Quantum Leap Healthcare Collaborative

Summary of Changes related to HER2- pembrolizumab (4 cycle) arm within the I-SPY2 TRIAL Master Protocol

Master Protocol	Date	Items related to pembrolizumab arm within I-SPY2 TRIAL Master Protocol
Amendment 1	8/1/2009	• Protocol development.
Amendment 2	11/13/2009	• Protocol development.
Amendment 3	2/1/2010	• Addition of drug regimen Appendixes and consents. • Administrative changes.
Amendment 4	4/27/2010	• Administrative changes. • Clarification of MammaPrint cutpoints.
Amendment 5	2/1/2011	• Administrative changes. • Inclusion of guidelines for substitution of epirubicin instead of doxorubicin in case of national shortage. • New participant materials.
Amendment 6	7/6/2011	• Administrative changes.
Amendment 7	11/15/2011	• Administrative changes. • Addition of sub-study “Quality of Life Measurement within the I-SPY 2 Study”, where paper-based Quality of Life questionnaire introduced.
Amendment 7.1	1/16/2012	• Administrative changes.
Amendment 8	4/10/2012	• Addition of co-study ACRIN6698 MRI Scan Protocol (a diffusion weighted MRI study).
Amendment 9	5/31/2012	• Addition of Section 5.4 “Concomitant Medication”. • Update to Section 11 “Reporting Adverse Events” regarding follow-up AE data collection.
Amendment 10	1/16/2013	• Administrative changes. • Updated Section 3.4 “Adaptive Randomization of Investigational Agents“ and Section 6 “Investigational Agent Information“ - Updated number of investigational agents from five to eight • Added new Section 8.6 “Evaluations for Premature Discontinuation of Investigational Agent(s)” - Participants who discontinue their randomized treatment assignment prematurely for any reason will be offered the option of remaining “on study”

		to complete the remaining study procedures and follow-up. Regardless of whether a participant chooses to stay on study, we will collect safety labs and assessment tests 30 days after last dose of investigational agent. • Update Section 4.1.2 "Inclusion Criteria for Treatment Phase of I-SPY 2 TRIAL" and Section 8.2 "Baseline Testing/Pretreatment Evaluation" - Defined parameters around allowable time to complete screening tests
Amendment 11	7/28/2014	• Administrative changes. • Update of non-profit sponsor from FNIH to QLHC.
Amendment 12	12/19/2014	• Administrative changes. • Addition of sub-study SURMOUNT (Surveillance Markers of Utility for Recurrence after Neoadjuvant Therapy for Breast Cancer). Added collection of circulating tumor cells (CTCs) in peripheral blood longitudinally and/or disseminated tumor cells (DTCs) in bone marrow at surgery/recurrence
Amendment 13	5/15/2015	• Update to Section 3.4 "Adaptive Randomization of Investigational Agents", 13 "Statistical Considerations", Appendix A "Statistical Considerations". Revised and updated with new language to reflect the change in minimum and maximum number of participants to be enrolled if an investigational agent is only open to either HER2 positive or HER2 negative participants (n=75 participant cap). • Update to Section 8.6 "Evaluations for Premature Discontinuation of Investigational Agents(s)" to clarify process for participants who discontinue study treatment prematurely and procedures that must still be followed (including S/AE and follow up period) • Update to Section 11 "Reporting Adverse Events" to clarify safety collection and follow up time period • Added Section 8.7 "Disease Progression" • Administrative changes.

		• Close accrual to co-study ACRIN6698.
Amendment 14	10/7/2015	• Introduction of pembrolizumab regimen in Appendix O. • Addition of pembrolizumab treatment informed consent form (ICF). • Administrative changes.
Amendment 15	3/18/2016	• Administrative changes.
Amendment 16 *(see redacted protocol)*	7/27/2016	• Formal replacement of Appendix A with an updated statistical model for pCR with adjustment for time trend. This model now includes a component that adjusts for possible time trends in the underlying pCR rate of each participant at the time of randomization. The change reflects the need to capture the effect of possible drifting pCR rates over time. • Updated Appendix O (Pembrolizumab), Table 2 and Section 2.6.1 Hepatic section to harmonize language for Hy's law events. • Section 5.1/5.8 "Addition of Paclitaxel Hypersensitivity Reaction Recommendation" (allows provision of nab-paclitaxel). • Informed consents removed from protocol to allow for independent amendments. • Administrative changes.
Amendment 17	3/10/2017	• Added Section 8.5 "Surgical Aspects" to provide guidance on nodal staging and axillary node surgery. • Added Section 12.5 Safety Working Group, new working group to address safety in addition to DSMB • Updated Appendix O (Pembrolizumab) – Section 2.1.1 Paclitaxel Premedication Regimen, addition of clause regarding use of anti-emetic medication during AC cycles, based on recommendations from Merck from other clinical trials; Section 2.2 Additional Eligibility Criteria, exclusion of patients with history of (non-infectious) pneumonitis that required steroids or current pneumonitis. Based on evidence from IB*; Section 2.5 Clinical Evaluation and Procedures, addition of expanded

		TSH testing, additional labs and surgical management guidelines for adrenal insufficiency, based on safety profiling of drug and additional measures to ensure safety of patients with adrenal conditions on this arm; Updated 3.2. Reported Clinical AEs and Potential Risks, update to section based on current IB v13 Feb 23 2017 • Administrative changes.
Amendment 18	10/10/2017	• Update to Appendix C to reflect pembrolizumab graduation from trial. • Update to Section 8.4 "Evaluations at Completion of Neoadjuvant Chemotherapy Treatment", to add in TSH safety testing for control participants at cycle 13, cycle 15, and 30 days post-surgery. Addition of blood collection table post chemotherapy. • Update to Section 8.6 "Post Surgery Follow-up", to update follow-up of new pertinent S/AEs for up to 12 months after surgery • SURMOUNT surveillance marker studies incorporated into main protocol

*Note no participants on the Pembrolizumab arm were assessed for this new eligibility as the accrual had closed on the arm November 5, 2016. As sites are blinded to accrual closures of an arm before participants reach surgery to prevent bias, the sites were not aware of arm closure until May 17, 2017. However, given site investigators were aware about the updated Merck IB in February 2017, changes were made to the protocol accordingly to not unblind the status of the arm.

Study Timeline:

1. August 7, 2015: Efficacy reports using the *time adjusted model* is evaluated by the DSMB to graduate arm regimens.
2. May 2, 2016: Time adjusted model is put into production in the randomization engine to randomize participants to drug regimens. Formally updated into the master protocol amendment 16, Appendix A.
3. November 26, 2015: Accrual to pembrolizumab activated in randomization engine.
4. November 5, 2016: Accrual to pembrolizumab deactivated in randomization engine.
5. May 17, 2017: Sites notified that accrual to pembrolizumab deactivated. Per the protocol, notification is provided after all expected pembrolizumab participants have completed surgery to prevent treatment bias (last patient to surgery May 16, 2017).
6. May 2017 to May 2022 (for select participants: May 2027): Follow-up for recurrence free survival (RFS), overall survival (OS), and distant metastasis-free survival (DRFS) after surgery is 5 years, except for those participants consented on the substudy SURMOUNT or reconsented to longer follow up in 2019, then follow up is 10 years.

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The I-SPY2 TRIAL is an ongoing standing platform trial with multiple drug arms, which enter and leave the trial at different times. New agents are selected and added to the trial as others leave the trial for success (graduate) or futility based on their efficacy in targeted patients. A drug arm may also leave the trial for safety concerns as recommended by the independent Data Safety Monitoring Board. A Master Protocol governs the entire trial with each drug arm protocol as a separate Appendix.

For this reason, we cannot share information or appendices of drugs which are still currently in the trial or in process for a peer-reviewed publication.

1) I-SPY2 TRIAL standing platform Master Protocol Amendment 16, July 27, 2016 (Redacted), the protocol at the time pembrolizumab (4 cycle) graduated from the trial including:
    a. Appendix A Statistical Considerations -further details of statistical design and analysis
    b. Appendix O Pembrolizumab, note the pembrolizumab (4 cycle) regimen was added to I-SPY2 TRIAL standing platform Master Protocol Amendment 14 October 7, 2015.

I-SPY 2 TRIAL

(**I**nvestigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**L**ecular Analysis **2**)

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IND#	**105,139**
Agent(s)/Supplier:	**See Appendices D−Q**
Protocol Version Date:	July 25, 2016
Protocol Revision or Amendment No.:	**16**

SCHEMA

Figure A: I-SPY 2 TRIAL(Investigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**L**ecular Analysis **2**)

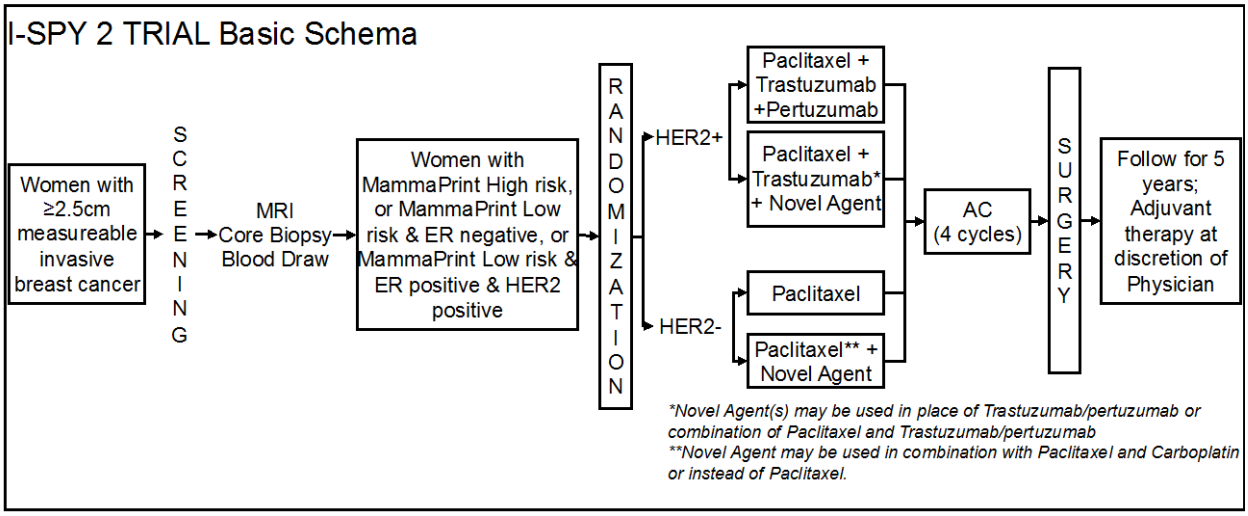


Figure B: I-SPY 2 Adaptive TRIAL Schema: Screening Tumor Eligibility & Randomization

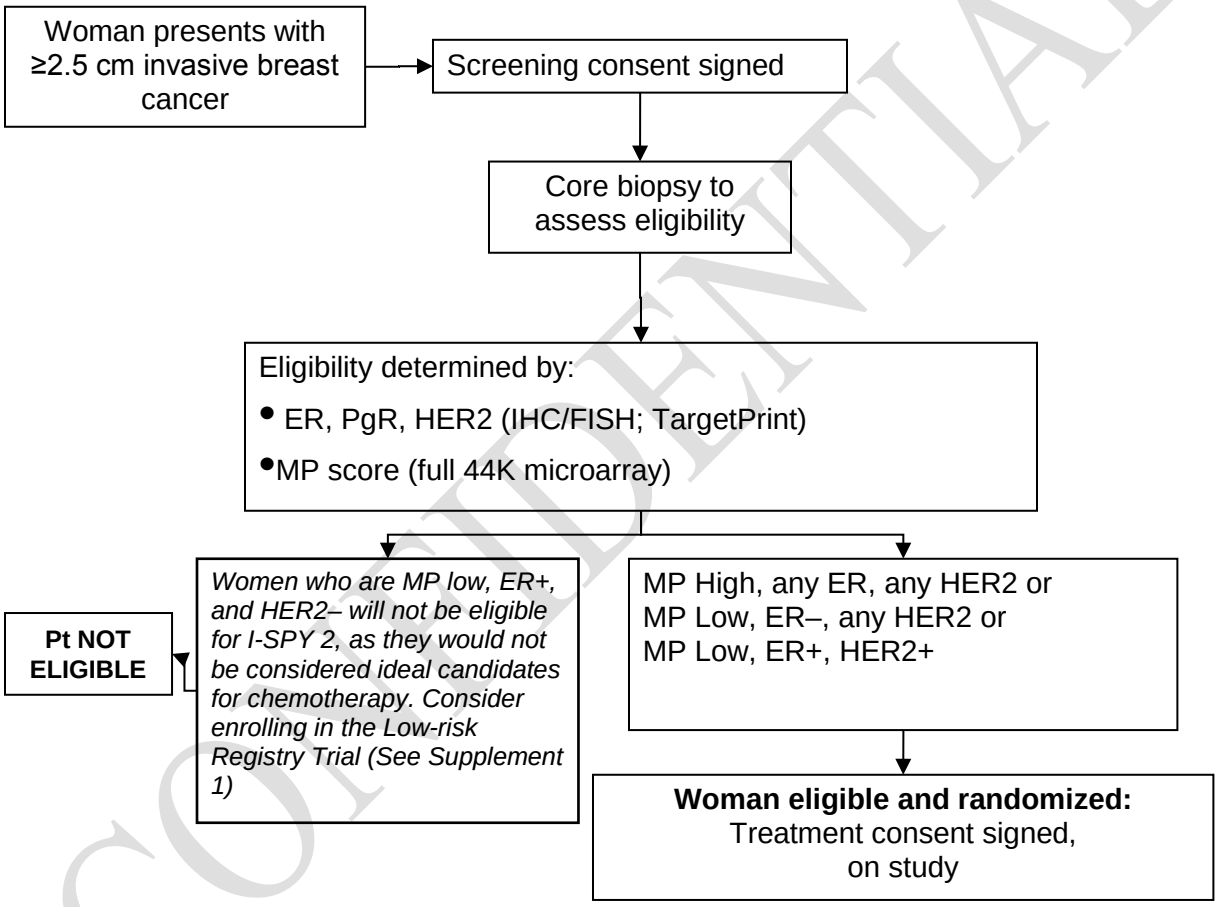
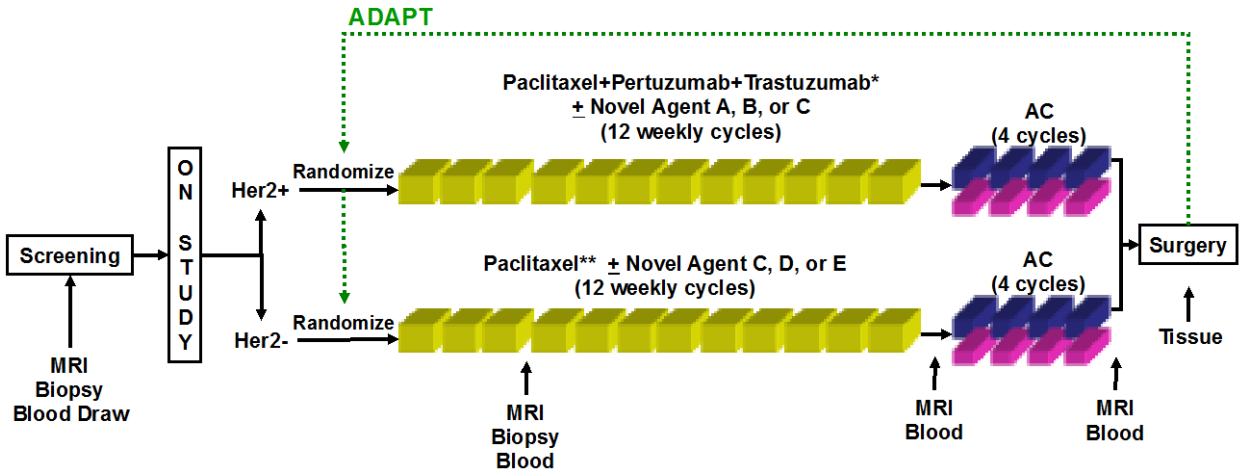


Figure C: Schedule of Study Procedures

Pretreatment (Time Point 0)	Early Paclitaxel (Time Point 1)	Inter-regimen (Time Point 2)	Pre-surgery/Surgery (Time Point 3)
MRI *(within 30 days prior to randomization)*	MRI *(end of week 3, prior to cycle 4)*	MRI *(prior to AC, at least 1 day after last paclitaxel)*	MRI *(prior to surgery, at least 2–3 weeks after AC)*
Core Biopsy *(prior to randomization)*	Core Biopsy *(end of wk 3, prior to cycle 4)*	---- ---- ----	Surgical Tissue *(at time of surgery)*
Blood Draw *(prior to randomization)*	Blood Draw *(end of week 3, prior to cycle 4)*	Blood Draw *(prior to AC, at least 1 week after last paclitaxel)*	Blood Draw *(prior to surgery, at least 2–3 weeks after AC)*

Abbreviations: AC = anthracycline; MRI = magnetic resonance imaging.

