligated to actively seek AE or SAE or other reportable safety events in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify Merck.

7.6.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see “note” below for exceptions)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization, but, based upon appropriate medical

and scientific judgment, may jeopardize the subject or may require intervention [e.g. medical, surgical] to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

Although overdose and cancer are not always serious by regulatory definition, these events should be reported on an SAE form and sent to Merck in an expedited manner. For purposes of this study, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Medical Monitor and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215-661-6229) utilizing Merck's Global Safety Intake Form.

NOTE: The following hospitalizations are <u>not</u> considered SAEs in Merck clinical studies:

- a visit to the emergency room or other hospital department for less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event);
- elective surgery, planned before signing consent;
- admissions as per protocol for a planned medical/surgical procedure;
- routine health assessment requiring admission for baseline/trending of health status (e.g. routine colonoscopy);
- medical/surgical admission for purpose other than remedying ill health state and was planned prior to entry into the study (appropriate documentation is required in these cases);

• admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).

Note that all pregnancies, regardless of outcome, must be reported to the sponsor on a Pregnancy Surveillance Form, not an SAE form. All pregnancies must be reported and followed to outcome, including pregnancies that occur in the female partner of a male study subject. See Section 8.4 for instructions on reporting pregnancies.

7.6.3 Non-serious Adverse Events

All adverse events that are not classified as serious.

7.6.4 Assignment of Adverse Event Intensity and Relationship to Investigational Product

All adverse events, including those that are serious, will be graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE), Version 5.0. The following categories and definitions of causal relationship to investigational product as determined by a physician should be used for adverse events:

• Definite: There is a reasonable causal relationship between the investigational product and the AE. The event responds to withdrawal of investigational product (de-challenge) and recurs with re-challenge when clinically feasible.

• Probable: There is a reasonable causal relationship between the investigational product and the AE. The event responds to de-challenge. Re-challenge is not required.

• Possible: There is reasonable causal relationship between the investigational product and the AE. De-challenge information is lacking or unclear.

• Not likely: There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.

• Not Related: There is not a temporal relationship to investigational product administration (too early or late or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease or circumstance and the AE.

The expression “reasonable causal relationship” is meant to convey in general that there are facts (e.g. evidence such as de-challenge/re-challenge) or other arguments to suggest a positive causal relationship.

7.6.5 Collection and Reporting

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the AE was administered, it should be recorded in the medical record. The investigator shall supply the sponsor and Ethics Committee with any additional requested information, notably for reported deaths of subjects.

7.6.6 Collection and Reporting for Serious Adverse Events

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause that occurs to any participant must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the participant to be excluded from the trial, or it is the result of a protocol-specified intervention, including, but not limited to, washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 1-year post surgery, any serious adverse event, or follow up to a serious adverse event, including death due to any cause whether or not related to the Merck product, must be reported within 24 hours to the Medical Monitor and within 2 working days to Merck Global Safety utilizing Merck's Global Safety Intake Form.
SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229
Additionally, any serious adverse event considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above or at any time outside of the time period specified in the previous paragraph also must be reported immediately to Merck Global Safety.
All participants with serious adverse events must be followed up for outcome.

A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

7.6.7 Handling of Expedited Safety Reports

In accordance with local regulations, Merck will notify investigators of all SAEs that are suspected (certainly, probably or possibly related to the investigational product) and unexpected (i.e. not previously described in the Investigator Brochure). In the European Union (EU), an event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Investigator notification of these events will be in the form of an expedited safety report (ESR).

Other important findings which may be reported by the sponsor as an ESR include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (e.g. animal) study, important safety recommendations from a study data monitoring committee or sponsor decision to end or temporarily halt a clinical study for safety reasons.
Upon receiving an ESR from Merck, the investigator must review and retain the ESR with the Investigator Brochure. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.
In addition, suspected serious adverse reactions (whether expected or unexpected) shall be reported by Merck to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

7.6.8 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI or follow up to an ECI that occurs to any participant must be reported within 2 working days to Merck Global Safety, if it causes the participant to be excluded from the trial or is the result of a protocol-specified intervention including, but not limited to, washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment or 30 days following cessation of treatment, if the participant initiates new anticancer therapy (whichever is earlier), any ECI or follow up to an ECI, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:

1. an overdose of Merck product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results;

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.6.9 Pregnancy

Sexually active WOCBP must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized (See appendix C). Before enrolling WOCBP in this clinical study, the investigator must review the guideline about study participation for WOCBP which can be found in the GCP Manual for Investigators. The topics include the following:

- General Information
- Informed Consent Form
- Pregnancy Prevention Information Sheet
- Drug Interactions with Hormonal Contraceptives
- Contraceptives in Current Use
- Guidelines for the Follow-up of a Reported Pregnancy.

Before study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion.
All WOCBP MUST have a negative pregnancy test within 72 hours before receiving study treatment. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive study treatment and must not be enrolled in the study.
In addition, all WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (e.g. dose tapering, if necessary, for subject safety). The investigator must immediately notify Merck of this event and record the pregnancy on the Pregnancy Surveillance Form (not an SAE form). Initial information on a pregnancy must be reported immediately to Merck, and the outcome information provided once the outcome is known. Completed Pregnancy Surveillance Forms must be forwarded to Merck according to SAE reporting procedures.
Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g. x-ray studies). Other appropriate pregnancy follow-up procedures should be considered, if indicated. In addition, the investigator must report to Merck and follow-up on information regarding the course of the pregnancy, including prenatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks.
Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety (Attn: Worldwide Product Safety; FAX 215-661-6229).

7.6.10 Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded in the medical record.

8.0 CORRELATIVES/SPECIAL STUDIES

Exploratory correlative studies will be performed to characterize the immunologic response of the intervention, to correlate immunologic laboratory parameters with clinical efficacy and toxicity, and to evaluate the utility of diagnostic imaging. Breast cancer tissue specimens, axillary lymph nodes specimens when available, peripheral blood samples, and optional fecal samples will be studied.

8.1 Peripheral blood correlatives

The rationale use of immunotherapy in the localized breast cancer setting requires a thorough assessment of potential predictive biomarkers. Potential biomarkers of pembrolizumab activity in the neoadjuvant HER2+ setting will be assessed by comparing blood-based immune markers among the three different arms of the study at baseline and on treatment.

Whole blood (8 mL) will be separated by centrifugation into a cellular fraction and plasma.

- The fraction of stem/progenitor cell, lymphocyte, and myeloid populations of total circulating mononuclear cells will be counted by flow cytometry using the following markers: CD3, CD4, CD8, CD14, CD25, CD34, CD45, CD56, CD127, and CD133.
- The following plasma biomarkers will be assessed: granulocyte-macrophage colony stimulating factor, interferon gamma, tumor necrosis factor alpha, interleukin-1b, IL-2, IL-6, IL-8, IL-10, and IL-12 heterodimer p70 (using a 9-plex Inflammatory Factor array).
- Surface molecules involved in immune recognition (PD-1 expression and other markers of differentiation, memory, and exhaustion) will also be assessed.
- Potential exploratory analyses will include:
  a) identification of circulating antigens,
  b) analysis of antibody response (quantitative antibody titer and qualitative antibody binding), and
  c) analysis of genomic or protein polymorphisms that may affect immune function and response to pembrolizumab.

In addition, circulating tumor DNA (ctDNA) will be assessed at baseline and on treatment utilizing a 16 gene next generation sequencing-based panel covering key breast cancer genes to describe therapy-induced genomic changes. A total of 20 mL of blood will be collected at each timepoint for ctDNA analysis. For those with detectable ctDNA, the mean allelic fraction will be calculated at baseline and on treatment.

Descriptive statistics will be used to summarize biomarker values at protocol-specific time points. The Wilcoxon ranked sum test will be used to evaluate the difference of baseline biomarker values between patients who did or did not experience a pathologic complete response.

2 (8 mL) CPT (Red/Green tiger top) tubes, 1 (6mL) red top tube, and 1 (5mL) Cyto-Chex BCT tube.

Other assays to be performed include:

- T-cell receptor DNA deep sequencing for clonal T-cell repertoire analysis.
- Serology Assays: to identify for humoral reactivity against putative TAAs such as NY-ESO-1 or MUC1. Microarray analyses may also be conducted to screen for novel TAA reactivity.
- Determination of cell counts, for example absolute lymphocyte count and eosinophil count.

8.2 Tumor specimen correlatives

Exploratory studies on core biopsy and surgical tissue to evaluate treatment response include, but are not limited to:

- Evaluation of cell proliferation by Ki-67;
- Expression of the PD-L1 inhibitory molecule (Ghebeh 2007);
- Lymphocyte infiltration including perivascular infiltration;
- Immunohistochemistry (IHC) staining to further define particular lymphocytic populations such as T-regulatory cells (Tregs) ($CD4^+CD25^+$*$FOXP3^+$)*, effector T-cells and the ratio between Tregs and effector T-cells ($CD4^+/CD8^+$);
- Immunohistochemistry (IHC) staining to identify subsets of dendritic cell (DC) populations (i.e. tolerogenic versus immunogenic) and to explore the impact of different treatment arms on the ratio between them;
- Sentinel or non-sentinel lymph node tissue, where available, will also be evaluated by immune profiling as described above;
- Tumoral DNA exome sequencing and RNA expression profiling to determine whether tumor mutational landscape correlates with immunologic response;
- T-cell receptor DNA deep sequencing for clonal T-cell repertoire analysis.

8.3 Optional Fecal Samples for Microbiome Analysis:

In conjunction with Dr. Stephen Shiao (Radiation Oncology, Cedars-Sinai), optional fecal samples will undergo microbiome analysis. Kits for sample collection will be provided to all participants who opt to participate in this optional part of the trial, upon registration to the trial. Each kit will be provided with a pre-labeled box to be shipped back by the patient for analysis to the laboratory of the CSMC PI who will be conducting this analysis:

Dr. Stephen Shiao
Cedars-Sinai Medical Center
110 N. George Burns Rd #D4094D
Los Angeles, CA 90048

All specimens will contain subject ID, study visit, and date of collection. The shipping and receipt of specimens will be tracked in a database and by emails sent to Dr. Shiao and the PI.

8.4 Sample Collection / Specimen Banking

Research bloods to be used for flow cytometry analysis, measurement of circulating cytokines, and TCR sequencing will be collected prior to initiation of neoadjuvant treatment, after two cycles and before surgery. Samples will be frozen and stored for subsequent analysis.

Tumor tissue will be obtained from a standard of care biopsy and surgery. Biopsy and collection will be conducted prior to C1D1 neoadjuvant treatment and from surgical specimen. For a fresh biopsy, at least 2 cores (1cm each) will be collected. One will be frozen for flow cytometry analysis and MerFISH and the other will be paraffin embedded for TIL score, mIHC, and TCR sequencing. For the ER+ cohort, if archived tissue is used in lieu of the first research biopsy, flow cytometry and MerFISH will not be conducted on this sample as fresh or frozen tissue is required for these studies. For archival tissue samples, a tissue block is preferred, but, if unavailable, 4 unstained slides and 1 H&E slide are acceptable.

All blood and tissue samples will need to be shipped to the laboratory who will be conducting this analysis. All specimens will need to be labeled according to subject ID, date of collection and study visit. The shipping and receipt of specimens will be tracked in a database and by emails sent to the lab and the PI. The corresponding study lab manual has further specimen labeling and shipping directions.

9.0 STATISTICAL CONSIDERATIONS

This is a phase 2 randomized 3 arm trial wherein eligible patients will be randomized in a 1:1:1 ratio between the 2 experimental arms of chemotherapy plus HER2 directed therapy and Pembrolizumab (arms B and C), or standard of care neoadjuvant chemotherapy and HER2 directed therapy (arm A). The primary endpoint is pathological complete response rate (pCR) in the breast and axilla (ypT0/Tis ypN0).

Because biomarkers/correlatives are presently not clearly defined in cancer immunotherapy studies, we have designed our study to allow a rigorous evaluation of serial pathology specimen collections. These serial evaluations collected in a randomized study will allow a unique opportunity to evaluate the impact of the proposed intervention by comparing pathology specimens for treated and untreated patients. These exploratory correlative studies are an integral component of this study and are expected to inform future studies.

9.1 Sample Size Estimation

The literature suggests that pCR rates in breast and axilla for patients treated with neoadjuvant trastuzumab, pertuzumab and chemotherapy ranges from 39% to 51.9%[16,17]. There is no data informing pCR rates on HER2 positive breast cancer patients treated with combination chemotherapy, HER2 directed therapy and pembrolizumab. In a different patient population, triple negative breast cancer, the addition of pembrolizumab to neoadjuvant chemotherapy increased pCR rates from 50% to 80%[30].

The rate of pCR in the breast and axilla in the control group (SCO THP Arm A) is expected to be 50% (per NeoSphere). We assume the treatment group will achieve a pCR rate of 73%. With a trial design 1:1:1 in THP+pembrolizumab (THP-K) vs. TH+pembrolizumab (TH-K) vs. THP, for alpha=0.05 and power=80%, 55 patients are required for each arm.

To account for a 5% loss to follow up during the trial, we decided to add 3 patients per arm (58 patients/arm). Due to the binary nature of the primary endpoint, patients who discontinue study therapy without undergoing surgery will be considered as a non-responder (non-pCR). A total of 174 patients will be enrolled in this trial.

9.2 Patients Clinical Characteristics

Patient's demographic and clinical characteristics at the baseline, including age, medical history and conditions, tumor descriptors (grade, histology, tumor size, nodal involvement, etc.) and any other study-appropriate data, such as date of initial diagnosis, will be summarized by each arm.

9.3 Study Outcomes

9.3.1 Primary Endpoints

The primary endpoint will be the pCR rate in the breast and axilla (ypT0/Tis ypN0). The rate will be calculated and compared between the modified intent-to-treat study groups and patient's clinical characteristics.

9.3.2 Secondary Endpoints

Second endpoints include event-free survival (EFS), Invasive Disease Free Survival (IDFS), Overall Survival (OS), pathological invasive and in situ complete response in breast and axilla (ypT0 ypN0), pathologic invasive complete response in the breast only (ypT0/is), residual cancer burden index, breast conserving surgery rates (proportion of patients who achieved breast conserving surgery out of the intent-to-treat population) and safety. These endpoints will be calculated and be compared between study groups. The proportion of patients who achieved breast conserving surgery will be tabulated.

9.3.3 Exploratory Analyses

We will perform the exploratory analyses to explore the potential findings for this trial. These analyses will be reported primarily using graphs, descriptive statistics and nonparametric tests where warranted. Specifically, we will characterize and evaluate the magnitude of immune effect as conferred by pre-operative pembrolizumab. Immune response in the experimental arm will be measured by the fold-increase of peripheral blood CD4+ T-cells expressing ICOS, a marker of T-cell activation, by flow cytometry. The potential associations between individual biomarkers and efficacy and safety outcomes will be summarized for all patients by treatment arm, and exploratory markers reported by subgroup, whenever possible.