Figure D: I-SPY 2 TRIAL, Adaptive Overall Study Schema



**Novel Agent may be used in place of Trastuzumab/pertuzuamb or combination of Paclitaxel and Trastuzumab/pertuzumab in Experimental Treatment Arm (see specific Novel Agent Appendix)*
***Novel Agent may be used in combination with Paclitaxel and Carboplatin in Experimental Treatment Arm or instead of paclitaxel (see specific Novel Agent Appendix)*

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LIST OF ABBREVIATIONS

AC	Doxorubicin/cyclophosphamide
ACRIN	American College of Radiology Imaging Network
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMPK	AMP-activated protein kinase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphatase
AUC	Area under the concentration-time curve
BCS	Breast-conserving surgery
BSI	Brief Symptom Inventory
BUN	Blood urea nitrogen
caBIG	Cancer Biomedical Informatics Grid
CALGB	Cancer and Leukemia Group B
CAPMM	Center for Applied Proteomics and Molecular Medicine
CBC	Complete blood count
CBIIT	Center for Biomedical Informatics and Information Technology
CDE	Common Data Element
cCR	Clinical complete response
cDNA	Complementary DNA
CGH	Comparative genomic hybridization
CHF	Congestive heart failure
CI	Confidence interval
CIS	Cancer *in situ*
CL	Clearance
CLIA	Clinical Laboratory Improvement Amendment
CRADA	Cooperative Research and Development Agreement
CRF	Case report form
CT	Computed tomography
CTA	Clinical Trials Agreement
CTDC	Clinical Trial Data Capture
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
CXR	Chest radiograph
DAPC	Data Access and Publications Committee
DCC	Data Coordinating Center
DFS	Disease-free survival
DLT	Dose-limiting toxicity
DSMB	Data Safety Monitoring Board
DT	Distress thermometer
EBC	Early breast cancer
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
FDA	Food and Drug Administration
FFPE	Formalin fixation and paraffin embedding
FISH	Fluorescence *in situ* hybridization
FNA	Fine-needle aspiration
FNIH	Foundation for the National Institutes of Health

List of Abbreviations *(continued)*

FTV	Functional tumor volume
FWA	Federalwide Assurance
5-FU	5-fluorouracil
GFR	Glomerular filtration rate
GMU	George Mason University
GWAS	Genome-wide association study
H&E	Hematoxylin and eosin
HADS	Hospital Anxiety and Depression Scale
HAHA	Human antihuman antibodies
HIPAA	Health Insurance Portability and Accountability Act
HER2	Human epidermal growth factor receptor
HNSCC	Head and neck squamous cell carcinoma
HNSTD	Highest non-severely toxic dose
HR	Hormone receptor (ER + PgR)
HRG	Heregulin
IASC	Independent Agent Selection Committee
ICH GCP	International Conference on Harmonisation Good Clinical Practice
IDE	Investigational Device Exemption
IGF	Insulin-like growth factor
IGF-1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor 1 receptor
IGFR	Insulin-like growth factor receptor
IgG_1	Human monoclonal antibody
IHC	Immunohistochemistry
ILD	Interstitial lung disease
IND	Investigational New Drug
IR	Insulin receptor
IRB	Institutional Review Board
I-SPY TRIAL	**I**nvestigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**L**ecular Analysis
K-M	Kaplan-Meier
LABC	Locally advanced breast cancer
LVEF	Left ventricular ejection fraction
LKB1	Liver kinase B1
LN	Lymph nodes
MAPK	Mitogen-activated protein kinase
MBC	Metastatic breast cancer
MCC	4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate
MDACC	MD Anderson Cancer Center
MP	MammaPrint
MP–	MammaPrint High1
MP+	MammaPrint High2
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
mTOR	Mammalian target of rapamycin
MUGA	Multigated acquisition scan
NCI	National Cancer Institute
NCCN	National Comprehensive Cancer Network

List of Abbreviations *(continued)*

NDA	New Drug Application
NIH	National Institutes of Health
NKI 70	Netherlands Cancer Institute 70-gene signature
NOAEL	No observed adverse effect level
NRH	Nodular regenerative hyperplasia
NSABP	National Surgical Adjuvant Breast and Bowel Project
NSCLC	Non-small cell lung carcinoma
OHRP	Office of Human Research Protections
OIVD	Office of *In Vitro* Diagnostics
ORR	Objective response rate
OS	Overall survival
PARP	Polyadenosine diphosphate ribose polymerase
pCR	Pathologic complete response
PD	Progressive disease
PE	Percent enhancement
PFS	Progression-free survival
PET	Positron emission tomography
PgP	P-glycoprotein
PgR	Progesterone receptor
pHER2	Phosphorylated HER2
PI	Principal Investigator
PI3K	Phosphatidylinositol-3-kinase
PK	Pharmacokinetics
PNET	Primitive neuroectodermal tumors
PR	Partial response
PSA	Prostate-specific antigen
QLHC	QuantumLeap Healthcare Collaborative
QOL	Quality of life
RCB	Residual cancer burden
RFS	Relapse-free survival
RPMA	Reverse phase protein microarray
ROC	Receiver operating characteristic
ROR	Risk of recurrence
ROR-S	Retinoid-related orphan receptors
SAE	Serious adverse event
SCC	Squamous cell carcinoma
SD	Stable disease
SER	Signal enhancement ratio
SI	Signal intensity
SMCC	Succinimidyl MCC
SNP	Single-nucleotide polymorphism
SPORE	Specialized Programs of Research Excellence
T4	Tumor growing into the chest wall or skin, including inflammatory breast cancer
TEAE	Treatment-emergent adverse event
TFAC	Paclitaxel (Taxol®), doxorubicin (Adriamycin™) and cyclophosphamide
TRANSCEND	**TRAN**slational Informatics **S**ystem to **C**oordinate **E**merging Biomarkers, **N**ovel Agents and Clinical **D**ata
UCSC	University of California, Santa Cruz
UCSF	University of California, San Francisco

List of Abbreviations *(continued)*

VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WBC	White blood cell

1. OBJECTIVES

1.1 Primary Objective

To determine whether adding investigational agents to standard neoadjuvant paclitaxel (with or without trastuzumab), and/or doxorubicin and cyclophosphamide, increases the probability of pathologic complete response (pCR) over standard neoadjuvant chemotherapy alone, for each biomarker signature established at trial entry, and to determine for each experimental agent used, the predictive probability of success in a subsequent phase 3 trial for each possible biomarker signature.

1.2 Secondary Objectives

1.2.1 Predictive and Prognostic Indices

To build predictive and prognostic indices based on qualification and exploratory markers to predict pCR and residual cancer burden (RCB).

1.2.2 Biological Specimen Resource and Imaging Data Base

To initiate the creation of a Biological Specimen Repository, consisting of tumor tissue, RNA, DNA, serum, and cells, as well as corresponding magnetic resonance (MR) and pathology images of these specimens for ongoing translational studies in genomics, proteomics, and imaging in order to establish their relationship to overall survival (OS).

1.2.3 Relapse-free Survival

To determine three- and five-year relapse-free survival (RFS) and OS among the treatment arms.

1.2.4 Investigational Agent Safety

To determine incidence of adverse events (AEs), serious adverse events (SAEs), and laboratory abnormalities of each investigational agent tested.

2. BACKGROUND

2.1 Breast Cancer

Breast cancer is diagnosed in almost 200,000 women annually in the United States alone; 45,000 women still die annually of this disease. Although many women now present with Stage I and II mammographically detected cancers and have excellent outcomes, 10–20% of newly diagnosed breast cancers present as locally advanced breast cancer (LABC) in which the risk of recurrence and death is significantly higher [1]. The absolute numbers of these cancers has not decreased over time [2] and successful treatment options remain limited. Women with LABC are distinct from those with screen-detected cancers. Up to 26% of LABC presents in women under the age of 40, prior to the age when screening is recommended [3]. In women who are being screened, the majority of women with LABC today (84%) present as "interval" cancers, where a palpable mass develops within one to two years of a normal screening mammogram [3]. Women with LABC represent a disproportionately large fraction of those who die from their disease. Since standard of care for these women increasingly includes neoadjuvant therapy prior to surgical resection, this population and setting represent a unique opportunity to learn how to tailor treatment for high-risk breast cancers.

The last decade of cancer research has shown breast cancer to be a heterogeneous disease, suggesting that directing agents to molecular pathways that characterize the disease in subsets of participants will improve treatment efficacy. Today, however, new breast cancer agents are first tested in phase 2 and 3 trials in the metastatic setting, followed by randomized phase 3 registration trials in the adjuvant setting. For the most part, these trials do not consider the specific molecular characteristics of the participant's disease. Moreover, adjuvant trials require long follow-up and many thousands of participants [4]. This process can typically take 15−20 years before marketing approval is gained for successful agents, and a substantial investment in time and resources is often put into agents that ultimately fail. The development and use of biomarkers for early measures of therapeutic response would facilitate the efficient evaluation of new agents in focused early clinical trials [5] and enable the development of more informed, smaller phase 3 trials. Although the use of biomarkers (molecular profiles, protein pathways, imaging, *etc.*) holds promise to enable the tailoring of agents to specific participant populations, developing translational approaches in clinical trials to predict agent response presents a major challenge.

2.1.1 Neoadjuvant Therapy for Breast Cancer

Development of multi-agent adjuvant chemotherapy regimens over the last two decades has substantially improved both disease-specific and OS outcomes for women with breast cancer [6]. The most effective adjuvant combination regimens include anthracyclines, such as doxorubicin or epirubicin (topoisomerase II inhibitors), the alkylating agent cyclophosphamide, and taxanes (currently docetaxel or paclitaxel), which are microtubule stabilizers. These different mechanisms of action often produce synergistic tumor shrinkage and avoid the development of resistance to single-agent treatment [7]. In the case of human epidermal growth factor receptor (HER2)-positive (HER2+) participants, a HER2 targeted monoclonal antibody, trastuzumab, has been shown to significantly improve survival when combined with a taxane-containing regimen [8]. Despite the gains in disease-free survival (DFS) and OS from these combination regimens, especially in hormone receptor-negative (HR–) participants[6], a substantial fraction of participants still relapse and die of breast cancer.

Although adjuvant therapy remains the mainstay of treatment for breast cancer, neoadjuvant chemotherapy is increasingly being used in women with large cancers or LABC. Several large trials have assessed the efficacy of neoadjuvant therapy when compared to standard adjuvant chemotherapy. A meta-analysis of 11 neoadjuvant trials was performed by the Early Breast Cancer Trialists Collaborative Group [9]. Preliminary results from this meta-analysis were presented at the National Cancer Institute (NCI)

neoadjuvant conference. Eleven randomized trials performed from 1981–1993, encompassing 4675 women, were included in the analysis. Preoperative therapy was associated with 18% fewer mastectomies and no significant difference in any breast cancer recurrence, breast cancer mortality, or death within 10 years of follow-up.

Two of these large randomized trials were undertaken by the National Surgical Adjuvant Breast and Bowel Project (NSABP) and provide the largest randomized data to date comparing preoperative to standard adjuvant chemotherapy. The NSABP B18 trial randomized 1523 women to either preoperative or postoperative doxorubicin/cyclophosphamide for a total of four cycles [10]. Breast tumor size was reduced in 80% of participants after preoperative therapy; 36% had a clinical complete response (cCR). The absolute pCR rate was 13%. Tumor size and clinical nodal status were independent predictors of cCR. Twenty-six percent of women with a cCR had a pCR. Clinical nodal response occurred in 89% of node-positive participants: 73% had a cCR and 44% of those had a pCR. There was a 37% increase in the incidence of pathologically negative nodes. Before randomization, lumpectomy was proposed for 86% of women with tumors ≤2 cm, 70% with tumors 2.1–5.0 cm, and 3% with tumors ≥5.1 cm. Clinical tumor size and nodal status influenced the physician's decision. Overall, 12% more lumpectomies were performed in the preoperative therapy group; in women with tumors ≥5.1 cm, there was a 175% increase. The NSABP-B27 trial was designed to determine the effects of adding docetaxel to preoperative doxorubicin and cyclophosphamide on breast cancer response rates and OS [11]. Women with operable breast cancer (n = 2411) were randomly assigned to receive preoperative doxorubicin and cyclophosphamide followed by surgery, doxorubicin and cyclophosphamide followed by docetaxel and surgery, or doxorubicin and cyclophosphamide followed by surgery and then docetaxel. Tamoxifen was initiated concurrently with chemotherapy. Median time on study for 2404 participants with follow-up was 77.9 months. Adding docetaxel to doxorubicin and cyclophosphamide did not significantly impact DFS or OS. There were trends toward improved DFS with the addition of docetaxel, which reduced the incidence of local recurrences as first events (p=0.0034). Preoperative, but not postoperative, docetaxel significantly improved DFS in participants who had a clinical partial response after doxorubicin and cyclophosphamide (hazard ratio = 0.71; 95% confidence interval (CI), 0.55–0.91; p=0.007). Thus, the primary benefit of neoadjuvant chemotherapy is to downstage tumors, thereby improving optimal surgical resection and increasing the probability of breast conservation [12].

However, an additional important finding in these studies was that the achievement of a pCR (*i.e.*, elimination of tumor in breast and axillary lymph nodes, as assessed at surgery) is a useful surrogate for prognosis in breast cancer participants overall, suggesting that chemotherapy sensitivity, in and of itself, is an independent predictor of DFS and OS. pCR, which occurred in 27% of participants in NSABP B-27, was doubled by addition of preoperative docetaxel, and was a significant predictor of OS regardless of treatment (hazard ratio = 0.33; 95% CI, 0.23–0.47; p<0.0001). Pathologic nodal status after chemotherapy was a significant predictor of OS (p <0.0001). The pCR rates of doxorubicin-containing regimens are in the range of 12%; combining doxorubicin and taxanes leads to pCR rates in the 25–27% range. However, the prognosis is still poor for those participants who present with very large tumors and those with significant residual disease in breast or lymph nodes [13–16]. Moreover, recent data that have incorporated molecular phenotyping into the classification of tumors undergoing neoadjuvant therapy have shown that response to treatment differs significantly by phenotype, as does the suitability of pCR as a useful surrogate [17]. Participants with strongly estrogen receptor-positive (ER+) tumors (those of the luminal A subtype) may have a low pCR rate, but subsequently have a favorable prognosis due to both the indolent natural history of the disease and the responsiveness of these tumors to anti-estrogen therapies. Conversely, many participants with triple-negative breast cancer (*i.e.*, ER/progesterone receptor (PgR)/HER2– by immunohistochemistry (IHC), or basal by molecular phenotyping) may have an excellent response to neoadjuvant chemotherapy, likely due to the high proliferative rate of these tumors. However, many of these participants subsequently relapse, and salvage strategies are lacking.

The optimal combination, sequencing, and schedule for neoadjuvant chemotherapy have not been established. In general, any regimen that is appropriate for *adjuvant* chemotherapy is also appropriate as a *neoadjuvant* regimen, and should be given in the same dose, combination, and schedule. For example, four cycles of doxorubicin/cyclophosphamide followed by paclitaxel is a common regimen for women with HER2/*neu* non-overexpressing LABC, and the addition of trastuzumab on a weekly schedule in combination with paclitaxel has been shown to be safe and effective [18]. However, several studies have examined variations to the standard regimen, with or without trastuzumab. The Eastern Cooperative Oncology Group (ECOG) 1193 trial compared doxorubicin followed by paclitaxel to the reverse sequence of paclitaxel followed by doxorubicin; no difference was observed in response rate [19]. ECOG subsequently performed a pilot trial in HER2 overexpressing early-stage breast cancer participants that added trastuzumab to weekly paclitaxel for 12 cycles followed by standard doxorubicin/cyclophosphamide (E2198). There was no safety signal suggesting increased cardiac or other toxicity with this sequence; the rate of clinical congestive heart failure was 3% [20]. Thus, clinical data to date suggest that the sequence of taxane and doxorubicin is of little importance. Interestingly, preclinical data have suggested that paclitaxel-induced inhibition of heat shock proteins and upregulation of topoisomerase II may lead to sensitization of tumor cells to doxorubicin [21].

2.2. The I-SPY TRIAL

The **I**nvestigation of **S**erial Studies to **P**redict **Y**our **T**herapeutic **R**esponse with **I**maging **A**nd mo**L**ecular analysis (I-SPY TRIAL) was designed to integrate clinical, laboratory and bioinformatics investigators in a new model to evaluate neoadjuvant chemotherapy in the setting of LABC—bringing together data from multiple molecular biomarker studies with imaging. The intent was to evaluate and develop biomarkers of early response to standard chemotherapy, and to develop a strategy to improve outcomes of women who do not have an optimal response to current standard therapy. The I-SPY TRIAL was designed as an inter-SPORE collaboration (NCI Specialized Programs of Research Excellence), with two cooperative clinical trial groups, the American College of Radiology Imaging Network (ACRIN), the Cancer and Leukemia Group B (CALGB), and the NCI Center for Biomedical Informatics and Information Technology (CBIIT) (protocols ACRIN 6657, CALGB 150007/150012). All participants received neoadjuvant chemotherapy to test a comprehensive panel of biomarkers, including MR imaging (MRI), for their ability to predict tumor response. Early endpoints being tested as predictors of three-year survival included MR changes (volume and longest diameter) and changes in gene expression. The intermediate endpoint was pCR (absence of invasive tumor in breast or lymph nodes at time of surgery), and the longer term endpoint was three-year RFS.

2.2.1. Results

Mechanics and demographics: Starting in the summer of 2002, 237 participants were enrolled in the I-SPY TRIAL. Of these, 216 successfully completed the trial and were considered eligible for evaluation. The participant population in I-SPY was diverse: 75% Caucasian, 19% African American, 4% Asian, and 2% other [22]. All treatment started with four cycles of doxorubicin; 95% of participants proceeded to a taxane regimen of four cycles. Serial biopsies, serum samples, and MR images were obtained prior to therapy, within two weeks of starting chemotherapy, between regimens, and at the time of surgical resection. We optimized methods of efficiently collecting and distributing tissue, and optimized the number and types of assays that could be performed. Expression array analysis was conducted on 149 participants on the Agilent 44K array, 118 participants on the Affymetrix U113A array, and 141 participants on a spotted complementary DNA (cDNA) array. Comparative genomic hybridization (CGH) arrays were conducted on 158 participants, and reverse-phase tissue protein lysate arrays on 149 participants[23]. Assay yield improved significantly by the end of the trial. Adding image guidance improved the yield of evaluable biopsies from 85% to 95%, depending on the type of imaging used [24].