9.3.4 Interim Analyses

Stopping rules for safety
To ensure the safety of the experimental regimens, interim analyses for safety will be performed. Specifically, safety will be evaluated after every 15th patient has received at least one dose of treatment on study in each of the experimental arms (i.e. in the safety population).

A serious adverse event (SAE) is defined as the occurrence of any of the following events:

1. a life threating adverse event,
2. death due to treatment or
3. a grade 3/4 AE necessitating a delay in surgery of ≥6 weeks after the last dose of study drug.

An experimental arm will be considered unsafe if there is statistical evidence that the rate of SAEs θ exceeds 10%. Specifically, an experimental arm is considered unsafe if the posterior probability that $\theta > 0.1$ given the data is more than 0.95;
$P(\theta > 0.1 \mid \text{data}) > 0.95$. A noninformative prior distribution for θ will be used.

If one of the experimental arms is deemed unsafe, the decision to continue enrollment to the other 2 arms will be made at the discretion of the PI together with the sponsor.
We will use a Bayesian sequential design by checking the decision rule to declare an arm as unsafe after 15, 30, and 45 patients are evaluable for safety. Table 8 gives the stopping rules for the design at each look and column 2 gives the maximum number of patients with SAE in order for the trial to proceed. For example, if 5 or more patients had SAEs after enrolling 15 patients, the arm is considered unsafe. The third column gives the probability of stopping the trial when in fact, the true $\theta = 0.10$. This is the equivalent of the Bayesian type I error probability. The target type I error probability was set at 0.05.

Number of Patients	Number to Continue	Probability to Stop	Cumulative Probability to Stop
15	4	0.013	0.013
30	6	0.019	0.032
45	8	0.016	0.048

Table 8. Stopping rules based on three interim looks. Number to continue is the maximum number of SAEs for not declaring an arm as unsafe.

Table 9 gives the design operating characteristics under selected values of the true rate of SAEs θ. It gives the probability of declaring an arm as unsafe under the alternative hypothesis, the expected sample size, and the average sample size given that the arm was declared unsafe. For example, if the true value of θ is 0.3, then there is a 96% chance that the trial is declared unsafe and the average sample size is about 25. On the other hand, there is a small chance of declaring the trial unsafe if θ is small; 4.8% chance of declaring the arm unsafe when in fact $\theta = 0.1$.

True Value of θ	Probability to Stop	Expected N	Expected N given that we Stopped
0.10	0.048	53.85	31.14

0.30	0.96	25.41	24.15
0.50	0.999	15.9	15.90

Table 9. Design operating characteristics under different scenarios for the true probability of SAE θ.

Arm C stopping rule for efficacy

The TH-K arm will be stopped early if the pCR rate is less than 40%. The expected pCR rate for the control arm A (THP) is 50%. The stopping rule will be assessed after the first 20 patients on the TH-K arm have completed surgery. Out of 20 patients, if 8 patients do not achieve pCR, regardless of hormone receptor-status, the TH-K arm will be stopped. The assessment will be rolling. Subjects can continue to enroll on the TH-K arm and treatment will not be stopped while the stopping rule is assessed. If the stopping rule is met, no additional patients will be randomized to the TH-K arm. Patients already on the TH-K arm will be permitted to continue.

9.4 Statistical Analyses

The primary endpoint pCR rate will be calculated and compared using the Cochran-Mantel-Haenszel test adjusting for the two stratification factors between the arms. Exact 95% confidence intervals for the difference in pCR rates between the arms will be constructed using the unconditional distribution of two binomials distributions[70]. A logistic regression model will be used to evaluate the treatment effect while adjusted for the factors (e.g. hormone receptor status, age, stage, LVI, grade).

The second endpoint rate will be compared using methods like those described for the primary endpoint. In addition, the second endpoint will also be analyzed using the survival analysis method. The survival rate and pattern will be compared using Kaplan-Meier and stratified log-rank test between the arms. The hazard ratio for each event and 95% confidence interval between each will be estimated using Cox hazard proportional regression model while adjusting the confounding factors.

The efficacy and safety outcomes for exploratory analyses will be summarized using descriptive statistical analyses.

All analyses will be performed for Intent-to-Treat (ITT), modified Intent-to-Treat (m-ITT), and Safety population based on the following definition:

- ITT population: all randomized subjects will be included in the ITT population.
- Modified ITT population: all randomized subjects that received any amount of the study drug will be included in the m-ITT population.
- Safety population: includes all subjects in the study who received at least 1 dose or any partial dose of pembrolizumab.

9.5 Randomization

Randomization will be conducted using a web-based randomization system. Randomization will be accomplished by the method of random permuted block. Eligible patients will be randomized in a 1:1:1 ratio between the experimental arm and control arm. Patients will be randomized and assigned a unique randomization number. As confirmation, the investigator will be provided a written verification of each patient's registration. Treatment should occur within 5 working days of the patient being randomized into the study. No patient may begin treatment prior to randomization and assignment of a medication number.

The patient randomization numbers are to be allocated dynamically in the order in which the patients are enrolled. The dynamic allocation will be stratified by hormone receptor status (positive or negative) and lymph nodes status (positive or negative).

10.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

10.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Pembrolizumab will be provided by Merck as summarized in Table 10.

Table 10. Product Description

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection

10.2 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

10.3 Clinical Supplies Disclosure

This trial is open-label, therefore, the participant, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

10.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.
Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

10.5 Returns and Reconciliation

The investigator and site are responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the participants and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

11.0 STUDY MANAGEMENT

11.1 Conflict of Interest

Any reportable conflict of interest will be disclosed to the IRB of record and will be outlined in the Informed Consent Form. Conflict of interest will be defined by a situation in which a study investigator is involved in an interest, financial or otherwise, which could affect the decision-making of that individual as it relates to this study.

11.2 Institutional Review Board (IRB) Approval and Consent

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.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s) and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

11.3 Registration Procedures

All subjects that sign informed consent will be assigned a subject number sequentially by their date of consent and listed on the study specific site dedicated screening log. Those subjects that do not pass the screening phase will be listed as screen failures on the site screening logs. The study team will also track all subjects who sign consent using OnCore.

Issues that would cause treatment delays after registration should be discussed with the Principal Investigator (PI). If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

Assignment of Subject ID: The study team will track all subjects who sign consent on a subject screening/enrollment log using a unique screening ID (S001, S002, etc.). Subjects found to be ineligible will be recorded as screen failures. Subjects found to be eligible will be registered in Clinical Studio and assigned a three-digit numeric ID.

A) Eligibility Verification

Prior to registration, all subjects must undergo eligibility verification by UTSW. The following documents will be completed and provided for review:

- Copy of applicable source documents (i.e. pathology report and cTNM staging redacted to remove PHI)
- Eligibility checklist (signed by investigator)
- Signed patient consent form with applicable HIPAA language.
- HIPAA authorization form

B) Registration

After eligibility is verified, registration is completed as follows:

Using the EDC system Clinical Studio, site investigator or staff will login and enter subject required information for registration and a study ID will be generated: Protocol #-AAA (site number)-BBB (unique subject ID);

- Notify the investigational pharmacy and treating physicians that a subject has gone on study and of the anticipated treatment start date.

Oversight by the site principal investigator is required throughout the entire registration process.

C) Randomization

This is an unblinded randomized comparison of standard neoadjuvant HER2 directed care versus neoadjuvant HER2 directed care and pembrolizumab. After eligibility is established and immediately after consent is obtained, patients will be registered and randomized within Clinical Studio.

Randomization will be conducted using a web-based randomization system within the EDC system Clinical Studio at the time of registration. Randomization will be accomplished by the method of random permuted block. Eligible patients will be randomized in a 1:1:1 ratio between the experimental arm and control arm.