Results: Of the 237 participants accrued in the I-SPY TRIAL, 215 participants had pathologic assessment available for analysis. RCB was calculated retrospectively on 201 participants (RCB calculator: http://www.mdanderson.org/breastcancer_RCB) [16]. RNA Agilent arrays were available on 149 participants. Participants that comprised the I-SPY population were biologically high risk, as defined by the Netherlands Cancer Institute 70-gene signature (NKI 70)-gene profile, with 91% of participants characterized as having a poor prognosis [22]. The mean tumor size was 6.0 cm and minimum size was 3.0 cm. The rate of pCR was 27%; 36% had RCB 0 or 1. Both pCR and RCB were predictive of three-year RFS with 3.9 years mean follow-up (p=0.04 and 0.01, respectively). HR and HER2/*neu* receptor (HER2) status were highly predictive of rates of pCR, ranging from 10% for HR+/HER2– to 50% for HR–/HER2–. The pCR rates for the intrinsic subtypes ranged from 2% to 50% as shown below in Table 2.1 [23].

Table 2.1 pCR, RCB, and Three-year RFS by Molecular Subtypes

IHC	Distribution (n = 194)	pCR (n = 188)	P-value	RCB (0 or 1) (n = 133)	P-value	3-yr RFS (n = 188)
HR+HER2-	48%	10%		21%		86%
HR+HER2+	12%	32%		48%		82%
HR-HER2+	12%	50%		79%		75%
HR-HER2-	28%	33%		41%		68%
Gene Profiles	**Distribution (n = 149)**	**pCR (n = 144)**	**P-value**	**RCB (0 or 1) (n = 133)**	**P-value**	**3-yr RFS (n = 144)**
Intrinsic Subtypes						
Luminal A	29%	2%		11%		97%
Luminal B	19%	15%		19%		80%
HER2-enriched	15%	52%		76%		90%
Basal	32%	34%		41%		59%
Normal-like	5%	43%	4.0 x 10-5	57%	7.5 x10-6	71%
ROR-S						
Low	26%	5%		19%		100%
Moderate	38%	22%		29%		80%
High	37%	40%	8.8 x 10-4	51%	0.0078	65%
NKI						
Good Outcome	9%	0%		18%		100%
Poor Outcome	91%	27%	0.038	37%	0.33	77%
Wound Healing						
Quiescent	23%	6%		30%		100%
Activated	77%	30%	0.0049	37%	0.52	73%
p53 Mutation Gene signature						
Wildtype	50%	11%		24%		93%
Mutation	50%	38%	3.7 x 10-4	46%	0.011	66%

The low-risk subsets had low rates of pCR and RCB, whereas high-risk subsets had high rates of pCR. DNA profiles showed similar findings in that high-risk subsets had high rates of pCR.

The most important finding is that pCR and RCB have very different abilities to predict outcome depending on the context of molecular profiles. For those participants showing favorable profiles, the outcome was good, regardless of RCB. For poor-risk profiles, pCR and especially RCB are predictive of outcome. This is true for the NKI 70-gene poor-risk profile, basal [24], wound healing (activated) signature (Figures 2.1, 2.2, and 2.3), and p53 mutation signature.

Figure 2.1: RCB as a Predictor of RFS Among NKI High-risk Participants

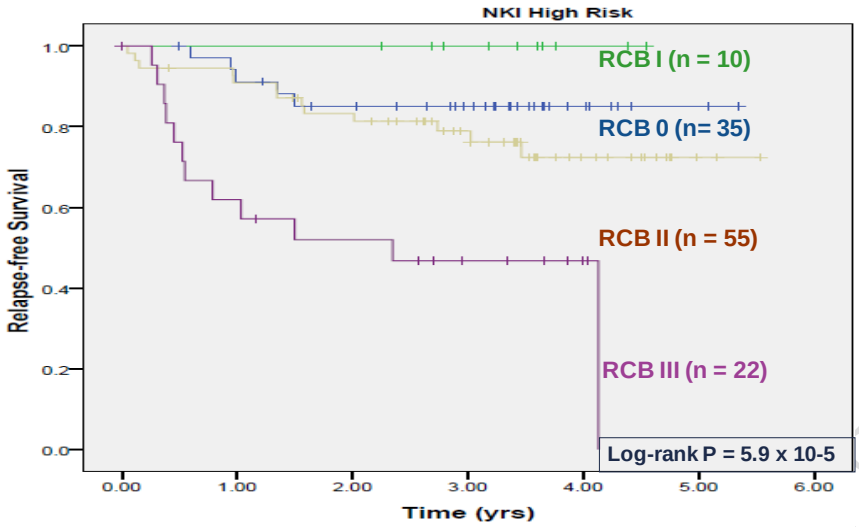


Figure 2.2: RCB as a Predictor of RFS Among Tumors with Wound Healing (Activated) Signature

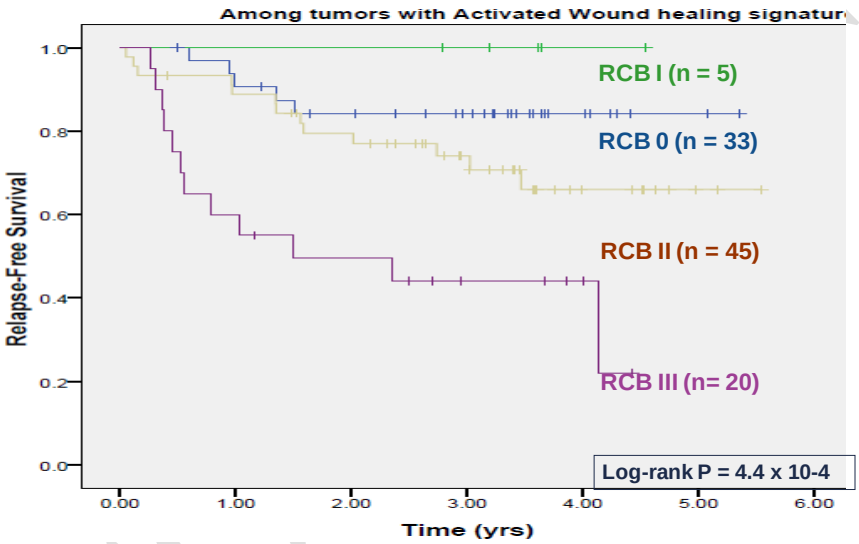
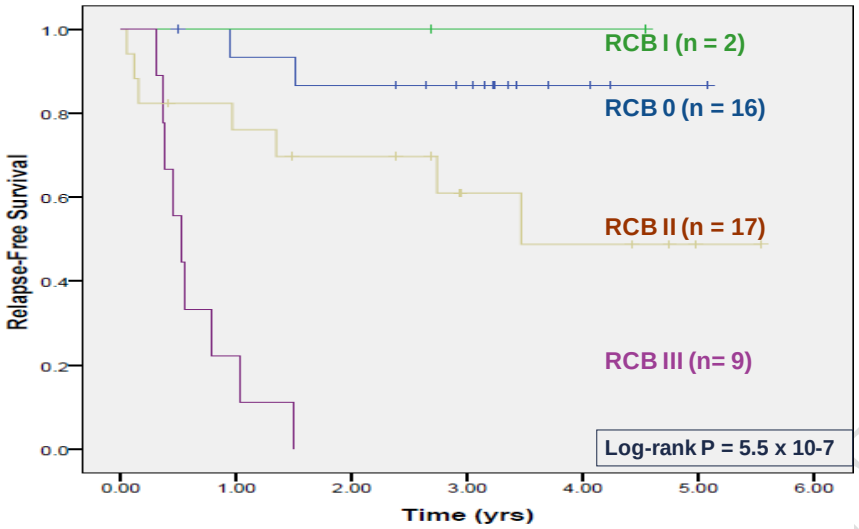


Figure 2.3: RCB as a Predictor of RFS Among Basal-like Tumors



There was considerable variation in how the RNA signatures classified participants as low- and high-risk. A composite signature to identify robust low- and high-risk subsets was developed, assigning a score of 1 or –1 to signatures with two categories (wound healing activated vs. quiescent; NKI 70-gene signature low-risk vs. high-risk) and a score of 1, 0, or –1 when three categories were defined (risk of recurrence (ROR)-S low vs. medium vs. high). The low-risk category has excellent outcomes, regardless of RCB. Intermediate and poor-risk molecular profile categories have significantly worse outcomes (Figure 2.4) (Van't Veer and Das, submitted).

Figure 2.4: Integrated Scoring as a Predictor of RFS

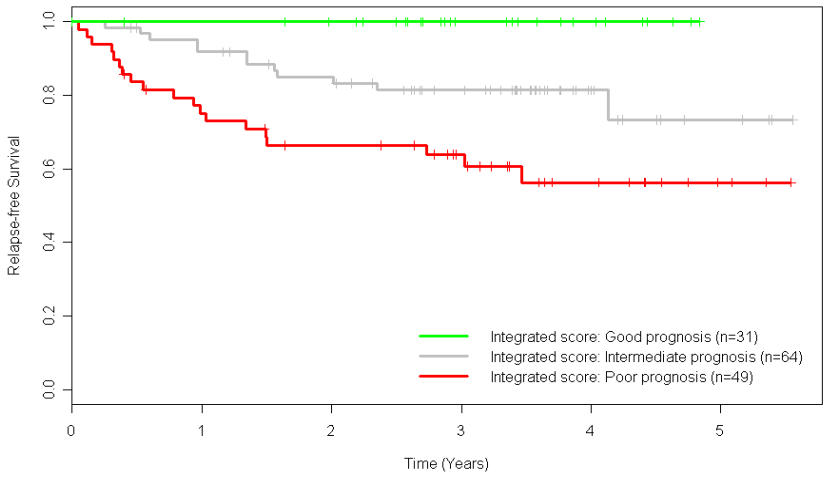
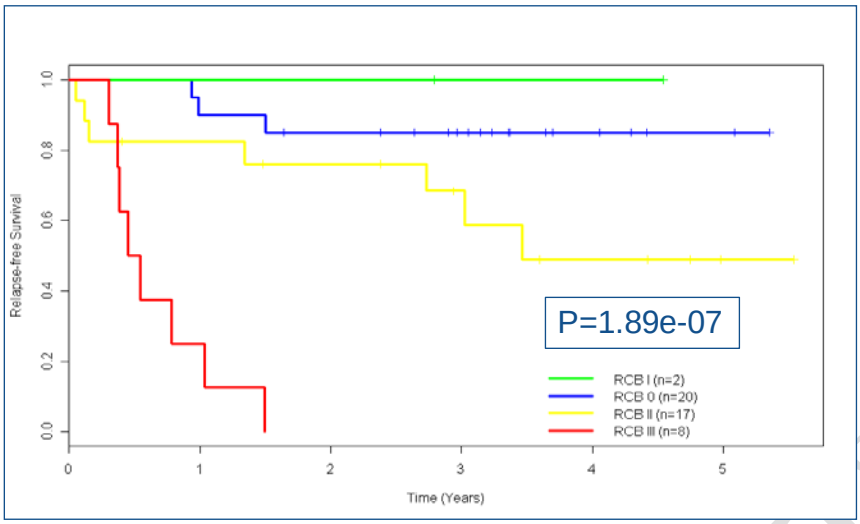


Figure 2.5: Integrated Score for Poor Prognosis Participants Associated with RCB



RCB in the poor-risk subsets is highly predictive of three-year RFS (Figure 2.5).

Given these data, we have elected to use the NKI 70-gene profile to exclude participants with a good prognosis. Those participants with a good prognosis by NKI 70-gene profile signature have an excellent outcome early on, and no participants with this profile had pCR. The selection of the poor-risk participants focuses on those participants in whom response to therapy is very predictive of outcome and who have a high risk for recurrence early in the course of their disease (Figure 2.5).

I-SPY 1 data show that improvement in pCR or RCB may be a rapid way to screen for the effectiveness of promising new targeted therapeutics. Moreover, it is anticipated that the most informative way to interpret the results will be by combining pCR and RCB evaluations with molecular subgroup analysis.

Of note, seven recurrences were reported among those who achieved pCR. Five of seven were HER2+ participants who were not treated with trastuzumab, since they were treated prior to the time that trastuzumab was clinically available in the adjuvant or neoadjuvant setting. An equivalent number of participants have now been given trastuzumab in the neoadjuvant setting, and there have subsequently been no early recurrences in the HER2+ group among participants with RCB = 0 or RCB = 1.

MRI volume is the imaging measure that best correlates with residual tumor in the breast, and change in MR volume (early and overall) is a strong predictor of pCR [25–27]. This supports the ACRIN 6657 (I-SPY TRIAL) hypothesis that MR volume can serve as a non-invasive measure of tumor burden and aid in evaluating response to therapy. Response to therapy was accurately captured by MR volume change among those participants who received trastuzumab in combination with taxane. Based on these data, we hypothesize that MR volume change can help us non-invasively determine response to investigational agents with chemotherapy.

The I-SPY TRIAL demonstrated that a collaborative group of investigators could effectively integrate biomarkers and imaging into the course of care by agreeing on standards for data collection, biomarker assessment, and MRI evaluations. The group also developed and shared methods to optimize assays; devised ways to obtain small amounts of frozen core biopsy material; agreed to use already established data-based tools for tissue tracking; as well as developed and utilized common platforms for information management and tissue repositories [2, 24, 28–30]. This robust infrastructure will be leveraged to support

this trial as further described in the **TRAN**slational Informatics **S**ystem to **C**oordinate **E**merging Biomarkers, **N**ovel Agents and Clinical **D**ata (TRANSCEND) Users Manual.

I-SPY 1 Infrastructure: The I-SPY TRIAL was one of the first prospective trials in which all data from participating laboratories was submitted electronically to a common database and centrally housed at the NCI CBIIT using a Cancer Biomedical Informatics Grid (caBIG) tool, caINTEGRATOR. The purpose of caINTEGRATOR was to enable cross-platform comparison among participants and to increase our understanding of the molecular heterogeneity of LABC for all assays performed [31]. We implemented a new strategy for biomarker discovery and data sharing, enabling access to the data for all investigators to accelerate the pace of learning. Once accrual of participants and samples was complete, outside investigators could submit requests to the I-SPY TRIAL Publications Committee (for samples or data) to conduct additional analyses and discoveries. All investigators were required to submit their data to caINTEGRATOR, thereby continuing the growth and enrichment of the data set. Over the course of three and a half years, 10 clinical centers accrued the participants. To date, over 15 laboratories have contributed data. Currently, clinical and laboratory data are submitted to caINTEGRATOR. The collection and aggregation of the data has enabled us to build upon our knowledge base as well as use novel analytical tools such as the Cancer Genome Browser [32].

2.3 Rationale: Neoadjuvant Adaptive Design Approach

Studies of neoadjuvant chemotherapy in Stage II and III breast cancer participants suggest that some have significant benefit from chemotherapy while others appear to derive much less value. Because breast cancer is a genetically and clinically heterogeneous disease, the ability to identify markers that predict early responders to standard chemotherapy and long-term survival would markedly improve the breast cancer treatment paradigm. A variety of histopathologic, genomic, proteomic, and imaging strategies have the potential to predict response to standard therapy and to provide a framework for testing investigational-targeted agents in the context of unique molecular subtypes of breast cancer. Rational matching of investigational agents with cohorts of participants whose disease characteristics suggest they might benefit from this “personalized” therapy requires an understanding of fundamental regulatory pathways that control breast cancer pathology as well as development of validated assay methods to reproducibly identify tissue or serum markers that predict response. The neoadjuvant setting provides the perfect opportunity to target agents to the biology of specific signatures and to rapidly assess the impact of these agents during the course of chemotherapeutic exposure, with confirmation of response at the time of surgical excision. Further, integrating imaging enables a non-invasive way of measuring response and accelerating learning about specific response to treatment.

The infrastructure of the I-SPY TRIAL enables us to take an important step toward systematically assigning phase 2 agents and rapidly learning about the impact of these agents on participants based on specific molecular characteristics (signatures) of their tumors. As stated previously, women who present with LABC are at high risk for recurrence; however, unlike women with metastatic disease, they are still potentially curable. A large number of phase 2 biologically targeted therapies need to be efficiently evaluated. Fortunately, most of them do not appear to be toxic, even in combination with chemotherapy. Further, emerging data suggests that these agents will be most efficacious in combination with chemotherapy. It is absolutely critical that we shorten the knowledge turns and lifecycle for evaluating new agents [33], and get the most promising agents to those most likely to benefit. Introducing these agents in the neoadjuvant setting, where we have a short-term (six-month) intermediate endpoint to assess efficacy, will provide the proper time horizon for agent evaluation. Furthermore, the introduction of these agents to women with curable high-risk disease carries the promise of improving survival rates in women most at risk of death due to their disease. However, the classic method of randomized trials evaluating one agent at a time for a set number of individuals is still inefficient, and will not allow us to rapidly learn for whom the new agents are most effective. To address this problem, the I-SPY 2 TRIAL team, in

collaboration with the NCI, Food and Drug Administration (FDA), and the Foundation for the National Institutes of Health (FNIH) Biomarker Consortium, collaborated to design an adaptive randomized phase 2 trial based on biomarker signatures where multiple agent classes can be evaluated simultaneously on a backbone of molecularly profiled participants. The trial process we present is explicitly intended to eliminate some of the enormous inefficiencies in our current trial designs.