11.4 Data Management and Quality Control and Reporting

The data will be entered into a HIPAA-compliant database. The Study Staff will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

11.5 Data and Safety Monitoring

11.5.1 Data Monitoring and Quality Assurance

The study project manager will perform the site evaluation visits for all potential sites approved by the sponsor. A study monitor will be assigned to the study.

The First Visit will occur within 8-12 weeks of 1st patient enrolled at the site. The study monitor with or without the Manager, Clinical Operations will arrange a visit to the site. This initial visit is intended to ensure that:

- The study is being conducted according to the protocol
- Confirm the following:
  - Eligibility of enrolled patients
  - Site has received all study supplies and staff understand re-ordering process
  - Study drug is properly stored and inventoried
  - The site staff understands how to complete the eCRFs and all required forms

The study monitor will schedule a site visit approximately every 16 weeks throughout the enrollment period and approximately every 24 weeks during the follow-up period. The visits may alternate between three and four days. Frequency may change depending on patient enrollment, deviations, safety concerns, quality of data and/or quantity of outstanding data. Interim visits will continue to occur after the last patient is enrolled at an interval based on the number of active patients and outstanding data per site. Monitoring intervals may also fluctuate by frequency based on necessity and efficiency of visits.

Remote monitoring will be conducted to review recruitment activity, ongoing data completion, to issue queries or resolve queries. If remote access to site EMR is not possible, sites will be required to send redacted source documents to study monitor. Remote monitoring will be determined by prior agreement amongst the site and sponsor.

Approximately every 2-4 weeks, the assigned project manager will review the EDC for completion and accuracy of critical safety data. The site is expected to upload de-identified source documents to the EDC, to allow for a satisfactory review of laboratory assessments,

physical exams, vitals, dosing information, adverse events and concomitant medications to ensure protocol compliance.

11.5.2 Safety Monitoring

Safety, data and progress oversight will be provided by the Lead PI with the assistance of Medical Monitor and an Institutional Data Safety Monitoring Committee (DSMC). The UTSW Simmons Comprehensive Cancer Center (SCCC) Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UTSW SCCC clinical trials. As part of that responsibility, the DSMC reviews all serious adverse events and UPIRSOs in real time as they are reported and reviews adverse events on a quarterly basis. The quality assurance activity for the Clinical Research Office provides for periodic auditing of clinical research documents to ensure data integrity and regulatory compliance. A copy of the DSMC plan is available upon request.

The SCCC DSMC meets quarterly and conducts annual comprehensive reviews of ongoing clinical trials, for which it serves as the DSMC of record. The Quality Assurance Coordinator (QAC) works as part of the DSMC to conduct regular audits based on the level of risk. Audit findings are reviewed at the next available DSMC meeting. In this way, frequency of DSMC monitoring is dependent upon the level of risk. Risk level is determined by the DSMC Chairman and a number of factors such as the phase of the study; the type of investigational agent, device or intervention being studied; and monitoring required to ensure the safety of study subjects based on the associated risks of the study. Protocol-specific DSMC plans must be consistent with these principles. UTSW will maintain continuous monitoring for the duration of the study by reviewing all subject data through in-person and remote monitoring visits (by study monitor) and spot check review by the project manager team.

11.6 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, monitoring/auditing logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. Study documents should be kept on file per local guidelines.

11.7 Adherence to Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, a planned deviation must be sent to

the Medical Monitor; the study shall be conducted exactly as described in the approved protocol.

11.7.1 Emergency Modifications

Investigators may implement a deviation from the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB or Sponsor (UTSW) approval.

11.7.2 Protocol Exceptions and Eligibility Waivers

An exception is an anticipated or planned deviation from the IRB-approved research protocol. This protocol does not allow for protocol exceptions: any conduct that does not follow the protocol is considered a deviation as stated above.

Eligibility Waivers (EW)
In general, subjects who do not meet the eligibility requirements should not be enrolled. For this protocol, eligibility waivers will not be granted.

11.7.3 Other Protocol Deviations

Logistical deviations from the protocol (e.g. minor changes to the study schedule for an individual subject) do not require prior IRB approval unless the deviation has the potential to affect the subject's safety. Such planned deviations that do meet this definition and do not affect the subject's safety should be noted in the subject's research record.

Unintentional deviations from the protocol that might affect subject safety or study integrity should be reported to the IRB per institutional standards.

11.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms and/or into a HIPAA-compliant study database. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12.0 REFERENCES

1) McGuire S et al. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr. 2016 Mar 15;7(2):418-9.
2) Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017 Jan;67(1):7-30. doi: 10.3322/caac.21387. Epub 2017 Jan 5.
3) Wolf AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol* 2013; **31:** 3997–4013.
4) AJCC (American Joint Committee on Cancer) Cancer Staging Manual, 8th edition, Amin MB, Edge SB, Greene FL et al (Eds), Springer, Chicago 2017.
5) Savas P, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth, MJ et al. Clinical relevance of host immunity in breast cancer: from TILs to clinic. Nat Rev Clin Oncol 2016;13:228-41.
6) Cimino-Mathews A, Thompson E, Taube JM, Ye X, Lu Y, Meeker A, et al. PD-L1 (B7-H1) expression and the immune microenvironment in primary and metastatic breast carcinomas. Human Pathol 2016;47:52-63.
7) Li Z, Li M, Lian Z, Zhu H, Kong L, Wang P, et al. Prognostic role of programmed death ligand-1 expression in breast cancer: a systematic review and meta-analysis. Target Oncol 2016;11:753-761.
8) Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med 2002; 347:1227.
9) Colleoni M, Sun Z, Price KN, et al. Annual Hazard Rates of Recurrence for Breast Cancer During 24 Years of Follow-Up: Results From the International Breast Cancer Study Group Trials I to V. J Clin Oncol 2016; 34:927.
10) Rastogi P, Anderson SJ, Bear HD, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol 2008; 26:778.
11) Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, Bonnefoi H, Cameron D, Gianni L, Valagussa P, Swain SM, Prowell T, Loibl S, Wickerham DL, Bogaerts J, Baselga J, Perou C, Blumenthal G, Blohmer J, Mamounas EP, Bergh J, Semiglazov V, Justice R, Eidtmann H, Paik S, Piccart M, Sridhara R, Fasching PA, Slaets L, Tang S, Gerber B, Geyer CE Jr, Pazdur R, Ditsch N, Rastogi P, Eiermann W, von Minckwitz G. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014 Jul 12;384(9938):164-72. doi: 10.1016/S0140-6736(13)62422-8. Epub 2014 Feb 14. Review.
12) Noone AM, Cronin KA, Altekruse SF, et al. Cancer Incidence and Survival Trends by Subtype Using Data from the Surveillance Epidemiology and End Results Program, 1992-2013. Cancer Epidemiol Biomarkers Prev 2017; 26:632.
13) Moja L, Tagliabue L, Balduzzi S, et al. Trastuzumab containing regimens for early breast cancer. Cochrane Database Syst Rev 2012.
14) Loibl S, Gianni L. HER2-positive breast cancer. Lancet. 2017 Jun 17;389(10087):2415-2429
15) Swain SM, Baselga J, Kim SB, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015; 372:724.
16) Schneeweiss A, Chia S, Hickish T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). Ann Oncol 2013; 24:2278.
17) Baselga J, Bradbury I, Eidtmann H, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 2012; 379:633.

18) Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012; 13:25.
19) Adams, S., et al. (2017). "Phase 2 study of pembrolizumab (pembro) monotherapy for previously treated metastatic triple-negative breast cancer (mTNBC): KEYNOTE-086 cohort A." Journal of Clinical Oncology 35(15_suppl): 1008-1008.
20) Ladoire S, Arnould L, Apetoh L, Coudert B, Martin F, Chauffert B, et al. Pathologic complete response to neoadjuvant chemotherapy of breast carcinoma is associated with the disappearance of tumor-infiltrating FOXP3+ regulatory T cells. Clin Cancer Res 2008;14:24;13-20.
21) Arnould L, Gelly M, Penault-Llorca F, et al. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? *Br J Cancer* 2006; 94: 259–67.
22) DeNardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Res 2007;9:212
23) Ribas T. Adaptive immune resistance: how cancer protects from immune attack. Cancer Discov 2015;5:915-9
24) McArthur HL. Checkpoint inhibitors in breast cancer: hype or promise? Clin Adv Hematol Oncol. 2016 Jun;14(6):392-5
25) Robert H. Vonderheide, Patricia M. LoRusso, Magi Khalil, Elaina M. Gartner, Divis Khaira, Denis Soulieres, Prudence Dorazio, Jennifer A. Trosko, Jens Rüter, Gabriella L. Mariani, Tiziana Usari and Susan M. Domchek. Tremelimumab in Combination with Exemestane in Patients with Advanced Breast Cancer and Treatment-Associated Modulation of Inducible Costimulator Expression on Patient T Cells. Clin Cancer Res. 2010 Jul 1;16(13):3485-94
26) McArthur HL, Diab A, Page DB, Yuan J, Solomon SB, Sacchini V, Comstock C, Durack JC, Maybody M, Sung J, Ginsberg A, Wong P, Barlas A, Dong Z, Zhao C, Blum B, Patil S, Neville D, Comen EA, Morris EA, Kotin A, Brogi E, Wen YH, Morrow M, Lacouture ME, Sharma P, Allison JP, Hudis CA, Wolchok JD, Norton L. A Pilot Study of Preoperative Single-Dose Ipilimumab and/or Cryoablation in Women with Early-Stage Breast Cancer with Comprehensive Immune Profiling. Clin Cancer Res. 2016 Dec 1;22(23):5729-5737.
27) Christopher R Heery, Geraldine O'Sullivan-Coyne, Ravi A Madan, Lisa Cordes, Arun Rajan, Myrna Rauckhorst, Elizabeth Lamping, Israel Oyelakin, Jennifer L Marté, Lauren M Lepone, Renee N Donahue, Italia Grenga, Jean-Marie Cuillerot, Berend Neuteboom, Anja von Heydebreck, Kevin Chin, Jeffrey Schlom, James L Gulley. Avelumab for metastatic or locally advanced previously treated solid tumours (JAVELIN Solid Tumor): a phase 1a, multicohort, dose-escalation trial. Lancet Oncol 2017; 18: 587–98.
28) Leisha A Emens, Fadi S Braiteh, Philippe Cassier, Jean-Pierre DeLord, Joseph Paul Eder, Xiaodong Shen, Yuanyuan Xiao, Yan Wang, Priti S Hegde, Daniel S Chen and Ian Krop. Abstract PD1-6: Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer. Cancer Res 2015;75(Suppl 9);abstr PD1-6.
29) Nanda, R., et al. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. Journal of Clinical Oncology 2016; 34(21): 2460-2467.
30) Nanda R et al. Pembrolizumab plus standard neoadjuvant therapy for high-risk breast cancer (BC): Results from I-SPY 2. Journal of Clinical Oncology 2017; 35 (Suppl): abstr 506.
31) Schmid P et al. Pembrolizumab (pembro) + chemotherapy (chemo) as neoadjuvant treatment for triple negative breast cancer (TNBC): Preliminary results from KEYNOTE-173. Journal of Clinical Oncology 2017; 35 (Suppl): abstr 156).
32) Leisha A Emens. Breast Cancer Immunotherapy: Facts and Hopes. Clin Cancer Res. 2017 Aug 11.
33) Adams S, Diamond JR, Hamilton EP, et al. Phase Ib trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer (mTNBC)J Clin Oncol 2016;34(Suppl):abstr 1009).

34) Tolaney S, Savulsky C, Aktan G, et al. Phase 1b/2 study to evaluate eribulin mesylate in combination with pembrolizumab in patients with metastatic triple-negative breast cancer. Presented at: 2016 San Antonio Breast Cancer Symposium; December 6-10, 2016; San Antonio, TX. Abstract P5-15-02.
35) L Arnould, M Gelly, F Penault-Llorca, L Benoit, F Bonnetain, C Migeon, V Cabaret, V Fermeaux, P Bertheau, J Garnier, J-F Jeannin, B Coudert. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? Br J Cancer. 2006 Jan 30; 94(2): 259–267.
36) Muraro E, Comaro E, Talamini R, Turchet E, Miolo G, Scalone S, Militello L, Lombardi D, Spazzapan S, Perin T, Massarut S, Crivellari D, Dolcetti R, Martorelli D. Improved Natural Killer cell activity and retained anti-tumor CD8(+) T cell responses contribute to the induction of a pathological complete response in HER2-positive breast cancer patients undergoing neoadjuvant chemotherapy. J Transl Med. 2015 Jun 27;13:204.
37) Matthew E. Wolpoe, Eric R. Lutz, Anne M. Ercolini, Satoshi Murata, Susan E. Ivie, Elizabeth S. Garrett, Leisha A. Emens, Elizabeth M. Jaffee and R. Todd Reilly. HER-2/neu-Specific Monoclonal Antibodies Collaborate with HER-2/neu-Targeted Granulocyte Macrophage Colony-Stimulating Factor Secreting Whole Cell Vaccination to Augment CD8$^+$ T Cell Effector Function and Tumor-Free Survival in Her-2/*neu*-Transgenic Mice. J Immunol August 15, 2003, 171 (4) 2161-2169.
38) Gang Chen, Richa Gupta, Silvia Petrik, Marina Laiko, James M. Leatherman, Justin M. Asquith, Maithili M. Daphtary, Elizabeth Garrett-Mayer, Nancy E. Davidson, Kellie Hirt, Maureen Berg, Jennifer N. Uram, Tianna Dauses, John Fetting, Elizabeth M. Duus, Saadet Atay-Rosenthal, Xiaobu Ye, Antonio C. Wolff, Vered Stearns, Elizabeth M. Jaffee and Leisha A. Emens. A Feasibility Study of Cyclophosphamide, Trastuzumab, and an Allogeneic GM-CSF–Secreting Breast Tumor Vaccine for HER2$^+$ Metastatic Breast Cancer. Cancer Immunol Res October 1 2014 2 (10) 949-961.
39) Peter S. Kim, Todd D. Armstrong, Hong Song, Matthew E. Wolpoe, Vivian Weiss, Elizabeth A. Manning, Lan Qing Huang, Satoshi Murata, George Sgouros, Leisha A. Emens, R. Todd Reilly, Elizabeth M. Jaffee. Antibody association with HER-2/*neu*–targeted vaccine enhances CD8$^+$ T cell responses in mice through Fc-mediated activation of DCs . J Clin Invest. 2008 May 1; 118(5): 1700–1711.
40) Stagg J, Loi S, Divisekera U, Ngiow SF, Duret H, Yagita H, Teng MW, Smyth MJ. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. Proc Natl Acad Sci U S A. 2011 Apr 26;108(17):7142-7.
41) Gennari R, Menard S, Fagnoni F, et al. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. Clin Cancer Res 2004;10:5650-5655.
42) Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987 Jan 9;235(4785):177-82.
43) Slamon DJ, Leyland-Jones B, Shal S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus monoclonal antibody against HER2 for metastatisc breast cancer that overexpresses HER2. N Eng J Med 344:783-792, 2001.
44) Gonzalez-Angulo AM, Litton JK, Broglio KR, et al. High risk of recurrence for patients with breast cancer who have human epidermal growth factor receptor 2-positive, node-negative tumors 1 cm or smaller. J Clin Oncol 2009; 27:5700.
45) Keefe DL. Trastuzumab-associated cardiotoxicity. Cancer 2002; 95:1592.
46) von Minckwitz G, du Bois A, Schmidt M, et al. Trastuzumab beyond progression in human epidermal growth factor receptor 2-positive advanced breast cancer: a german breast group 26/breast international group 03-05 study. J Clin Oncol 2009; 27:1999.
47) Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006; 355:2733.