Using Bayesian methods of adaptive randomization [34], agents will be assigned to participants who have higher probability of efficacy. Therefore, agents which show the appropriate beneficial changes within a specific molecular signature will be preferentially assigned within that signature and move through the trial more rapidly. Agents that do not show the likelihood of improved pCR rate in any predefined biomarker signature will be dropped from the trial. Each agent's Bayesian predictive probability [34] of being successful in a phase 3 confirmatory trial will be calculated for each possible signature. Agents will be dropped from the trial for futility when this probability drops sufficiently low for all signatures. Agents will be graduated at an interim point should this probability reach a sufficient level for one or more signatures. Those agents with high Bayesian predictive probability of being more effective than standard therapy will graduate along with their corresponding biomarker signatures, allowing these agent-biomarker combinations to be tested in smaller phase 3 trials. At graduation, an agent's predictive probability will be provided to its sponsoring company for all signatures tested. Depending on participant accrual rates, new agents can be added at any time during the trial to replace the agents that are dropped or graduated.

The Biomarkers Consortium of FNIH supported the development plan for this adaptive design protocol in breast cancer based on promising results to date of the I-SPY TRIAL. FNIH was established by the US Congress to support the mission of the National Institutes of Health (NIH). The mission of FNIH is to foster public health through scientific discovery, translational research, and dissemination of research results through specially configured, high-impact public-private partnerships consistent with NIH priorities. The Biomarkers Consortium is a collaborative public-private partnership managed by FNIH in an effort to create fundamental change in how healthcare research and medical product developments are conducted. This expectation is being accomplished by bringing together leaders from biotechnology and pharmaceutical industries, government, academia, and non-profit organizations to work together to accelerate identification, development, and regulatory acceptance of biomarkers.

2.4 Study Agent Rationale

The I-SPY 2 TRIAL investigational agents are described in appendices D–x. As investigational agents are added to the trial, they will appear as subsequent appendices.

2.4.1 Agent Selection

The initial process of candidate agent review began with the I-SPY 2 Agent Selection Working Group. This group consisted of I-SPY oncologists, phase 1 and 2 trialists, and interested industry representatives, who generated an initial list of agents that would be potentially appropriate for I-SPY 2. The list included agents targeting biologic pathways thought to be upregulated in breast cancer, including HER2 (*e.g.*, HER2 monoclonal cytotoxin conjugates, pan-Erb2 inhibitors), insulin-like growth factor (IGF)-1 receptor, phosphatidylinositol-3-kinase (PI3K), mTOR, cMET, apoptosis (BCL-2 inhibitors), angiogenesis inhibitors, DNA damage repair mechanisms (polyadenosine diphosphate ribose polymerase [PARP] inhibitors), and the death receptor pathway (Apo2L/TRAIL agonists). The list was evaluated in a series of teleconferences during 2008 and the agents under development were reviewed for their potential to improve breast cancer outcomes. From an initial nominated list of >65 agents within at least 10 major molecular pathway target classes, 20 priority agents were selected for further detailed assessment. A summary list of the agents, their targets, and molecular signature targets (*e.g.*, HER2+ *vs.* HER2–) was

made available in a follow-up meeting with additional pharmaceutical company representatives, and further comments on the selections were invited. Literature reviews were prepared and current development status details, including efficacy and safety information and ongoing or planned clinical trial data, were solicited from the relevant pharmaceutical companies. Data were assembled into worksheets on each agent. First consideration was given to agents that had completed phase 1 safety testing in combination with a taxane, and clinical evidence or preclinical rationale for activity against breast cancer. The non-industry members of the Agent Selection Working Group conducted a complete review and the committee members recommended to either Approve (high, medium or low priority) or Reject (secondary to safety) the selections. These agents were then sent to the Independent Agent Selection Committee (IASC).

Final prioritization of Tier 1 agents is made by the IASC, whose members come from industry, academia, research institutions, foundations, and patient advocacy groups not directly involved in the trial. An agreement at the outset of the design process was made to test or select only one agent within a therapeutic class (*e.g.*, only one IGF receptor (IGFR)-1 antibody-based inhibitor will be tested).

2.4.2 Designation of Tier 1 *versus* Tier 2 Agents for the I-SPY 2 TRIAL Process

Over the course of the trial, agents will be dropped or graduated and new agents will be needed to replace them. For this reason, agents need to be qualified for the trial over the course of the study period. Those agents ready for use when the trial opens are designated as Tier 1 agents. Tier 1 agents must have appropriate safety data alone and in combination with a taxane. Tier 2 agents are promising agents in the process of going through phase 1 testing alone or in combination with taxanes that will be evaluated for appropriateness during the course of the trial as data in combination with paclitaxel become available.

2.4.3 I-SPY 2 Investigational Tier 1 Agents

The Tier 1 agents being tested include agents that target HER2, IGF-IR, angiogenesis pathways, DNA damage repair mechanisms, and death receptors. After each agent, the signature being targeted is listed (See Appendix C–x). For HER2 targeted agents or agent combinations, if there are phase 2 data suggesting efficacy equal to or greater to that of trastuzumab or a combination of paclitaxel plus trastuzumab, the investigational agent or agent combination will be used in place of trastuzumab or paclitaxel plus trastuzumab; without such data, agents or agent combinations will be used in addition to paclitaxel plus trastuzumab.

For each Tier 1 agent, we have organized the presentation of data to include:

- MECHANISM
- IN VITRO STUDIES
- ANIMAL STUDIES
- HUMAN STUDIES, including phase 1 data and phase 1 data in combination with taxanes
- ONGOING STUDIES
- TOXICITY and SAFETY
- PHARMACOKINETICS

Each Tier 1 agent has an assigned Chaperone and co-Chaperone. Chaperones are assigned by the Agent Selection Working Group chairs, and are selected based on their preclinical and/or clinical experience with a specific pathway and/or agent. Chaperones are responsible for overseeing the agent/agent combination within the trial (*i.e.*, participate in the development and maintenance of the agent appendix and overall safety of the participants receiving the agent).

Please refer to Appendices D–x for detailed information for the I-SPY 2 tier 1 investigational study agents.

Example: Agent X, Specifications for Use

Target: e.g., *HER2, angiogenesis pathway, etc.*
Signature: e.g., *HER2+, ER+, – – –*
Schedule: *Given weekly with paclitaxel*
Trastuzumab: e.g., *Used in place of trastuzumab or in addition to trastuzumab*
Manufacturer: *List pharmaceutical manufacturer*

2.5 General Approach in Evaluating Agent(s) and Biomarkers

I-SPY 2 TRIAL will examine the efficacy of at least ten investigational agents/agent combinations in women with locally advanced Stage II or III breast cancer. The randomization and agent assignments will be based on the MammaPrint 44K Array, HR, and HER2 status. Each participant randomized to an experimental arm will be assigned to one investigational agent (plus paclitaxel/trastuzumab) until protocol completion or removal; re-randomization is not planned under the current proposed design. An important objective of the study is to identify MR imaging and molecular characteristics predictive of pCR and survival in these participants. The goal is to determine an optimal biomarker profile for each experimental regimen being considered, and to graduate these regimens from the trial into phase 3 pivotal studies. Regimens will be dropped for a specific profile if they are not sufficiently effective from that profile.

The Master Investigational New Drug (IND) application will be amended to include additional investigational agents/agent combinations as updated safety and efficacy information becomes available and initial treatment groups are dropped for futility or graduated. Investigational Device Exemptions (IDEs) for Agendia's MammaPrint 44K Array, Agendia's TargetPrint HER2 44K Array, and for Hologic's MR volume Aegis software are included as part of the Master IND. Additional biomarker assessment methods proposed for qualification include protein assays such as IHC/fluorescence *in situ* hybridization (FISH) as well as reverse phase protein microarrays (RPMA) specific for targeted pathways (to identify pathways driving participant's tumor); mRNA array assay for agent and prognostic predictors; and Affymetrix or Agilent gene expression arrays (*e.g.*, paclitaxel (Taxol®), doxorubicin (Adriamycin™) and cyclophosphamide (TFAC) RCB) for prediction of response. Under the proposed study plan, IDEs for these biomarkers may also be prepared for submission to the Office of *In Vitro* Diagnostics (OIVD) as the data are generated. Other possible exploratory biomarkers include DNA methylation, DNA sequencing, genome-wide association studies (GWAS), pharmacogenomics, and microRNAs, as well as blood/serum/plasma/cell-based assays evaluating tumor cell or proteins in circulation.

Access to I-SPY 2 TRIAL data and biospecimen repository is governed by the I-SPY 2 Data Access and Publications Committee (DAPC). Researchers interested in obtaining access to the I-SPY 2 dataset for analysis should submit a completed concept sheet to the DAPC. Concept sheets can be obtained at www.ispy2trial.org. Those researchers interested in evaluating a biomarker platform in the I-SPY 2 TRIAL will be designated the Platform Chaperone once their concept sheet is approved by the DAPC. The Platform Chaperone will have continued involvement with other I-SPY 2 researchers interested in utilizing their platform; however, the Platform Chaperone will not own the data obtained by the other I-SPY 2 researchers. Requests for biospecimens are sent to the I-SPY 2 Biomarker Committee who will review and recommend requests for biospecimens to the I-SPY 2 DAPC for final approval.

3. SUMMARY OF STUDY PLAN

3.1 Screening Phase

I-SPY 2 TRIAL is a neoadjuvant trial making use of adaptive design to identify successful treatment regimens for Stage II/III breast cancer. Women with ≥2.5 cm invasive breast cancer by palpation or imaging are eligible for study screening (see Schema, Figures A and B). Tumor ER and PgR status, and HER2 by community IHC and/or FISH conducted at a local laboratory, will be done as part of a routine diagnostic work-up. After the participant consents to be screened, a core biopsy will be performed, and sections will be sent to Agendia for MammaPrint score and TargetPrint HER2 gene expression assay using the Agendia 44K full genome microarray (MammaPrint 44K Array Low and High scores are determined per FDA label for the cleared MammaPrint device). A tumor will be considered HER2+ if any one of the three assays (IHC, FISH, TargetPrint) is positive (as defined in §7.3). Participants will also undergo a pretreatment MRI for determination of maximum tumor dimension.

Women who are low risk by MammaPrint 44K Array and also ER+ and HER2– will be excluded from the trial as shown in Table 3.1.

Table 3.1 Table of Eligibility for Randomization

	MammaPrint Low*		MammaPrint High*	
	ER+	ER–	ER+	ER–
HER2+	Eligible	Eligible	Eligible	Eligible
HER2–	Not Eligible	Eligible	Eligible	Eligible

*MammaPrint 44K Array Low and High are determined per FDA label for the cleared MammaPrint device

NOTE: Participants not eligible to participate in the treatment phase of I-SPY 2 because they are ER+, HER2-, MammaPrint Low are eligible to participate in the Low-risk Registry Trial (see Supplement 1).

Participants eligible for randomization will be recategorized according to biomarker profiles shown in Table 3.2. A participant's ER and PgR status are used to determine their HR status. For instance, ER+ and PgR– is HR+; ER– and PgR+ is HR+. Additionally, a participant's MammaPrint 44K Array Low/High score is adjusted to either the MammaPrint High1 or High2 class. MammaPrint High1 (MP–) and High2 (MP+) classes were determined by the pre-defined median cut-point of I-SPY 1 participants who fit the eligibility criteria of I-SPY 2.

Table 3.2 Biomarker Profiles for Treatment

	MammaPrint High1*		**MammaPrint High2***	
	HR+	HR–	HR+	HR–
HER2+	Eligible	Eligible	Eligible	Eligible
HER2–	Eligible	Eligible	Eligible	Eligible

*MammaPrint High1 and MammaPrint High2 are determined by the predefined median cut-point of I-SPY 1 participants who fit the eligibility criteria of I-SPY 2.

These biomarker profiles are used for randomizing each participant to a treatment arm (see Schema, Figure D). For every participant that is randomized, there is a 20% chance the participant will be

randomized to the control arm (paclitaxel or paclitaxel plus trastuzumab and pertuzumab if HER2+), regardless of how many investigational agents are in the study. Each investigational agent will have an initial biomarker signature that will determine which participants will be randomized to that investigational agent. A biomarker signature as predefined by the agent manufacturer can range from a maximum of all participants to a more limited signature (see §13.2). For example, participants with tumors considered to be HER2+ will receive paclitaxel plus trastuzumab (Herceptin) and pertuzumab (Perjeta) as part of the control arm, and HER2-directed agents in the experimental arm, where they will receive paclitaxel plus trastuzumab plus new agent A; paclitaxel plus trastuzumab plus new agent B; or paclitaxel plus trastuzumab plus new agent C. If a new agent being considered has phase 2 data showing equivalent or improved efficacy to trastuzumab or paclitaxel plus trastuzumab, the new agent will replace trastuzumab or paclitaxel plus trastuzumab in the experimental arm. The HER2– group will be randomized to receive either weekly paclitaxel alone or weekly paclitaxel plus new agent C; paclitaxel plus new agent D; or paclitaxel plus new agent E.

Due to the accelerated approval of pertuzumab in the neoadjuvant setting, the HER2+ control arm (paclitaxel plus trastuzumab) has been temporarily closed in accordance with the recommendations of the I-SPY 2 DSMB and Investigators. Patients with HER2+ disease randomized to control will receive paclitaxel plus trastuzumab and pertuzumab, this arm is known as the "Bridging control arm."

3.2 Treatment Phase

Investigational agents will be given in 12 weekly intervals or at other intervals over a 12-week period. After a participant completes three weekly cycles or one three-week cycle of therapy, she will undergo a repeat MRI, core biopsy of the tumor, and blood draw. She will continue treatment for nine more weekly cycles (or nine weeks for a total of 12), and undergo a third MRI and blood draw. She will then receive four cycles of doxorubicin and cyclophosphamide at two- or three-week intervals prior to surgery. The participant will have an MRI and blood draw prior to surgery, and tumor tissue will be collected at surgery. The primary endpoint is pCR (defined as absence of invasive tumor in breast or lymph nodes at the completion of all neoadjuvant chemotherapy). A more complex and detailed pathologic evaluation, RCB [16], will be used to evaluate surgical specimens. RCB is estimated from routine pathologic sections of the primary breast tumor site and the regional lymph nodes after completion of neoadjuvant therapy. Six variables are included in a formula including tumor bed size, cellularity, and extent of the disease in the breast and nodes; it is calculated using automated software (www.mdanderson.org/breastcancer_RCB). RCB is potentially a better predictor of five-year RFS [16].

3.3 Biomarkers

MRI will be used as a noninvasive serial measurement of response during the course of treatment; MRI longest diameter and volume will be measured at each time point and will be used for early evaluation of response. MRI volume measured just prior to surgical resection has been correlated with residual tumor; change in MRI volume was well correlated with pCR in I-SPY 1. MRI volume is an automated measurement which will be obtained directly from the MRI workstation using a CAD software system by Hologic. As part of a joint R-01-Small Business Innovation Research Grant under the auspices of Dr. Nola Hylton (Principal Investigator, ACRIN 6657, I-SPY TRIAL), MR software workstations will be placed at all participating I-SPY 2 sites.

Peripheral blood samples will be collected pretreatment, early in paclitaxel treatment (end of week 3), inter-regimen, and presurgery. Tissue samples will be collected from core biopsies done pretreatment and early in paclitaxel treatment (end of week 3), and surgical tissue will be collected at the time of surgery if sufficient tumor remains. Tissue obtained during the screening phase of the trial will be used to generate molecular profiles for initial randomization assignment and to generate further qualifying biomarkers,

including gene and protein measurements by Agilent 44K and Affymetrix arrays, mRNA arrays, and RPMA assays. These molecular characteristics (biomarkers/pathways) will be used to correlate with pathologic, imaging, or RCB response measures in the neoadjuvant setting with the investigational therapeutic agents. Tissue and blood will be used for exploratory research and to generate molecular data on next-generation technology platforms. Finally, participants will be followed post surgery for five years for recurrence-free and overall survival.

Over the course of the trial, additional qualifying biomarkers will be put forward and tested for their ability to predict tumor response to specific classes of investigational targeted therapeutics, and exploratory biomarkers that have future promise for better stratifying tumor type and response will also be incorporated into the trial (see §7 for details).

3.4 Adaptive Randomization of Investigational Agents

Agents will be assigned to all signatures where they might be effective. The control arm will apply to all profiles. Randomization probabilities are determined based on the accumulating data about all agents in the trial. The trial is designed to study over time which profiles predict response to each agent. Each agent's probability of being successful in a phase 3 confirmatory trial will be calculated:

- Agents will be graduated at an interim point should one or more of these probabilities reach a sufficient level.
- Agents will be dropped from the trial for futility when probabilities drop sufficiently low.

If the maximum sample size of 120 participants assigned to a regimen (over all biomarker types) is reached, assignments to the regimen will end. (With the exception if assignment of a regimen is to be restricted to patients with tumors that are either HER2+ or HER2– then the maximum total sample size for that regimen is 75.)

If an investigational agent reaches a threshold for graduation, the Data Safety Monitoring Board (DSMB) will review the findings and make a recommendation to Study Principal Investigators (PIs) for final approval. During the review by the DSMB and PIs, participants will continue to be randomized to the regimen or agent. Once the agent graduates, no additional participants will be randomized to that agent. Participants currently receiving that agent will continue on the regimen until they complete the entire course of treatment.

If the maximum sample size of 120 participants (75 patients if a regimen is restricted to patients with HER2+ or HER2-) is reached, no additional participants will be randomized to that agent. Participants currently receiving the agent will continue on the regimen until they complete the entire course of treatment.

If an agent is found not to reach a specified threshold of improvement in response, it may be dropped for futility; the DSMB will review the findings, and if they agree, will recommend to the PIs that the agent be dropped from the trial. During review by the DSMB and PIs, no participants will be randomized to that regimen or agent. Participants who have not completed the course of the agent will continue to receive the agent until a determination is made. Once an agent is dropped from the trial, the option to continue or drop the agent will be at the discretion of the participant and her treating physician. Participants who do not continue on the agent will continue on-study but will revert to the standard/control regimen; their outcomes will remain part of the arm to which they were assigned.

If an investigational agent is removed from the trial due to serious side effects from the agent, use of that agent for all participants will be stopped. Participants will continue on-study but will revert to the standard/control regimen and their outcomes will remain part of the arm to which they were randomized.

The above assignment and stopping rules for randomization apply to all regimens in the trial irrespective of study entry.

Up to eight investigational agents will be active at any given time; the first set of agents is currently being selected. The number of agents considered will be restricted by the ability to "process" the trial agents expeditiously in order to give companies timely information concerning the potential role of the agent in treating breast cancer. Trial data will also be used to test, qualify, and validate biomarkers as predictors of response to specific therapeutic agents. This trial is an opportunity to integrate information from emerging biomarkers and thereby accelerate identification of optimal therapies for women at highest risk of progression.

Randomization will be adaptive to maximize information about better-performing therapies and minimize the time it takes to identify optimal biomarker profiles. Using MRI results as a biomarker during various stages of treatment, our design will build a longitudinal model of tumor response. Such a model is critical for the adaptive aspect of the trial to enable early assessment of therapeutic benefit. The number of participants required to evaluate each investigational agent will range from a of 20 participants to a maximum of 120 (75, if assignment of a regimen is restricted to patients with tumors that are either HER2+ or HER2–).
We anticipate evaluating at least 10 investigational agents or combinations of agents over the course of the I-SPY 2 TRIAL process.

3.5 Trial Informatics

The bioinformatics system (caBIG-based collaborative interface) developed and applied by the I-SPY TRIAL will continue. The infrastructure put in place from the I-SPY TRIAL study consortium and NCI is intact and includes web-based participant registration and randomization as well as data sharing and analysis. The adaptive trial software bundle developed as part of the TRANSCEND project includes software for tissue tracking using caTISSUE, the caBIG biospecimen management system, among others. After qualifying to participate in the I-SPY 2 TRIAL process, all sites are trained to use the software tools (available at https://login.salesforce.com/) and provided a user manual (see TRANSCEND User Manual).

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria Overview

The I-SPY 2 TRIAL will enroll women through a two-stage process that includes the initial screening phase followed by the treatment phase for those women eligible for enrollment. Separate consent forms will be used for each phase, and eligibility for each phase is detailed below.

4.1.1 Eligibility Criteria for Initial Screening Phase of I-SPY 2 TRIAL

A. Histologically confirmed invasive cancer of the female breast. Histologic confirmation can be obtained by fine-needle aspiration (FNA), core needle biopsy, or incisional biopsy (allowed if residual tumor is 2.5cm). Metaplastic and inflammatory carcinomas are eligible, and synchronous bilateral primaries are eligible if the more advanced tumor meets staging criteria. Participants who have an FNA for diagnosis must have histological documentation of invasive carcinoma by the start of chemotherapy.

B. Clinically or radiologically measureable disease in the breast after diagnostic biopsy, defined as longest diameter greater than or equal to 25 mm (2.5 cm).If a tumor meets this criteria by clinical exam only, the tumor must also be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm (2 cm) with conventional techniques (positron emission tomography (PET), computed tomography (CT), MRI, ultrasound, or x-ray) or as >10 mm (1 cm) with spiral CT scan. All tumor measurements must be recorded in metric notation.

C. Prior therapy: No prior cytotoxic regimens are allowed for this malignancy. Participants may not have had prior chemotherapy, other targeted anticancer therapies, or prior radiation therapy to the ipsilateral breast for this malignancy. Prior bis-phosphonate therapy is allowed.

D. Age ≥18 years: Because no dosing or AE data are currently available on use of experimental trial agents for participants <18 years of age, children are excluded from this study.

E. Performance status: ECOG performance status 0−1.

F. Core biopsy: Willing and able to undergo core biopsy of the primary breast lesion to assess baseline biomarkers to determine eligibility for treatment phase of I-SPY 2 TRIAL.

G. Nonpregnant and non-breastfeeding: Effects on a developing human fetus of phase 2 agents under study at the recommended therapeutic dose are unknown. For this reason and because these agents may be teratogenic, women of child-bearing potential must agree to use adequate contraception (double barrier methods of birth control or abstinence) prior to study entry and for the duration of study treatment phase. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

If a participant is of child-bearing potential (women are considered not of childbearing potential if they are at least one year postmenopausal and/or surgically sterile), she must have documented negative serum or negative urine pregnancy tests within 14 days of entry to screening phase.

H. No ferromagnetic prostheses: Participants who have metallic surgical implants that are not compatible with an MRI machine are not eligible. Otherwise eligible participants should be asked if they have any heart valves, aneurysm clips, orthopedic prosthesis, or any metallic fragments anywhere in their body prior to enrolling in the study.

I. Ability to understand and willingness to sign a written informed consent document (I-SPY 2 TRIAL Screening Consent).

4.1.2 Inclusion Criteria for Treatment Phase of I-SPY 2 TRIAL

Participants successfully enrolled on the screening phase of I-SPY 2 TRIAL will be evaluated for eligibility for the treatment phase of I-SPY 2 based on the results of several tumor biomarker assays. In addition to the eligibility criteria outlined in §4.2, participants who have completed the Initial Screening phase must meet the following eligibility criteria:

A. Eligible breast tumors must also meet one of the following criteria:

- Stage II or III
- T4, any N, M0, including clinical or pathologic inflammatory cancer
- Regional Stage IV, where supraclavicular lymph nodes are the only sites of metastasis, will be evaluated at the time of surgery.

B. Breast Hormone status: Any tumor ER/PgR status, any HER2/*neu* status as measured by local hospital pathology laboratory, and meets any tumor assay profile described in 4.1.2 F. Tumors will be considered positive when:

- ≥5% tumor staining for ER and/or PgR is seen.
- Any one of the following three conditions for HER2 are met:
  - IHC 3+;
  - Overexpression by FISH (as defined by FDA-cleared/approved tests used at each institution). When an increase in CEP17 copy number is observed by FISH (*i.e.*, polysomy), the participant will be considered HER2+ if the ratio of HER2 signals/nucleus is greater than 6;
  - TargetPrint HER2+.

C. Normal organ and marrow function as defined below (test results can be used if done within 30 days of consenting to treatment phase):

- Leukocytes ≥3000/μL
- Absolute neutrophil count ≥1500/μL
- Platelets ≥100,000/μL
- Total bilirubin within normal institutional limits, unless participant has Gilbert's disease, for which bilirubin must be ≤2.0 × ULN
- Aspartate aminotransferase (AST) (SGOT) or alanine transaminase (ALT) (SGPT) ≤1.5 × institutional ULN
- Creatinine <1.5 × institutional ULN
- For agent-specific criteria, see Appendix C §2.

D. No uncontrolled or severe cardiac disease (history of diagnosis of unstable angina, myocardial infarction, symptomatic congestive heart failure, serious uncontrolled cardiac arrhythmia [including atrial flutter/fibrillation], requirement for inotropic support or use of

devices for cardiac conditions [pacemakers/defibrillators]). Baseline ejection fraction (by nuclear imaging or echocardiography) must be ≥50%.

E. No clinical or imaging evidence of distant metastases by CT with or without PET, PA and lateral chest radiograph (CXR), radionuclide bone scan, and/or LFTs including total bilirubin, ALT, and AST within ranges defined in §4.1.2 C(test results can be used if done within 30 days of consenting to treatment phase).

F. Breast tumor assay profile must include one of the following:

- MammaPrint High, any ER status, any HER2 status
- MammaPrint Low, ER– (<5%), any HER2 status
- MammaPrint Low, ER+, HER2/*neu* positive by any one of the three methods used (IHC, FISH, TargetPrint). See §3, Table of Eligibility

G. Ability to understand and willingness to sign a written informed consent document (I-SPY 2 TRIAL Treatment Consent)

H. All additional applicable investigational agent-specific eligibility criteria can be found in §2 of Appendix C. Each investigational agent-specific eligibility criteria can also be found in §2.2 of each investigational agent-specific appendix.

4.2 Exclusion Criteria

A. Use of any other investigational agents within 30 days of starting study treatment.

B. History of allergic reactions attributed to compounds of similar chemical or biologic composition to Study Agent or accompanying supportive medications.

C. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, diabetes, or psychiatric illness/social situations that would limit compliance with study requirements.

D. Sentinel lymph node dissection/biopsy on the nodes draining from the study index tumor site is not allowable prior to the start of chemotherapy. Any participant who has undergone sentinel lymph node dissection/biopsy procedure on the side of the study index tumor prior to start of chemotherapy is not eligible. Clinical evaluation of the axilla and FNA and/or core biopsy of any suspicious nodes detected clinically or radiologically should be performed prior to starting chemotherapy.

4.3 Inclusion of Women and Minorities

This study will be carried out in women. In the I-SPY 1 TRIAL, there was 19% participation of African American women, 4% Asian, and 2% other. If, during the initial phases of accrual, this distribution is not achieved, the study will specifically recruit participants through the usual channels for medical center research participant advertising (newsletter, posters) to achieve the desired participant mix. The following information will be reported in compliance with FDA annual reporting requirements.

4.4 Recruitment and Retention Plan

Participant eligibility will be systematically assessed at each of the participating I-SPY 2 study sites. A screening log will be kept in TRANSCEND documenting the review of potentially eligible participants as well as reasons for non-enrollment. Sites will provide detailed information to all relevant treating physicians on the conduct of the trial to optimize physician participation. Monthly conference calls will review recruitment at each site so that sites not meeting recruitment goals can be identified early and interventions to improve recruitment can be instituted.

The I-SPY 2 TRIAL is listed on the NIH website Clinicaltrials.gov to enable referring physicians to identify local sites for participant referral (NCT01042379). Participants will also be able to find the I SPY-2 TRIAL sites through breastcancertrials.org, a clinical trial matching web site. Women diagnosed with breast cancer can go to this national-service web site and enter their information to find clinical trials appropriate for them. Once they find a trial, they can contact a research site and send their information through TRIAL CONNECT, which includes their contact information and their eligibility screened against the trial eligibility. In addition, the study will work with advocate groups across the country to improve awareness.

A large, organized cadre of experienced participant advocates will participate within community participant support services locations to help educate participants about the trial and assist in the recruitment and retention process. These advocates will be experts on the trial design and conduct and will assist potentially eligible participants in understanding the informed consent process as well as assisting those enrolled in navigating the various steps within the trial assessment and treatment process.

5. CHEMOTHERAPY ADMINISTRATION

5.1 Standard Chemotherapy Treatment Plan for Control Arm, HER2– Tumors

Participants randomized to the standard chemotherapy treatment arm who are HER2– will receive 12 cycles of paclitaxel at 80 mg/m^2 once every seven days (q1w) ± 1 day. For paclitaxel, Filgrastim can be used at investigators discretion. A minimum of seven days after completing the paclitaxel regimen, participants will receive four cycles of doxorubicin at 60 mg/m^2 plus cyclophosphamide at 600 mg/m^2 once every 14 days (q2w) ± 1 day or once every 21 days (q3w) ± 1 day at physician discretion. For AC, Filgrastim or Pegfilgrastim can be used at investigator's discretion. **All treatment doses should be based on actual body weight and not ideal body weight. If participant's body weight increases or decreases by ≥10% from baseline during the course of the treatment phase, the body surface area and agent dose should be recalculated.**

Table 5.1 T (q1w) followed by AC (q2w or q3w) Administration

Agent	Dose	Route	Cycle
Paclitaxel*	80 mg/m^2	IV	1–12
Doxorubicin	60 mg/m^2	IV	13–16
Cyclophosphamide	600 mg/m^2	IV	13–16

*If participants have a hypersensitivity reaction to Paclitaxel that cannot be ameliorated with supportive care, Nab-paclitaxel may be administered at 100mg/m^2 weekly for the remainder of the 12 weeks.

5.2 Standard Chemotherapy Treatment Plan for Arm, HER2+ Tumors

Due to accelerated approval of pertuzumab, the paclitaxel plus trastuzumab control arm has temporarily been closed in accordance with the recommendations of the I-SPY 2 DSMB and Investigators. Paclitaxel plus trastuzumab and pertuzumab will serve as a "Bridging control arm" in the time machine as described in the amended statistical plan (Appendix A). Randomization will be 4:1 for the investigational arms relative to the pertuzumab containing arm.

Participants randomized to the standard chemotherapy treatment arm who are HER2+ will receive 12 cycles of paclitaxel at 80 mg/m^2 q1wk ± 1 day plus trastuzumab q1w and pertuzumab q3w ± 1 day. Trastuzumab is given every week at a loading dose of 4 mg/kg for cycle 1 and maintenance dose of 2 mg/kg for cycles 2–12. Pertuzumab is given every 3 weeks at a loading dose of 840 mg for cycle 1 and maintenance dose of 420 mg for cycles 4, 7 and 10. For paclitaxel, Filgrastim can be used at investigators discretion. A minimum of seven days after completing the paclitaxel regimen, participants will receive four cycles of doxorubicin at 60 mg/m^2 plus cyclophosphamide at 600 mg/m^2 q2w ± 1 day or q3w ± 1 day at physician discretion. For AC, Filgrastim or Pegfilgrastim can be used at investigator's discretion. **All treatment doses should be based on actual body weight and not ideal body weight. If participant's body weight increases or decreases by ≥10% from baseline during the course of the treatment phase, the body surface area and agent dose should be recalculated.**

Table 5.2.1 TH (q1w) x12 weeks Followed by AC (q2w or q3w) Administration (Original Control)

Agent	Dose	Route	Cycle
Paclitaxel*	80 mg/m^2	IV	1–12
Trastuzumab	4 mg/kg	IV	1
Trastuzumab	2 mg/kg	IV	2–12
Doxorubicin	60 mg/m^2	IV	13–16
Cyclophosphamide	600 mg/m^2	IV	13–16

Table 5.2.2 TH (q1w) and Pertuzumab (q3w) x12 weeks Followed by AC (q2w or q3w) Administration (Bridging Control)

Agent	Dose	Route	Cycle[b]
Paclitaxel*	80 mg/m^2	Iv	1–12
Pertuzumab[a]	840 mg (loading dose) 420 mg (thereafter)	Iv	1, 4, 7, 10
Trastuzumab	4 mg/kg (loading dose) 2 mg/kg (thereafter)	Iv	1 2–12
Doxorubicin	60 mg/m^2	Iv	13–16
Cyclophosphamide	600 mg/m^2	Iv	13–16

[a] Pertuzumab is administered 1 hour before the delivery of weekly paclitaxel. On days that all three drugs are given, trastuzumab infusion is given first followed by pertuzumab followed by paclitaxel infusion

[b]Note that each cycle for paclitaxel combinations = one week, each cycle for AC = two or three weeks.

* If participants have a hypersensitivity reaction to Paclitaxel that cannot be ameliorated with supportive care, Nab-paclitaxel may be administered at 100mg/m^2 weekly for the remainder of the 12 weeks.

5.3 Investigational Agent Treatment Plan

Participants randomized to an investigational agent may receive 12 cycles of paclitaxel at 80 mg/m^2 q1wk ± 1 day in addition to the investigational agent; however, for administration details of the investigational agent regimen, refer to the specific agent appendix(D–x). A minimum of seven days after completing the investigational agent regimen, participants will receive four cycles of doxorubicin at 60 mg/m^2 plus cyclophosphamide at 600 mg/m^2 q2w ± 1 day or q3w ± 1 day at physician discretion. For AC, Filgrastim or Pegfilgrastim can be used at investigator's discretion. **All treatment doses should be based on actual body weight and not ideal body weight. If participant's body weight increases or decreases by ≥10% from baseline during the course of the treatment phase, the body surface area and agent dose should be recalculated.**

5.4 Premedications for Paclitaxel, Trastuzumab, and Doxorubicin plus Cyclophosphamide

It is recommended that the treating physician follows National Comprehensive Cancer Network guidelines (www.nccn.org). The guidelines are provided in Appendix B.

5.5 Concomitant Medication

ER positive participants ONLY:

Any form of ovarian ablation is prohibited (*e.g*., gonadotropin-releasing hormone (GNRH) agonist, oophorectomy, radiation, *etc*.) before surgery.

For all participants:
Birth control usage and an oophorectomy at the time of primary breast surgery are acceptable. Aromatase inhibitor for limited use to harvest oocytes prior to starting neoadjuvant chemotherapy is allowed.

5.6 Toxicity Management and Dose Modifications

Toxicity management and dose modifications for standard therapy are outlined in Appendix B (for paclitaxel and AC) and Appendix K (for the combination of pertuzumab and trastuzumab).
Toxicity management and dose modifications for investigational agents are outlined in each specific agent appendix (Appendices D–x).