48) Blackwell KL, Burstein HJ, Storniolo AM, et al. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 Study. J Clin Oncol 2012; 30:2585.
49) Krop IE, Kim SB, Martin AG, et al. Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. Lancet Oncol 2017; 18:743.
50) Investigator's Brochure, Pertuzumab (rhuMAb 2C4), *Eighth Version, February 2009.*
51) Lenihan D, Suter T, Brammer M et al. Pooled analysis of cardiac safety in patients with cancer treated with pertuzumab. Ann Oncol 2012;23:791–800.
52) Baselga J[1], Gelmon KA, Verma S, Wardley A, Conte P, Miles D, Bianchi G, Cortes J, McNally VA, Ross GA, Fumoleau P, Gianni L. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. J Clin Oncol. 2010 Mar 1;28(7):1138-44
53) Swain SM, Baselga J, Kim SB, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015; 372:724.
54) Smyth LM, Iyengar NM, Chen MF, Popper SM, Patil S, Wasserheit-Lieblich C, Argolo DF, Singh JC, Chandarlapaty S, Sugarman SM, Comen EA, Drullinsky PR, Traina TA, Troso-Sandoval T, Baselga J, Norton L, Hudis CA, Dang CT. Weekly paclitaxel with trastuzumab and pertuzumab in patients with HER2-overexpressing metastatic breast cancer: overall survival and updated progression-free survival results from a phase II study. Breast Cancer Res Treat. 2016 Jul;158(1):91-7.
55) Rastogi P, Anderson SJ, Bear HD, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. *J Clin Oncol* 2008; **26:** 778–85.
56) www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&applno=125409.
57) von Minckwitz G, Procter M, de Azambuja E, et al. Adjuvant Pertuzumab and Trastuzumab in Early HER2-Positive Breast Cancer. N Engl J Med 2017; 377:122.
58) Bianchini G, Pusztai L, Pienkowski T, Im YH, Bianchi GV, Tseng LM, Liu MC, Lluch A, Galeota E, Magazzù D, de la Haba-Rodríguez J, Oh DY, Poirier B, Pedrini JL, Semiglazov V, Valagussa P, Gianni L. Immune modulation of pathologic complete response after neoadjuvant HER2-directed therapies in the NeoSphere trial. Ann Oncol. 2015 Dec;26(12):2429-36.
59) Loi S, Giobbe-Hurder A, Gombos A, Bachelot T, Hui R, Curigliano G, Campone M, Biganzoli L, Bonnefoi H, Jerusalem G, Bartsch R, Rabaglio-Poretti M, Kammler R, Maibach R, Smyth MJ, Di Leo A, Colleoni M, Viale G, Regan MM, Andre F International Breast Cancer Study Group and Breast International Group. Phase Ib/II study evaluating safety and efficacy of pembrolizumab and trastuzumab in patients with trastuzumab-resistant HER2-positive metastatic breast cancer: Results from the PANACEA (IBCSG 45-13/BIG 4-13/KEYNOTE-014) study. Breast Cancer Res Treat, 2017, Abstract GS2-06.
60) Cimino-Mathews A, Foote JB, Emens LA. Immune targeting in breast cancer. Oncology (Williston Park). 2015 May;29(5):375-85.
61) Denkert C, Loibl S, Noske A, et al: Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 28:105-13, 2010
62) Loi S, Sirtaine N, Piette F, et al: Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 31:860-7, 2013
63) Zitvogel L, Galluzzi L, Kepp O, et al: Type I interferons in anticancer immunity. Nat Rev Immunol 15:405-14, 2015
64) Tan AH, Goh SY, Wong SC, et al: T helper cell-specific regulation of inducible costimulator expression via distinct mechanisms mediated by T-bet and GATA-3. J Biol Chem 283:128-36, 2008
65) Chen KH, Boettiger AN, Moffitt JR, et al: RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. Science 348:aaa6090, 2015

66) Iida N, Dzutsev A, Stewart CA, et al: Commensal bacteria control cancer response to therapy by modulating the tumor microenviroment. Science 342:967-70, 2013.
67) Sivan A, Corrales L, Hubert N, et al: Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 350:1084-9, 2015.
68) Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, Assad L, Poniecka A, Hennessy B, Green M, Buzdar AU, Singletary SE, Hortobagyi GN, Pusztai L (2007) Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 25:4414–4422
69) Investigator's Brochure, Keytruda®, Fifteenth Version, September 2017.
70) Chan IS, Zhang Z. 1999. Test-based exact confidence intervals for the difference of two binomial proportions. Biometrics, 55(4):1202-9.

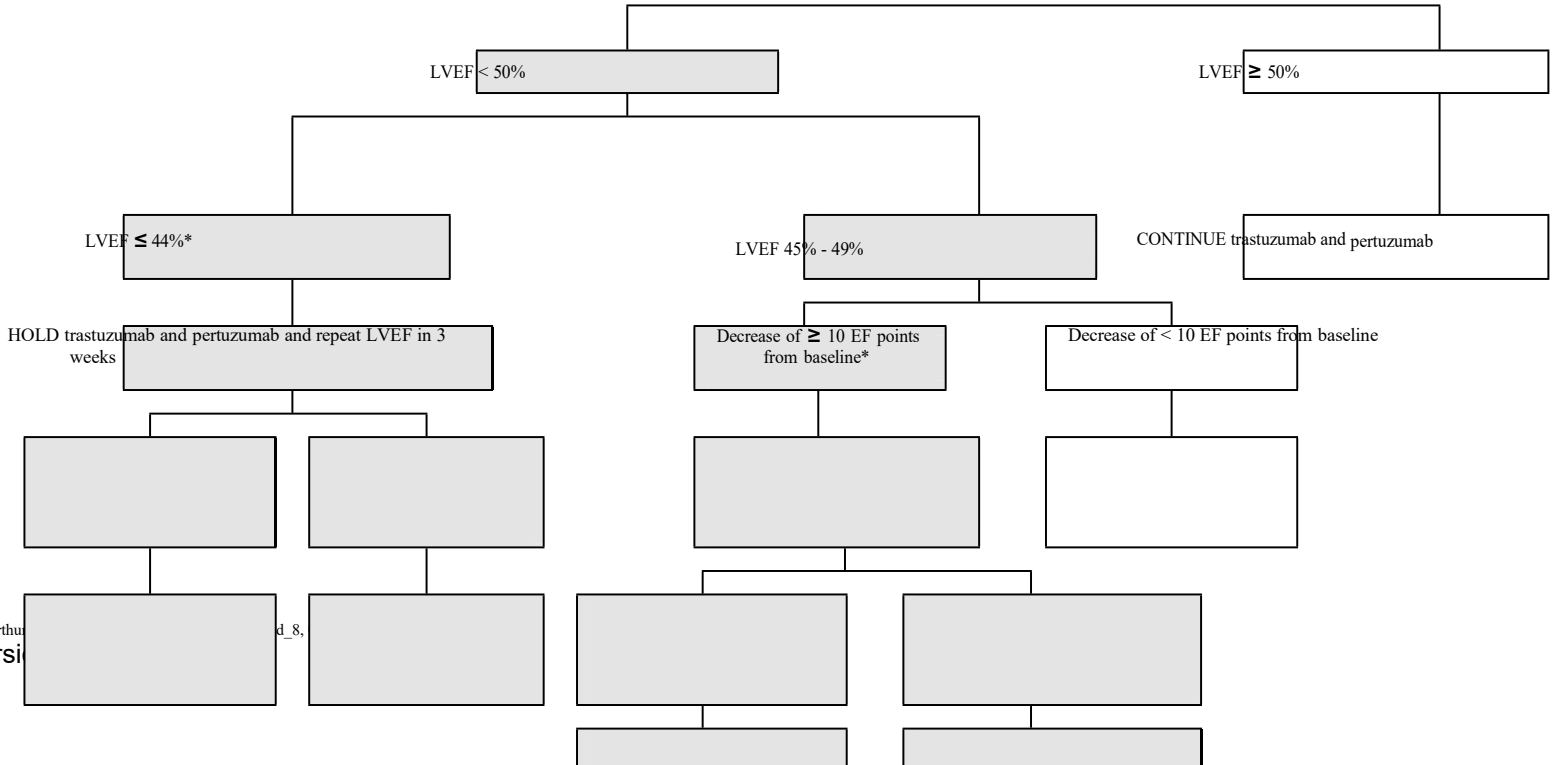
13.0 APPENDICES

Appendix A: Criteria for New York Heart Association Functional Classification

Functional capacity (four classes)

Class I:	No limitation of physical activity. Ordinary physical activity does not cause fatigue, palpitation or dyspnea.
Class II:	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnea.
Class III:	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation or dyspnea.
Class IV:	Unable to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency may be present even at rest. If any physical activity is undertaken, discomfort is increased.

Appendix B Algorithm for Continuation and Discontinuation of Trastuzumab and Pertuzumab Based on LVEF Assessment in Asymptomatic Patients



LVEF ≤ 44% OR LVEF 45% - 49% and decrease of ≥ 10 points from baseline

LVEF 45% - 49% and a decrease of < 10 points from baseline OR LVEF > 49%

HOLD trastuzumab and pertuzumab and repeat LVEF in 3 weeks

CONTINUE trastuzumab and pertuzumab and repeat LVEF in 3 weeks

STOP trastuzumab and pertuzumab**

RESUME trastuzumab and pertuzumab

LVEF ≤ 44% OR LVEF 45% - 49% and decrease of ≥ 10 points from baseline

LVEF 45% - 49% and decrease of < 10 points from baseline OR LVEF > 49 %

STOP trastuzumab and pertuzumab**

RESUME trastuzumab and pertuzumab

EF = ejection fraction; LVEF = left ventricular ejection fraction.

* Report as AE – Reporting term 'Left ventricular systolic dysfunction'

** Report as AE (eCRF AE eform) and Non-Serious Event of Special Interest (SAE form) Reporting term 'Left ventricular systolic dysfunction'

Note: LVEF assessment results must be available before/or on the day of the next scheduled trastuzumab and pertuzumab/placebo equivalent administration.

Appendix C: Contraceptive Guidance and Pregnancy Testing

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP:

- **Premenarchal**
- **Premenopausal female with 1 of the following:**
  - **Documented hysterectomy**
  - **Documented bilateral salpingectomy**
  - **Documented bilateral oophorectomy**

Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

- **Postmenopausal female**

  A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

  A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.

  Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants:

Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame in section 5.6:

- **Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent**
- **Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 11 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.**

Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception that has a low user dependency consistently and correctly as described in Table 11 during the protocol-defined time frame in Section 5.6.

Table 11 Highly Effective Contraceptive Methods That Have Low User Dependency

Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable contraceptive implants are limited to those which inhibit ovulation.

Appendix D: SUMMARY OF CHANGES

Amendment 1 Protocol Version 2 dated 10Jan2019:

changes made to version 1 dated 25Sep 2018

1. Title page:
    a. updated co-investigators to include Dr. Stephen Shiao and removed Dr. Kollman and Wachsman.
    b. NCT number added
2. List of abbreviations: EDC added
3. Eligibility, 4.1 Inclusion Criteria:
    a. Clarification of criteria #2
    b. Type of criteria #4 to state greater than or equal to 18 years of age
    c. Criteria #8 clarified from provided tissue to confirmation of adequate tissue. Section 8.1 reference added.
    d. Criteria #9 removed the ECOG requirement of completion within 7 days of randomization
4. Eligibility, 4.2 Exclusion Criteria:
    a. #8 clarified to specify that a malignancy other than the current breast cancer diagnosis
    b. #13 updated to include specific hepatitis panel tests to confirm eligibility
5. Section 5.1 Treatment Dosage and Administration: Pertuzumab administration clarified to state that the observational period following infusion is recommended (per FDA approved guidelines), but not required and may be performed per institutional standards.

    Subsequent cycle observation period was removed.
6. Section 5.2 Dose Modifications and Delays: Clarified dosing delays and timing regarding holds and restarting or discontinuing treatment
7. Section 5.3 Method of Assigning Subjects to Treatment Groups: updated to include the new EDC Clinical Studio. Clarification of working days as business days.
8. Section 5.4 Excluded Therapies updated to include Proton Pump Inhibitor guidelines during treatment
9. Section 5.5 Other Modalities, etc.: Surgery timing added to confirm procedure at 5 weeks (+/-1 week) following completion of protocol treatment.
10. Section 6.0 Study Procedures:
    a. Screening time clarified to 28 days
    b. CMP labs clarified to state tests that must be included
    c. Coagulation test added to screening
    d. Research blood and optional fecal sample moved from screening to C1D1
    e. Clarification of the timing of research blood and optional fecal samples during treatment
    f. Research blood specimen tube and timing updated
    g. Timing of CBC added as drawn weekly days 1, 8 and 15 (+/- 3 days) for every cycle during treatment
11. Table 3, Time and Events Table: updated to reflect the changes listed above.
    a. Clarified that safety labs utilized for screening do not need to be repeated on C1D1 if completed within 7 days of treatment initiation

b. Clarified that pregnancy test will need to be repeated if completed over 72 hours prior to treatment initiation
c. Breast imaging added at screening, surgery and follow up
d. Staging imaging timepoints clarified
e. Vitals added to screening, before dosing visits, surgery and during follow up
f. Footnotes clarified throughout chart

12. Section 6.3 Follow up Procedures: SOC f/u visit to occur every 3 months (+/- 1 week)
13. Section 7.5.2 Expedited Reporting: updated to include coordinating center/outside contracted CRO, Oncotherapeutics and contact information
    a. Section 7.5.2.2: clarified that IRB must be notified per institutional standard
14. Section 7.6.2 SAEs: the following language was added: Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.
15. Section 7.6.2 and 7.6.6 udpated to include Merck global safety reporting to include PI, Oncotherapeutics and Merck as recipients in overdoes and SAE and other safety information reports utilizing the Merck global safety intake form.
16. FDA MEDWATCH reporting requirements for IND reporting removed. This study is IND exempt
17. Section 8.0 Correlatives:
    a. Blood collection tubes updated as: 2 (8 mL) CPT (Red/Green tiger top) tubes, 1 (6mL) red top tube, and 1 (5mL) Cyto-Chex BCT tube.
    b. Sample instructions added: All research blood (and tissue) samples will need to be shipped to the laboratory of the CSMC PI who will be conducting this analysis. All specimens will need to be labeled according to subject ID, date of collection and study visit. The shipping and receipt of specimens will be tracked in a database and by emails sent to Dr. Shiao and the PI. The corresponding study lab manual has further specimen labeling and shipping directions.
    c. Archival tissue samples updated to state: For archival tissue samples, a tissue block is preferred, but it unavailable 4 unstained slides and 1 H&E slide is acceptable.
18. Section 11.3 Registration Procedures: updated to include screening log, and removal of review procedures of log by investigator and CRC. Clinical studio EDC added as registration tool. Registration/Randomization procedures and eligibility verification updated to reflect new procedures with Oncotherapeutics and EDC, Clinical Studio.
19. Section 11.5 Data and Safety Monitoring: Language updated to reflect the data monitoring plan and safety review by Oncotherapeutics.
20. Section 11.7 Adherence to protocol, the following modifications were made: Emergency modifications, protocol exceptions and planned deviations request must be sent to PI and Medical Monitor. Medical Monitor contact information included. Eligibility waivers updated to reflect PI policy that none will be granted. Protocol amendments process removed from protocol. Site specific deviation language was removed.
21. Formatting updates and changes throughout the document