5.7 Doxorubicin Shortage Guidelines

Due to ongoing supply issues, shortages of doxorubicin may occur intermittently. The following guidelines should be followed with regard to doxorubicin administration:

Participant Status when Doxorubicin Shortage is Experienced	When to use Epirubicin	Epirubicin Dosage	Dose Modification and Monitoring for Epirubicin	Doxorubicin Supply Restored
Currently Receiving Doxorubicin	• Switch to epirubicin at the next scheduled cycle • Continue treatment per study schedule	Starting dose: 90mg/m^2	•Epirubicin dose modification can be made using percentage adjustments specified in Appendix B, Table 3 of the protocol. •All aspects of therapy and monitoring should be followed as specified in the protocol. •Document in research record that substitution was due to doxorubicin shortage	Return to using doxorubicin at an equivalent dose to the previously administered epirubicin.
Not Yet Receiving Doxorubicin • Site cannot guarantee at least 2 cycles of doxorubicin at time of initiating anthracycline treatment	• Use epirubicin for all 4 cycles • Continue treatment per study schedule	Starting dose: 90mg/m^2	•Epirubicin dose modification can be made using percentage adjustments specified in Appendix B, Table 3 of the protocol. •All aspects of therapy and monitoring should be followed as specified in the protocol	Continue with epirubicin for all cycles
• Site can guarantee at least 2 cycles of doxorubicin	• Use doxorubicin as instructed in §5 of the protocol. • If shortage occurs, follow guidelines above for participants receiving doxorubicin			

NOTE: As participants in I-SPY 2 receiving neoadjuvant therapy are potentially curable, and since epirubicin has been shown to be an effective agent for breast cancer with no additional side effects, the change to epirubicin can be made immediately to avoid withholding chemotherapy treatment in this potentially curable participant population.

5.8 Paclitaxel Hypersensitivity Reaction Recommendations

Patients who have paclitaxel hypersensitivity reactions are eligible to stay on their assigned I-SPY treatment arm. In the case of paclitaxel hypersensitivity that cannot be ameliorated with supportive care, Nab-paclitaxel at 100 mg/m2 IV weekly may be used in substitution. Nab-paclitaxel should be administered according to standard practice and may be co-adminstered with investigational agents. All protocol procedures should continue as normally prescribed and patients will remain on study treatment.

5.9 Adjuvant Treatment Recommendations

There are no adjuvant treatment requirements for this trial. Adjuvant therapy is the discretion of the treating physician and participant. However, it is recommended that participants receive the standard of care following neoadjuvant chemotherapy and surgery, including hormonal therapy for a minimum of five years if HR+, one year of trastuzumab if HER2+, and radiation therapy if indicated.

6. INVESTIGATIONAL AGENT INFORMATION (See Appendices C and D–x)

The I-SPY 2 TRIAL protocol and IND are structured to enable the seamless addition and release of investigational agents over the course of the trial. When an investigational agent is added or released from use in this trial, only relevant appendices require updating, specifically Appendix C and the corresponding investigational agent's appendix.

Appendix C: Overview of all investigational agents in the study
Appendix D–x: All investigational agent-specific information (one investigational agent per appendix). As investigational agents are added to the trial, they will appear as subsequent appendices.

Each investigational agent falls into one of three categories as described in Appendix C: 1) Agents approved, pending activation for randomization, 2) Agents approved, activated for randomization, and 3) Agents graduated or dropped, no longer activated for randomization.

Adding an Investigational Agent to the Trial
To add a new investigational agent or (new dose or combination of agents) when eight arms are active, the trial team prepares the protocol amendment containing: 1) the new investigational agent's appendix, 2) the corresponding supplemental informed consent, and 3) an updated Appendix C showing the new agent in Table 1.1 (Investigational Agents Approved, Pending Activation for Randomization).

The protocol amendment will be considered a major modification to the protocol and will require a full IRB committee review; however, it will not require stopping accrual to the trial because there will be no change to Table 1.2 (Investigational Agents Approved, Activated for Randomization). New investigational agents will remain in this category until all trial sites have received IRB approval and there is space in the randomization engine for the new agent.

When the randomization engine has room for a new agent to be added, a protocol amendment will be generated updating Appendix C by moving the new investigational agent from Table 1.1 (Investigational Agents Approved, Pending Activation for Randomization) to Table 1.2 (Investigational Agents Approved, Activated for Randomization).

The protocol amendment will be considered a minor modification to the protocol and will require an expedited review by the IRB, which should take about 1 to 2 weeks. The trial will not have to stop accruing during this period, because participants will not be randomized to the new investigational agent until all the sites have IRB approval for the agent.

In summary, two steps must occur at the site level to add and use an investigational agent to the trial when eight arms are active:

1) A protocol amendment to ***add*** the new investigational agent must be submitted and approved by the full IRB committee at each site
2) A second protocol amendment to ***activate*** the new investigational agent for randomization must be approved by expedited IRB review at each site

When fewer than eight arms are active, it is possible to add and activate an agent in one subsequent amendment. In that case, the trial team prepares the protocol amendment containing: 1) the new investigational agent's appendix, 2) the corresponding supplemental informed consent, and 3) an updated Appendix C showing the new agent in Table 1.2 (Investigational Agents Approved, Activated for Randomization). After the protocol undergoes full IRB committee reviews and approval is obtained by all sites, the arm is considered activated.

Releasing an Agent from the Trial
When an investigational agent is graduated or dropped from the trial, a protocol amendment will be generated. Appendix C will be updated moving the agent from Table 1.2 (Investigational Agents Approved, Activated for Randomization) to Table 1.3 (Investigational Agents Graduated or Dropped, No Longer Active for Randomization).

The protocol amendment will be considered a minor modification to the protocol. The protocol will be submitted concurrently to all the study site's IRBs and will only require expedited IRB review and approval, which should take 1 to 2 weeks. The trial will not have to stop accruing to the other treatment arms during this period.

7. BIOMARKERS FOR ELIGIBILITY, STRATIFICATION, AND RESPONSE MONITORING

7.1 Tissue and Blood Specimens for Biomarker Assessment

As standard of care, a diagnostic pretreatment core will be evaluated by a local pathologist according to local histopathologic standards. This includes a hematoxylin and eosin (H&E) stain and FDA-cleared/approved IHC/FISH assessment for ER, PgR, and HER2.

Tissue will be acquired at three time points during the trial for this study. Pretreatment (time point 0) and at the end of the third cycle of paclitaxel (time point 1), four 16-gauge core needle biopsies will be taken using an image-guided 16-gauge biopsy device. At the time of surgery (time point 3), a representative sample of tumor will be collected and cut into two pieces, only if sufficient tumor remains. The surgical specimen will be assessed using standard histopathologic parameters and the RCB technique to evaluate the extent of residual disease by the site pathologist. The surgical assessment will be used to determine the primary endpoint, pCR.

Pretreatment cores will serve to assess biomarkers that are used to evaluate eligibility as well as stratification in the trial (§7.2). The remainder of the pretreatment cores, as well as all tissue samples from time points 1 and 3, will be used to perform qualifying biomarker assays (§7.3) and for further research (exploratory biomarkers, §7.4).

All I-SPY 2 TRIAL tissue specimens will be embedded in OCT in a cryostat mold and frozen at –80°C, then sent to the I-SPY Lab at University of California, San Francisco (UCSF) on dry ice (§10). When applicable, a frozen core can be thawed and processed for formalin fixation and paraffin embedding (FFPE). If there is no tumor on the research tissue samples, a surgical pathology block will be provided as needed.

Blood samples will be drawn pretreatment (time point 0), early paclitaxel (time point 1), inter-regimen (time point 2), and before surgery (time point 3); see ‘Schema’ section, Figure C. Serum, plasma, and buffy coat cells will be stored at –80°C for research (§10). Samples will be shipped batch-wise to the I-SPY Lab at UCSF.

7.2 MRI and MRI Functional Tumor Volume (FTV) for Biomarker Assessment

All MRI exams will be performed as described in Figure C, Schedule of Study Procedures. Each participant should have all MRI exams performed using the same magnetic configuration (manufacturer; field strength; breast coil model).

7.2.1 MRI Scan Protocol

The breast MRI protocol includes a T2-weighted sequence, diffusion-weighted imaging (DWI) sequence, and dynamic contrast-enhanced (DCE) series using a bilateral, 3D, fat-suppressed, and T1-weighted sequence with 80–100 second temporal resolution.

7.2.1.1 General Requirements

- 1.5T or 3.0T whole body MRI scanner
- Dedicated breast radiofrequency coil
- Participant scanned in prone position with in-dwelling IV catheter
- Contrast agent injection with FDA-approved gadolinium-based contrast agent; **the**

same contrast agent brand should be used for all MRI exams for the same participant.

- The MRI exam will include a localization scan, a T2-weighted sequence, and a diffusion-weighted sequence, followed by a contrast-enhanced T1-weighted series:
    - T2-weighted sequence performed before contrast
    - Diffusion-weighted sequence performed before contrast
    - T1-weighted sequence with 80–100 second temporal resolution, performed once pre-contrast and multiple times post-injection using identical sequence parameters (see MOP §Imaging procedures); transmit and receive **gain settings should remain constant** for pre- and post-contrast T1-weighted imaging
    - Pre-contrast T1 images should be checked prior to contrast injection to confirm acceptable fat-suppression
    - Post-contrast imaging should continue for at least 8 minutes following contrast agent injection
    - Care should be taken to **select the smallest field of view (FOV) and slice coverage that completely encompasses both breasts and axilla**

NOTE: For guidelines on the specific pulse sequence parameters, see MOP §Imaging procedures.

7.2.1.2 ACRIN 6698 Co-study for Participating Sites—Closed to Accrual

- Sites participating in ACRIN 6698 will use the MRI protocol as specified in the ACRIN 6698 protocol. Language in the model consent about the ACRIN 6698 co-study is only included for those sites participating in this co-study.

NOTE: The study is closed to accrual. It has met its accrual goals.

7.2.2 MRI Functional Tumor Volume Assessment

All MR volume measurements will be automated and measured using software on the Hologic work station. Directly following each MRI examination, image data will be transferred to the local Aegis workstation for processing by the radiology technologist or study coordinator, who places rectangular regions-of-interest on cranio-caudal and medial-lateral projection views surrounding the tumor, in order to restrict the volume of calculation.

Data acceptability for processing will be assessed by the technologist based on several quality factors (success of contrast injection, absence of participant motion, or other artifacts). Tumor volume measurements will be computed according to the signal enhancement ratio (SER) method developed at UCSF [35]. The subsequent calculation of FTV is automated as part of the Aegis with SER plug-in software, with verification by the study radiologist.

Specifically, using the series of high-resolution T1-weighted images acquired before and following the injection of gadolinium-based contrast agent, the percent enhancement (PE), defined as the change in signal intensity at 2.5 minutes post-contrast relative to pre-contrast signal intensity (or $PE = [(S_1–S_0)/S_0] \times 100$, where S_0 and S_1 represent the signal intensities [SI] of each voxel in the pre-contrast and first post-contrast images) will be measured at every pixel in the image. Tumor volume will be calculated as the sum of all pixels meeting a predefined threshold of 70%*. Tumor pixels will be further characterized by their SER value, defined as the ratio of early enhancement to late enhancement $(S_1–S_0)/(S_2–S_0)$, where S_0 and S_1 are as defined previously and S_2 represents the signal intensity in the late post-contrast image.

Tumor volume will be segmented into sub-volumes with high, moderate, and low SER values. MRI FTV is computed by summing all eligible voxels with SER values above a threshold value of 0.9*.

Following verification by the study radiologist, tumor volume measurements will be sent to the I-SPY 2 electronic clinical trial data capture system (TRANSCEND) in the MRI volume case report form.

*Threshold may vary by imaging site depending on equipment and site-specific protocol variations.

7.3 Incorporation of Established Biomarkers

IHC markers based on community standards (FDA-cleared/approved tests at each site's participating pathology laboratory) will be collected from a participant's diagnostic breast core biopsy as part of the standard of care. These markers include the expression of tumor ER, PgR, and HER2, complemented by HER2 FISH as appropriate (for HER2 IHC 2+ cases, perform FISH testing as indicated by FDA). A study pretreatment frozen core will be sectioned to generate one H&E section. Appropriate sections will be further processed by Agendia Inc for RNA expression assessment of the TargetPrint HER2 and MammaPrint using the Agendia MammaPrint 44K full genome microarray manufactured by Agilent Technologies. A tumor will be considered ER+ and/or PgR+ if there is five percent or greater positive tumor staining. A tumor will be considered HER2+ if any one of the following three conditions are met: a) HER2 IHC 3+; b) overexpression by FISH (as defined by FDA-cleared/approved tests used at each institution); or c) TargetPrint HER2+. When an increase in CEP17 copy number is observed by FISH (*i.e.*, "polysomy"), the participant will be considered HER2+ if the ratio of HER2 signals/nucleus is greater than 6.

Women who are low-risk by MammaPrint and ER+ as well as HER2– will not be eligible for this trial (see eligibility scheme). Eligible women who are MammaPrint high-risk or MammaPrint low-risk and ER–, or MammaPrint low-risk and ER+ and HER2+ will be stratified based on their HER2, HR, and MammaPrint expression and subsequently randomized according to the adaptive randomization scheme (see §3).

In addition to pCR, MR volume change will also be used to inform the adaptive randomization.
The established biomarkers used will fulfill FDA regulatory requirements, including FDA clearance, or FDA IDE. The IDEs filed with the Master IND will include additional technical details.

7.4 Incorporation of Qualifying Biomarkers

Pretreatment (time point 0) and early paclitaxel (time point 1) biopsies will be used to generate qualifying biomarkers. Qualifying biomarkers are defined as those assays with promise to predict response to standard chemotherapy and investigational agents. These will include gene and protein (pathway) measurements by Agilent 44K and Affymetrix arrays, mRNA arrays, and RPMA assays to determine if molecular characteristics (biomarkers/pathways) correlate with pathologic, imaging, or RCB response measures in the neoadjuvant setting using the investigational therapeutic agents. Qualifying biomarkers will be performed under Clinical Laboratory Improvement Amendment (CLIA) conditions and have the potential to be used during the course of the trial for participant stratification, for which they then need to acquire IDE status.

Over the course of the trial, additional qualifying biomarkers will be put forward and tested for their ability to predict tumor response to specific classes of investigational targeted therapeutics, in addition to validating molecular profiles proposed by investigators that predict pCR to standard anthracycline and taxane-containing neoadjuvant chemotherapy [36–39].

Cell Line Agent Response Profiling: All agents being used in the trial will be tested against a 60-cell-line panel using an *in vitro* system created by Joe Gray, PhD, Professor, Laboratory Medicine and Radiation Oncology, UCSF; Director, Division of Life Sciences, Lawrence Berkeley National Laboratory. Gene expression patterns found on Affymetrix arrays that identify likelihood of response or resistance to a given agent will be transferred to a high throughput mRNA array assay [40].

RPMA: Five 8-micron sections of tissue from a core will be used for protein lysate arrays. The cut sections of the frozen core will be used for pathway analysis, *e.g.*, total HER2 and phosphorylated HER2 (pHER2) assay by RPMA by George Mason University [41].

TFAC/RCB predictor: A gene expression profile predicting likelihood of response to standard chemotherapy, TFAC, has been established. Sections of a frozen core will be processed for mRNA expression analysis on Affymetrix arrays to validate this predictor by MD Anderson Cancer Center (MDACC) Molecular Laboratory [42].

Phase 2 agent response biomarkers: Biomarker response to the various investigational agents will be tested on the appropriate biospecimen, *e.g.*, tumor tissue, blood, and will include gene expression patterns derived from single gene/protein assessments or will encompass patterns/pathways as captured by multiple index assays. For the scientific rational for the biomarkers selected, see specific investigational agent appendix.

Pharmacogenomics by single-nucleotide polymorphism (SNP) and GWAS: Paclitaxel toxicity has been correlated to gene variant alleles that affect metabolism of the agents. Specific SNPs will be evaluated on DNA extracted from blood samples, as well as by GWAS analysis.

Candidate gene SNPs: In addition to the GWAS approach to toxicity, we will utilize the Illumina Human 610-Quad BeadChip technology to evaluate SNPs. SNP data will be generated to examine candidate SNPs, SNP signatures, and haplotypes predicting response to other standard agents being utilized, as well as investigational agents being tested in the I-SPY 2 TRIAL. For example, CYP3A4 and glutathione-*S*-transferase polymorphisms previously shown to modulate response to cyclophosphamide [43] will be correlated to imaging response to the anthracycline/cyclophosphamide regimen in all participants receiving this therapy. In addition, an interleukin-6 haplotype signature associated with early relapse in ER+ participants will be examined in those participants with ER+ disease [44]. Generation of genome-wide SNP data will enable additional germline SNP signatures to be identified for the investigational agents being tested in I-SPY 2 as exploratory biomarkers.

7.5 Incorporation of Exploratory Biomarkers

Additional tissue will be used to enable molecular assays to be performed using next generation technology platforms. Exploratory biomarkers for response prediction can be evaluated in a pure research setting. Likely assays include sequencing, methylation, microRNA, *etc.*; new technologies will be explored once they become available and are judged valuable. Once the new assays are performed, they can be compared to molecular assays already performed on the Agilent platforms and reverse phase tissue arrays, as well as by IHC.

Peripheral blood samples: At various time points as indicated in the trial scheme, peripheral blood sampling will be performed. Blood samples will be processed to enable further biomarker research employing different techniques. Serum, plasma, and buffy coat will be stored in multiple aliquots.

Circulating tumor cells: Methods will be chosen that are compatible with the FNIH Biomarker Consortium circulating tumor cell trial (http://www.fnih.org).

7.6 Repository for Storing, Analyzing, and Comparing Assay Results

All specimens and specimen transformation will be recorded and tracked using caTISSUE, the web-based tissue tracking and shipping tool developed by the NCI. Specimen tracking numbers will be used to track and store assay results. Assay results will be stored in caINTEGRATOR , the NCI data repository built for cross-platform analysis. In addition, we will employ other analytic tools such as the University of California-Santa Cruz (UCSC) Cancer Genome Browser.