Amendment 2 Protocol Version 3 dated 03Mar2020:

changes made to version 2 dated 10Jan2019

1. List of abbreviations: added missing abbreviations.

2. Her2 changed to HER2 throughout the document for consistency.
3. Typo corrections, formatting updates, and changes throughout the document.
4. Study schema: clarified use of additional therapy for biopsy proven residual disease.
5. Study summary: clarified study duration, study centers, and safety and efficacy analyses.
6. Background and rationale:
    a. Changed “programmed death ligand 1” to “programmed cell death ligand 1”;
    b. Changed “programmed death 1” to “programmed cell death protein 1”;
    c. Changed “cytotoxic T lymphocyte-associated antigen 4” to cytotoxic T lymphocyte-associated protein 4”.
7. Section 2.4.2 Secondary Efficacy Endpoints: changed “Any evidence of ipsilateral or contralateral disease in sit will not be identified as progressive disease.” to “All in situ cancer events (ipsilateral or contralateral DCIS, ipsilateral or contralateral LCIS and all in situ cancer of non-breast sites) are excluded as an event in the endpoint.”.
8. Section 2.4.3 Exploratory Endpoints: changed serial peripheral blood and optional fecal sample collection to “as per schedule in Table 3”.
9. Section 3.1 Overview of the Study Design:
    a. Added “Patients with biopsy-proven residual disease after completing the study treatment may receive additional chemotherapy (i.e. four cycles of adriamycin + cyclophosphamide administered every two weeks) prior to surgery at the treating physician discretion and should be seen in follow-up at each additional treatment prior to surgery.”;
    b. Clarified follow up visit schedule to every 3 months until 36 months after surgery and every 6 months in year 4 and 5, then annually until year 10 after surgery.
10. Section 5.1 Treatment Dosage and Administration: Paclitaxel administration clarified to state that additional premedication should be administered as per standard practice.
11. Section 5.4 Concomitant Medication and Treatment:
    a. Removed acetaminophen from Allowed Therapies because same as paracetamol (duplication);
    b. Added corticosteroids for immune related adverse events, thyroid medications, and gonadotropin-releasing hormone analog/antagonists to the Allowed Therapies;
    c. Clarified excluded anti-cancer therapies to allow exception for ddAC administration in patients with residual disease before surgery;
    d. Removed steroids and corticosteroids from the Excluded Therapies.
12. Section 6.0 Study Procedures:
    a. Clarified screening/baseline procedures;
    b. Added carbon dioxide as an option within CMP labs;
    c. Hepatitis B, Hepatitis C and HIV tests removed;
    d. TSH + free T3 and T4 changed to THS to reflex T4 or TSH with T4 per institutional standards;
    e. Modified section 6.2.1 Subjects randomized to control arm to include ddAC chemotherapy for patients with residual disease after Cycle 4;
    f. Research blood and optional fecal sample moved from C2D1 to C3D1;
    g. Added optional fecal sample collection at 30and 60 days after surgery;
    h. Clarified Echo and MUGA timing.
    i. Clarified long-term follow-up schedule
    j. Table 3, Time and Events Table updated to reflect the changes listed above and footnotes clarified throughout chart.
13. Table 4: clarified guideline for paclitaxel use in case of grade 4 hepatic toxicity.
14. Table 7: clarified guidelines for premedication at subsequent doses of pembrolizumab in case of grade 2 infusion reaction.

15. Section 8.1 Peripheral blood correlatives: updated to list specific blood correlative assays to be conducted.
16. Section 9.3.4 Interim Analyses: added Arm C stopping rule for efficacy.
17. Section 11.3: added cTNM staging to source documents to obtain for eligibility verification.
18. Section 11.5.2 Safety Monitoring: Language updated to reflect the additional data and safety monitoring by an Institutional Data Safety Monitoring Committee with a frequency of 6 months.
19. Appendix C: Table 9 changed to Table 11.
20. Removed Performance Status at surgery in Schedule of Events.
21. Clarified duration of toxicity assessment through 1-year post surgery.
22. Updated acceptable forms of trastuzumab throughout the document.
23. Added allowance of nab-paclitaxel.
24. Increased long-term follow-up to year 10.

Protocol Version 4 dated 08DEC2020:

1. Addition of Torrance sub-investigators.
2. Clarified TSH per PI to read: TSH with reflex to T3 and free T4 **OR** TSH with T3 and free T4.
3. Removed section 7.5.3

Protocol Version 5 dated 24MAR2021:

1. Revise Exclusion Criteria #5 (vaccines) per Merck revised Protocol Template.
2. Revise Exclusion Criteria #10 (pneumonitis) to include interstitial lung disease per Merck revised Protocol Template.
3. Revise Section 5.2.3 and Table 6 per Merck revised Protocol Template.
4. Added Optional fresh breast biopsies for analysis (Cedars patients only) at baseline and week 6.

Protocol Version 5.1 dated 16JUN2021:

Cover page: Principal Investigator changed from Dr. Heather McArthur to Dr. Reva Basho.

Protocol Version 6.0 dated 23May2022:

Cover page- updated to reflect UTSW as lead site

Section 11.5.2 – Updated to reflect UTSW SCCC DSMC standard template language for safety monitoring

Minor clarifications about lost to follow up contact and vital sign collection during long term follow up

Protocol Version 7.0 dated 22Feb2023:

Cover page updated to reflect current protocol version and date

Signature page updated to reflect current protocol version and date

Study Summary: Addition of New Mexico Cancer Center as subsite

Inclusion Criterium 10: Removal of language regarding screening lab collection time period to align with schedule of events

Section 6.2.1 Removal of clinical breast exam and mammogram language after completion of cycle 4 of treatment

Protocol Version 8.0 dated 14Dec2023:

1. Cover page updated to reflect current protocol version and date
2. Signature page updated to reflect current protocol version and date
3. Section 7.5.2 Removal of VP of Oncotherapeutics as contact for SAE reporting and updated SAE reporting table
4. Section 7.6.2 Removal of Oncotherapeutics as contact for SAE reporting
5. Section 7.6.6 Removal of coordinating center Oncotherapeutics as contact for overdose reporting
6. Section 8.4 Updated sample collection to remove incorrect laboratory address
6. Section 11.3 Replacement of Oncotherapeutics with UTSW
7. Section 11.5 Replacement of Oncotherapeutics with IIT project manager; replacement of CRA with Study Monitor; replacement of Oncotherapeutics with Sponsor; removal of 'and follow specifications deemed appropriate by both Sponsor and Oncotherapeutics.'
8. Section 11.5.2 Removal of Oncotherapeutics; replacement of CRO with UTSW; replacement of CRA with study monitor
9. Section 11.7 Removal of Oncotherapeutics
10. Section 11.7.1 Removal of Oncotherapeutics