8. CLINICAL EVALUATIONS AND PROCEDURES

8.1 Schedule of Events

Table 8.1 Study Calendar

Evaluation/ Procedure	Registration & Screening (Pre-randomization)	Paclitaxel Regimen[a]	AC Regimen	Pre-Surgery	Follow-up
Informed Consent	X	X (post-randomization)			
Assess Eligibility	X				
Medical History	X				
Physical Exam	X	X	X	X	X
Laboratory Blood Tests[b]	X	X	X		
Pregnancy Test	X				
Investigational Agent-specific Laboratory and Assessment Tests[c]	X	X	X	X	
Metastatic Evaluation	X				
Breast MRI	X	X (end of week 3)	X (pre-AC)	X	
ECHO/MUGA	X	X[c] (end of week 12)	X[d] (post-AC)		X[e]
Study Biopsy/Tissue Collection	X	X (end of week 3)		X (at time of surgery)	
Study Blood Draw for Serum, Plasma, and Buffy Coat	X	X (end of week 3)	X (pre-AC)	X	
Clinical Assessment	X	X	X	X	X
Administration of Investigational Agent		X			
Adverse Event Collection		X	X	X	X (30 days, 6 and 12 months post-surgery)

[a]For participants randomized to an investigational arm that does not include paclitaxel, see §2.5 of each agent-specific appendix for modifications to standard procedures(Appendices D-N).
[b]See§8.3.
[c]For investigational agent-specific evaluations, refer to the appropriate Appendix.
[d]ECHO or MUGA performed on HER2+ participants (*i.e.*, receiving trastuzumab/pertuzuamb and/or specific investigational agents). For investigational agent-specific evaluations, refer to the appropriate Appendix.
[e]ECHO or MUGA performed once every 3 months for as long as HER2+ participant continues on trastuzumab or pertuzumab post-surgery as standard of care, *e.g.*, 9–12 months.

8.2 Baseline Testing/Pretreatment Evaluation

The following procedures will be done before a participant is randomized for the study:

- Medical history and physical exam (including collection of height, weight, ECOG score)
- Histologically confirmed invasive breast cancer (including hormone evaluation by local pathology laboratory)
- Laboratory blood tests including (if applicable, within ranges defined in §4):
  - CBC with differential, including:
    - White blood cell (WBC) count
    - WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)

        - Platelet count
        - Hemoglobin
        - Hematocrit
    - Electrolyte panel, including:
        - Sodium
        - Potassium
        - Chloride
        - Total carbon dioxide
        - Anion gap
    - Liver function tests, including:
        - Total bilirubin
        - ALT
        - AST
        - ALP
    - Kidney function tests, including:
        - Blood urea nitrogen (BUN)
        - Creatinine
    - Pregnancy test for participant of child-bearing potential within 14 days of entry to screening phase
- Other laboratory and assessment tests as needed based upon investigational agent requirements; see Appendix C §2
- ECHO or multigated acquisition scan (MUGA) evaluation
- Metastatic evaluation (tests can be used if done within 30 days of consenting to treatment phase)—required testing for this trial to rule out distant metastatic disease (as defined in §4.1.2. E); includes any of the following:
    - PA and lateral CXR
    - CT with or without PET
    - Radionuclide bone scan
    - LFTs including total bilirubin, ALT, and AST within ranges defined in §4.1.2 C
- Breast MRI (to be completed within 30 days of starting study treatment)
- Study breast core biopsy (used for MammaPrint, TargetPrint HER2, qualifying, and exploratory biomarkers)
- Study blood draw

8.3 Evaluations During Neoadjuvant Chemotherapy Treatment

The following procedures will be done during the participant's paclitaxel regimen:

- Paclitaxel, if given based upon randomization, administered weekly
- Trastuzumab and pertuzumab, if given based upon randomization, administered weekly and every three weeks respectively
- Investigational agent, if given based upon randomization, administered on agent's specific dosing schedule
- Clinical assessment, breast MRI, blood draw, and core biopsy at the end of week 3, prior to the fourth paclitaxel infusion
- ECHO/MUGA evaluation post-paclitaxel and trastuzumab/pertuzumab for all HER2+ participants (every three months in conjunction with standard of care)
- Laboratory blood tests q1w, in conjunction with paclitaxel dosing (can be done within 2* days of paclitaxel administration):
    - CBC with differential, including:
        - White blood cell (WBC) count

        - WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
        - Platelet count
        - Hemoglobin
        - Hematocrit
    - Electrolyte panel, including:
        - Sodium
        - Potassium
        - Chloride
        - Total carbon dioxide
        - Anion gap
    - Liver function tests, including:
        - Total bilirubin
        - ALT
        - AST
        - ALP
    - Kidney function tests, including:
        - Blood urea nitrogen (BUN)
        - Creatinine

* NOTE: For C1D1 of paclitaxel treatment, labs drawn during the screening phase can be used if done within 3 weeks (21 days) of treatment start.

- Other laboratory and assessment tests as needed based upon investigational agent requirements, see investigational agent appendices (§2.5).
- AE reporting every three cycles in conjunction with the participant's weekly clinic visit.

Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation

The following procedures will be done during the participant's AC regimen:

- Clinical assessment, breast MRI and blood draw prior to starting AC treatment
- AC administered q2w or q3w at physician discretion
- Standard laboratory blood tests q2w or q3w, in conjunction with AC dosing (can be done within 2 days of AC administration):
    - CBC with differential, including:
        - WBC count
        - WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
        - Platelet count
        - Hemoglobin
        - Hematocrit
    - Electrolyte panel, including:
        - Sodium
        - Potassium
        - Chloride
        - Total carbon dioxide
        - Anion gap
    - Liver function tests, including:
        - Total bilirubin
        - ALT
        - AST
        - ALP
    - Kidney function tests, including:
        - BUN

        - Creatinine
    - AE reporting every cycle of AC in conjunction with participant's two- or three-week clinic visit
    - ECHO/MUGA evaluation post-AC for all HER2+ participants (every three months in conjunction with standard of care)

Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation

8.4 Evaluations at Completion of Neoadjuvant Chemotherapy Treatment

The following procedures will be done following completion of the participant's neoadjuvant chemotherapy:

- Breast MRI prior to surgery
- Study blood draw
- Tissue collected for the study at the time of surgery, if tumor remains on gross examination and sampling will not impact the final diagnostic evaluation

8.5 Postsurgery Follow-up

The participants will be followed for five years following the date of surgery for survival and recurrence. Follow-up will be collected every six *(± one month)* months for five years. New S/AEs will be collected up to 30 days *(± one week)* following the participant's surgery; continuing S/AEs will be monitored until resolution/baseline or 12 months postsurgery, whichever occurs first (See table 8.2 for specifics). For HER2+ participants receiving trastuzumab or pertuzumab postsurgery, ECHO or MUGA will be performed once every three months for 9–12 months during the follow-up period as standard of care.

Table 8.2: Follow-up S/AE Collection Criteria

Related S/AEs: Follow until one of the following occurs:	**Unrelated severe or life threatening S/AEs: Follow until one of the following occurs:**
– Resolved or improved to baseline	– Resolved or improved to baseline
– Relationship is reassessed as unrelated	– Severity improved to Grade 2
– Death	– Death
– Start of new anti-cancer regimen	– Start of new anti-cancer regimen
– Investigator confirms that no further improvement can be expected	– Investigator confirms that no further improvement can be expected
– Clinical or safety data will no longer be collected, or final database closure	– Clinical or safety data will no longer be collected, or final database closure

The final outcome of each adverse event must be recorded on the eCRF

Since OS is a study endpoint, AEs with a fatal outcome more than 30 days following surgery will be collected only as survival status (not as an SAE), unless there is evidence suggesting a causal relationship between the protocol treatment and the event with a fatal outcome. See specifics regarding progression in section 8.7.

8.6 Evaluations for Premature Discontinuation of Study Treatment

Since this is an intent-to-treat trial participants who discontinue their randomized treatment assignment prematurely for any reason will remain "on study" to complete the remaining study procedures and follow-up, including the following:

- Breast MRI, blood draw, and core biopsy at the end of week 3, prior to the fourth weeks of treatment
- Clinical assessment, breast MRI and blood draw prior to starting AC treatment
- Clinical assessment, breast MRI, and study blood draw prior to surgery
- Tissue collection at the time of surgery, if tumor remains on gross examination and sampling will not impact the final diagnostic evaluation
- For HER2+ participants continuing to receive trastuzumab or pertuzumab, ECHO or MUGA performed once every three months for 9–12 months during the follow-up period as standard of care
- All new S/AEs will be collected up to 30 days following the last administration of study treatment.
    - SAEs resulting from a study procedure (breast MRI, core biopsy, blood draw) will continue to be collected until surgery.
- Ongoing S/AEs will be monitored until resolution/baseline or 12 months, whichever occurs first. (See table 8.2 for specifics).
- For participants that were randomized to an investigational agent:
    - Collect safety labs and assessment tests 30 days after last dose of investigational agent, see investigation agent appendices (§2.5).

- For participants who choose to forgoe any of the above described procedures, a protocol deviation should be filed.

If a participant withdraws from the study prematurely, we will collect new adverse events, safety labs and assessment tests 30 days after last dose of study treatment; see specific investigational agent appendices (D-N).

8.7 Disease Progression

If progression of the primary tumor or evidence of metastasis requires discontinuation from study medication, then local practice should be followed. For participants who progress on-study treatment, new S/AEs will be collected 30 days following last study treatment administration (paclitaxel and/or investigational agent, or AC); continuing S/AEs will be monitored until resolution/baseline or 12 months following last study agent administration, whichever occurs first. See table 8.2

Progression should not be reported as an adverse event if it is clearly consistent with the suspected progression of the underlying cancer. Hospitalization due solely to the progression of underlying malignancy should not be reported as a serious adverse event. If there is any uncertainty about an adverse event being due only to the disease under study, please contact the DCC at (855)-889-5170 for further guidance on reporting.

9. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

9.1 Primary Endpoint

To assess objective response rates, as measured by pCR, of investigational agents in combination with standard neoadjuvant paclitaxel, doxorubicin, and cyclophosphamide. This measurement will occur after the end of chemotherapy during pathologic assessment of residual disease.

pCR is defined as no residual invasive cancer in the breast (at the time of definitive surgical resection)or in the lymph nodes (no invasive tumor by H&E). A study-trained pathologist will evaluate pCR. The Study Lead Pathologist will make the final determination on any indeterminate or contested results.

9.2 Secondary Endpoints

Additional information on the response to paclitaxel plus or minus a targeted agent will be obtained by measuring the change in MRI volume from the baseline MRI to the MRI performed following the completion of paclitaxel based therapy and by measuring RCB at time of pathologic assessment of residual disease.

9.2.1 MRI FTV

Measured from dynamic contrast-enhanced (DCE) MR images of the breast, FTV calculation is based on the SER breast MRI technique developed at UCSF and used in the ACRIN 6657 multi-center clinical trial [45–47]. High spatial resolution contrast-enhanced MRI is performed using a three-time point method that acquires images before, immediately following contrast injection, and in the late phase of contrast passage, using a small molecular weight gadolinium-containing contrast agent, administered intravenously. DCE-MRI data are transferred to the Hologic Aegis workstation for FTV processing immediately following the MRI examination. The measurement is verified by the study local radiologist.

9.2.2 Residual Cancer Burden

RCB was proposed as a determinant of the extent of residual disease in the post-treatment surgical resection specimen of participants with breast cancer who received preoperative chemotherapy [16]. Six variables are included in the formula. An RCB index value can also be calculated and involves the categorization into one of four RCB classes (RCB 0 or pCR, RCB I or near pCR, RCB II, RCB III). The calculation formula and detailed description can be found at: www.mdanderson.org/breastcancer_RCB. In brief, the variables include cross-sectional dimensions of the residual tumor bed (d1 and d2), estimate of the proportion of that residual tumor bed area that is involved by cancer (% CA), estimate the proportion of the cancer that is *in situ* component (% CIS), number of positive lymph nodes (LN), and measure of the diameter of the largest nodal metastasis (dmet). RCB variables will be collected by the study local pathologist, who will be trained in the technique. The Study Lead Pathologist will make the final determination on any indeterminate and contested results.

9.2.3 Three- and Five-year Relapse/RFS and OS

Three- and five-year RFS and OS will also be assessed. RFS is defined as local/regional invasive recurrence, invasive ipsilateral breast tumor recurrence, distant recurrence, inoperable (meaning no surgery because of progression), and/or death from breast cancer RFS will be calculated from the time of

treatment to event. OS is defined by death from breast cancer, non-breast cancer, unknown, or any other cause and will be calculated from the time of study entry to event [48].

9.3 Off-Investigational Agent Criteria

Off-investigational agent criteria is specified in the agent-specific appendix. Participants may stop taking study agent for the following reasons: toxicity; inadequate agent supply; or participant preference. This is an intent-to-treat trial, so participants will continue to be followed in order to continue to collect study data according to the schedule of events. See MOP for instructions on how to document participant's preference on discontinuing the investigational agent early (see section 8.6).

If an investigational agent reaches a threshold for graduation or is dropped for futility, no additional participants will be randomized to that agent. Participants currently receiving that agent will continue on the regimen until they complete the entire course of treatment (for graduating agents), or the option to continue or drop the agent will be at the discretion of the participant and her treating physician (for agents dropped for futility). In the later instance, participants will continue on-study but will revert to the standard/control regimen and will remain part of the arm to which they were assigned. See §3.4 for additional details regarding participant treatment options when an agent leaves the trial.

9.4 Off-study Criteria

Participants may go off-study either because the protocol intervention and any protocol-required follow-up period is completed or because the participant withdraws consent. See the individual agent appendices for further instruction. See MOP for further instructions for participants withdrawing consent.

9.5 Study Termination

QuantumLeap Healthcare Collaborative (QLHC) as the study Sponsor has the right to discontinue the study at any time.

10. SPECIMEN MANAGEMENT

10.1 Central Laboratories

All study samples will be sent to the I-SPY Laboratory, as part of the UCSF CLIA facility, for processing of the samples in a timely manner. The frozen cores will be sectioned to determined presence of tumor and tumor density by H&E stain (results will be available in caTISSUE for all users to access). A core with sufficient tumor will be sectioned and distributed to appropriate labs listed in §10.3. A frozen core with tumor cells present will be FFPE, with sections distributed to appropriate labs listed in §10.3.

Blood samples will be aliquoted for distribution as listed in §10.3.

All quality and quantity specimen data will be stored in caTISSUE, as well as shipment tracking information. Each laboratory involved in the analysis of samples will be responsible for entering appropriate assay data.

10.2 Specimen Collection, Handling, and Shipping Procedures

Table 10.2 lists the study speciment collections. Details regarding specimen collection, processing, tracking and shipping can be found in the I-SPY 2 MOP.

Table 10.2

Sample Type	Amount	Time point(s)	Notes
Core biopsies	Four (4) - 16-gauge core biopsies	Time point 0 and 1 (week 3)	If there is no tumor on the research tissue samples, a surgical pathology FFPE block will be provided as needed.
Whole Blood Serum aliquots	One (1) 5 ml marble/tiger-top vacutainer	Time point 0, 1 (wk 3), 2 (pre-AC), 3 (pre-surgery)	• Containing no anticoagulant, only serum separator • 1.0 ml of serum per 2 ml cryovial, for a total of three cryovials
Whole Blood Plasma and Buffy coat aliquots	Two (2) 5–10 ml EDTA/ lavender-top tubes	Time point 0, 1 (wk 3), 2 (pre-AC), 3 (pre-surgery)	1.8ml of plasma per 2 ml cryovial, for a total of three cryovials

10.3 Ancillary Laboratories

The following laboratories will receive the following study samples for analysis from the I-SPY Lab at UCSF:

10.3.1 Agendia

Agendia will receive sections of tumor for RNA isolation and analysis on Agilent 44K microarray. The raw 44K array data and normalized 44K array data will be made available on caARRAY for other Investigators to access. MammaPrint and TargetPrint HER2 results will be available in caIntegrator2. Samples will be sent to the Huntington Beach facility at the address below. The Agendia Netherlands Main Office information is provided for reference only; samples will not be sent to this facility unless instructed:

Agendia, Inc
22 Morgan
Irvine, CA 92618
United States
Main Office:1-888-321-2732
Local contact: George Pounds

Agendia BV
Science Park 406
1098XH, Amsterdam
The Netherlands
Main Office: +31 (20) 462-1500
Local contact: Arno Floore

10.3.2 George Mason University (GMU)

The Center for Applied Proteomics and Molecular Medicine (CAPMM) at GMU will receive five frozen 8-micron sections of tissue for RPMA analysis. Samples will be sent to the following address:

Dr. Julie Wulfkuhle
George Mason University
Bull Run Hall Room 351
10900 University Blvd.
Manassas, VA 20110
Phone: 703-993-4114
Lab contact: Dr. Julie Wulfkuhle

10.3.3 UCSF I-SPY Laboratory

UCSF I-SPY laboratory will receive two 8-micron sections of FFPE core for analysis of the mRNA array assay to predict agent sensitivity. Samples will be sent to the following address:

Drs. Joe Gray and Laura van't Veer
UCSF I-SPY Lab
2340 Sutter Street, Room S441
San Francisco, CA 94115
Lab Contact: Sarah Davis, MS
Phone: 415-885-7490
FAX: 415-353-7503

10.3.4 UCSF CLIA Pharmacogenomics

CLIA Pharmacogenomics laboratory will receive 400 nanograms of germline and tumor DNA (TBD) for Genome-wide SNP analysis. Samples will be sent to the following address:

Dr. Kathy Giacomini
UCSF
1550 4th Street
Mission Bay, RH 581
San Francisco, CA 94158
Phone: 415-514-4363
FAX: 415-514-4361

10.3.5 MDACC Molecular Laboratory

MDACC CLIA molecular laboratory will receive 1 microgram of isolated tumor RNA for analysis on Affymetrix U133A microarray and TFAC/RCB predictor. The raw array data and normalized array data will be made available on caARRAY. Samples will be sent to the following address:

MDACC
Attn: Dr. Fraser Symmans
8515 Fannin Street
Room NAO1.053
Houston, TX 77054-2512
Lab contact: Feng Lin
Phone: 713-792-2512

10.3.6 Investigational Agent Response Biomarkers – IGFR Inhibitor

Circulating bioactive IGF biomarkers will be evaluated in 100 µL of serum for those participants treated with an IGFR Inhibitor. The biomarker test includes IGF-IR and IGF-2. Samples will be sent to the following address:

Dr. Mary Hixon
Brown University Molecular Medicine Labs
Attn: Caitlin Brown
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Phone: 401-863-6125
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10.3.7 Investigational Agent Response Biomarkers – Pan ErbB Inhibitor

Biomarkers that will be assessed for participants treated with a Pan ErbB Inhibitor. The biomarker test includes AKT-S308, PTEN, Stathmin by IHC, and HER2 by serum analysis. Samples will be sent to the following address:

TBD

10.3.8 Additional Samples

Additional samples will be approved for other qualifying and exploratory assays. The I-SPY 2 Biomarker Committee will receive, review, and recommend requests for samples to the I-SPY 2 Data Access and Publication Committee for final approval.

Biomarker data will be made available through the I-SPY 2 Data Portal (caINTEGRATOR), as indicated by the I-SPY 2 Data Access and Publication Guidelines.

11. REPORTING ADVERSE EVENTS

Definition: An AE is any untoward medical occurrence in a study participant that does not necessarily have a causal relationship with the treatment or study participant. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with participation in a study, whether or not related to that participation.

A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in the specific agent appendix as noted under §6.0, Investigational Agent Information, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

Baseline symptoms will be collected once a participant signs the screening informed consent. All AEs are reported whether or not related to a study procedure or study treatment.

11.1.2 AE Data Elements

- AE reported date
- AE verbatim term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as an SAE
- Action taken with the study agent
- Outcome of the event
- Whether or not the participant dropped from the study due to AE
- Comments

11.1.3 Severity of AEs

Identify the AE using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE 4.0 provides a grading scale for each AE listed. A copy of the CTCAE 4.0 can be found at http://ctep.cancer.gov.

AEs will be assessed for severity according to the CTCAE 4.0, which provides unique clinical descriptions of grade for each AE term. These are based on the following general guidelines, which can be used to assess the severity of AE terms not listed in the CTCAE 4.0:

Grade 0 No AE
- Sign/symptom within normal limits

Grade 1 Mild AE
- Asymptomatic **or**
- Mild or minor symptoms **or**
- Marginal clinical relevance **or**
- Clinical or diagnostic observations only **or**
- Intervention not indicated **or**

- Non-prescription intervention indicated

Grade 2 Moderate AE
- Intervention indicated **or**
- Minimal, local, noninvasive intervention (*e.g.*, packing, cautery) **or**
- Limiting instrumental activities of daily living (*e.g.*, shopping; laundry; transportation; ability to conduct finances)

Grade 3 Severe AE
- Medically significant but not life-threatening **or**
- Inpatient or prolongation of hospitalization indicated **or**
- Important medical event that does not result in hospitalization but may jeopardize the participant **or** may require intervention either
  - to prevent hospitalization **or**
  - to prevent the AE from becoming life-threatening or potentially resulting in death
- Disabling
- Results in persistent or significant disability or incapacity **or**
- Limiting self care activities of daily living (*e.g.*, getting in and out of bed; dressing; eating; getting around inside; bathing; using the toilet)

Grade 4 Life-threatening AE
- Life-threatening consequences
- Urgent intervention indicated
- Urgent operative intervention indicated
- Participant is at risk of death **at the time of the event** if immediate intervention is not undertaken
- Blindness or deafness (need to decide if unilateral or bilateral)

Grade 5 Fatal AE
- Death

All AEs and SAEs will be coded using MedDRA version 12.0 for reporting to the FDA, DSMB, and Institutional Review Boards (IRBs), as required.

11.1.4 Assessment of Relationship of AE to Treatment

The possibility that the AE is related to study agent will be classified as one of the following: unrelated, unlikely, possible, probable, and definite as described below:

- Unrelated (There is no evidence of causal relationship). Previous term was "Not Related."
- Unlikely (There is *little* evidence to suggest there is a causal relationship (*e.g.*, the event did not occur within a reasonable time after administration of the trial medication). There is *another reasonable explanation* for the event (*e.g.*, the participant's clinical condition, other concomitant treatments).
- Possible (There is *some* evidence to suggest a causal relationship (*e.g.*, the event occurred within a reasonable time after administration of the trial medication). However, the influence of *other factors may have contributed* to the event (*e.g.*, the participant's clinical condition, other concomitant events).
- Probable (There *is evidence* to suggest a causal relationship, and the influence of other

factors is *unlikely).*

- Definite (There is *clear* evidence to suggest a causal relationship and other possible contributing factors can be *ruled out*).

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices, until resolution/baseline or 12 months, whichever occurs first. These events will be collected based on clinic visits planned for months six and 12 (± one month).

11.2 Serious Adverse Events

11.2.1 SAE Definition

Fed. Reg. 75, Sept. 29, 2010 defines an SAE as an event, occurring at any dose, which meets any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon the appropriate medical judgment, they may jeopardize the participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed.

11.2.2 Reporting Serious Adverse Events to QLHC

The organizations will report SAEs on the I-SPY 2 TRIAL SAE Report Form. The DCC safety department must be notified within 24 hours of knowledge of the event. The organization must submit the SAE report form within 48 hours of knowledge of the event. Please refer to the I-SPY 2 Manual of Operations for completion and submission guidelines.

Include the following information when calling:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call-back phone number
- Affiliation/Institution conducting the study
- Protocol number, title of protocol
- Description of the SAE, including reason serious and attribution to drug(s)

DCC will triage the reported information and inform the study interim Medical Monitor, below:

Sausan Abouharb, MD
Assistant Professor, Breast Medical Oncology, The University of Texas MD Anderson Cancer Center
15151 Holcombe Boulevard, Unit 1354
Houston, TX 77030 USA
Phone: (713) 745-3543
Email: sabouharb@mdanderson.org

The Medical Monitor and safety/regulatory staff will determine which SAEs require expedited FDA submission as safety reports. SAEs and AEs will be communicated to the relevant agent manufacturer per their individual safety reporting requirements regarding timing, frequency, and format.

All investigational sites will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

Follow-up of SAE: Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the study-specific SAE Report Form in the appropriate format. Follow-up information should be sent to the DCC Safety Department as soon as available. Ongoing SAEs will be followed until resolved or up to 12 months. These events will be collected in the study database.

12. STUDY OVERSIGHT AND MONITORING

This study is sponsored by QLHC, a 501(c)3 dedicated to the delivery of innovative healthcare solutions. Other partners involved in the trial's design and development include the FDA, University of California, San Francisco (UCSF), Berry Consultants, LLC and other collaborating regulatory agencies and investigators. I-SPY 2 Project Oversight Team ensures the effective, efficient and ethical conduct of the trial on behalf of QLHC and all participating agencies and sectors. The I-SPY 2 Executive Operations Group is responsible for the scripting and execution of the study protocol, as well as oversight of the trial operations. The I-SPY Committees (Agents, Biomarkers, Informatics, Pathology, Publications, Regulatory and Site Operations) in conjunction with the I-SPY 2 Project Management team will guide the trial's conduct both in the United States and internationally. Strict monitoring guidelines for phase 2 trials will apply and the utmost effort will be paid to the collection of data that is in compliance with FDA and other participating regulatory agency guidelines.

12.1 Data Management

This study, based on the established I-SPY 2 TRIAL Data Access and Publication Guidelines, will collect and report clinical trial data using the NCI-sponsored TRANSCEND platform managed by UCSF. The Clinical Trial Data Capture (CTDC) application, a component of TRANSCEND, will be the database of record for the clinical trial data participant for the sponsor and FDA to audit. Due to the complex nature of the statistical design, a randomization engine with webservice was developed and is included as a component of TRANSCEND. In order to perform the required calculations for decision making and to perform randomization, all data relevant to the statistical modeling will be de-identified and flow from the CTDC application to the randomization engine via webservice; results will be returned to the CTDC. All participant case report forms will be submitted according to Table 12.1 in §12.2 and the DCC will review data in real time. All application users will be trained to use the system and will comply with the instructions in the protocol-specific "User Manual" provided by the UCSF/TRANSCEND team as well as applicable regulatory requirements such as 21 CFR Part 11. De-identified study data will be available in the caINTEGRATOR analysis portal for approved users, as outlined in the I-SPY 2 TRIAL Data Access and Publication Guidelines posted on the I-SPY 2 website:. http://www.ispytrials.org/

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRFs) utilizing cancer data standards registry and repository (caDSR) common data elements (CDEs). Study staff will enter data into the web-based eCRF in TRANSCEND; see submission schedule in Table 12.1. Instructions on how to use the TRANSCEND data capture system are provided in the TRANSCEND user's manual as part of the MOP.

The ability to randomize a participant and to ensure the randomization engine is updated with the most current response data is dependent on timely completion of the CRFs listed in Table 12.1. For a detailed submission schedule, refer to the MOP §TRANSCEND User Manual, I-SPY 2 Case Report Form Instructions.

Table 12.1 Case Report Form Submission Schedule

Form	When form is to be completed
Pre-eligibility Checklist	Completed for each participant considered for I-SPY 2 (also used to track pre-screen failures)
Registration Form	For each participant signing a screening consent form.
Menopausal Status Form	For each participant signing a screening consent form.
Tissue Specimen Form	For each core biopsy done for the study (pre-treatment, early paclitaxel treatment, surgery).
On-study Eligibility Form	Once before randomization.
On-study Pathology Form	Once before randomization.
MammaPrint Form	Agendia will complete before randomization.
MRI Volume Form	For each MRI done for the study (pretreatment, early paclitaxel treatment, inter-regimen, presurgery).
Blood Specimen Form	For each blood collection done for the study (pretreatment, early paclitaxel treatment, inter-regimen, presurgery).
Response Evaluation Form	Before baseline core biopsy, before first AC infusion, presurgery.
Baseline Symptom Form	Within 2 weeks after signing screening consent.

Form	When form is to be completed
Lab and Test Form	Baseline: Laboratory blood test and ECHO/MUGA Treatment: Laboratory blood test for each paclitaxel*,AC cycle. ECHO/MUGA every 3 months for HER2+ participants (or as required for investigational agents).
Randomization Form	Once after participant has been randomized
AE Form	Paclitaxel Regimen*: cycle 1, 4, 7, 10 AC Regimen: cycle 1, 2, 3, 4 (study cycles 13–16). Surgery: within 2 weeks before surgery. Follow-up:30 days, 6 and 12 months post-surgery.
Chemo Treatment Form	Every treatment: Paclitaxel*: cycle 1–12 AC: cycle 1-4 (study cycles 13-16)
Chemo Summary From	Once after neoadjuvant chemotherapy is complete
Post Surgery Summary Form	After primary surgery following neoadjuvant chemotherapy; update RCB if second surgery has tumor present.
Follow-up Form	Every 6 months for 5 years from date of initial surgery and at death.
Lost to Follow-up Form and No Longer Lost to Follow-Up Form	As needed.
Off-study Form	As needed.
Protocol Violation Form	As needed.

*For participants randomized to an investigational arm that does not include paclitaxel, refer to agent-specific appendix for additional submission guidelines. (Appendices D-N)

NOTE: If a participant withdraws from the Screening Phase, complete all screening CRFs up to the point of withdrawal and complete the Randomization CRF indicating why the participant withdrew (see TRANSCEND user's manual in MOP for more details).

NOTE: If a participant is approached for the study but declines to join the study, see TRANSCEND user's manual in MOP for how to document this.

12.3 Source Documents

All source documents will be maintained at the investigational sites in the TRANSCEND clinical trial data capture system, as specified in the TRANSCEND user's manual. Participants' research charts or electronic medical records containing the source documents, including laboratory records for verification of eligibility and data to confirm molecular classification, as well as other data which will be entered into the eCRFs. As instructed, source documents will be de-identified to maintain participant confidentiality, digitized, and electronically stored in the eCRF as part of the TRANSCEND database. The source documents will be used for off-site quality control and verification by the DCC.

12.4 Data and Safety Monitoring Plan

A DSMB has been formed to assure participant safety in this clinical trial. As outlined in the I-SPY 2 TRIAL DSMB Charter, DSMB members will also have additional responsibility for assurance that the trial is conducted to a high standard, and they may be involved in conduct and interpretation of data analyses for efficacy in addition to their primary responsibility for participant safety. The responsibilities of this group include reviewing quantitative recruitment and compliance progress for the study, and recommending modifications of the trial protocol and/or administrative structure in the event these goals are not met. The committee will also review tabulated aggregate toxicity and endpoint data. The committee will submit written recommendations on the progress of the study to the study Principal Investigator and Biomarkers Consortium Project Team.

The DSMB will include a panel of experts recruited from outside of the institutions involved in this study. The DSMB will meet monthly during the study.

12.5 QLHC or FDA Monitoring

QLHC (or their designee), the lead clinical site (UCSF), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc.*), as well as IRB records and other regulatory documentation, will be retained by the investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), FDA regulations and guidances, and NIH requirements unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). QLHC will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the FDA. If any part of the study is done outside of the US, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Clinical Trials Agreement

Investigational agents are provided under a Clinical Trials Agreement (CTA) between Agent Manufacturer and the QLHC.

Data, Intellectual Property, and publications are available in the I-SPY 2 Project Plan available on the I-SPY 2 website: http://www.ispytrials.org/

13. I-SPY 2 STATISTICAL CONSIDERATIONS

13.1 Sample Size/Accrual Rate

For any given agent, a minimum of 20 and a maximum of 120 participants will accrue prior to agent being dropped or graduated from the trial. (If assignment of a regimen is to be restricted to patients with tumors that are either HER2+ or HER2– then the maximum total sample size for that regimen is 75). Under a Master IND, new agents will replace the agents that leave the trial. 20% of participants will be randomized to a control arm. Up to 25 sites will participate with an anticipated minimum monthly accrual of 15–20, or 180–240 per year. We anticipate that I-SPY 2 will be a standing trial constantly replacing agents leaving the trial with new agents.

13.2 Randomization and Stratification Using Biomarker Signatures

Upon entry to the trial, participants will be categorized according to their disease subtypes based on three standard biomarkers: hormone receptor status (both ER– and PgR–, either ER+ or PgR+, HER2 status (normal [–], positive [+]), and MammaPrint status High1 [MP–] or High2 [MP+]). MammaPrint High 1 and High 2 are determined by the predefined median cut-point of I-SPY 1 participants who fit the eligibility criteria for I-SPY 2. Therefore, there are eight (= 2x2x2) subtypes possible. The goal of the trial is to determine for which disease subtypes—if any—each experimental regimen is an improvement over control therapy. Table 1 shows the eight possible subtypes with prevalences observed in I-SPY 1.

Table 13.1 Prevalence of Eight Disease Subtypes in I-SPY 1

	MammaPrint High 1 (MP–)		**MammaPrint High 2 (MP+)**	
	Hormone Receptor+	**Hormone Receptor–**	**Hormone Receptor+**	**Hormone Receptor–**
HER2+	**16%**	**7%**	**4%**	**10%**
HER2–	**23%**	**6%**	**6%**	**28%**

Limited sample sizes and low prevalences make it impossible to carry out inferences within disease subtypes considered separately [34, 49]. For example, it would be impossible to decide that a regimen benefits participants with specifically HR+, HER2+, MP+ tumors, because the prevalence of such tumors in I-SPY 1 was only 4%. Moreover, such a small subpopulation would not be of marketing interest to any collaborating pharmaceutical company. Further, if a regimen were effective in HR+, HER2+, MP– tumors, HR–, HER2+, MP– tumors, and HR–, HER2+, MP+ tumors, it would almost certainly be effective in HR+, HER2+, MP+ tumors. A limited number of subsets of disease subtypes are of scientific and marketing interest; we call these "biomarker signatures." We consider 10 signatures, listed in Table 13.2, with prevalences estimated from I-SPY 1. "X" indicates that the respective disease subtype is included in each signature.

Table 13.2 Ten Candidate Biomarker Signatures

Biomarker Signature	Types (Hormone Receptor, HER2, MP)								Estimated prevalence
	+++	++–	+–+	+– –	–++	–+–	– –+	– – –	
1 (All)	X	X	X	X	X	X	X	X	100%
2 (HR+)	X	X	X	X					49%
3 (HR–)					X	X	X	X	51%
4 (HER2+)	X	X			X	X			37%
5 (HER2–)			X	X			X	X	63%
6 (MammaPrint+)	X		X		X		X		48%
7 (– –)*							X	X	34%
8 (–+)					X	X			17%
9 (++)	X	X							20%
10 (+–)			X	X					29%

*Signature #7 is called "triple negative" because ER, PgR, and HER2 are all negative.
Estimated prevalences are from I-SPY 1 (Table 1).

Some signatures overlap. Indeed, some signatures are subsets of other signatures. Signature #1 contains every other signature as a subset. Signature #10 is relatively small and is contained in Signatures #2 and #5 as well as #1.

A participant's category is the subtype of her tumor—the eight possible subtypes (for driving treatment assignment in this trial) shown in Table 13.1. Assignments to therapy will be on the basis of subtype using the current information about the efficacy of the various regimens for that subtype (see analysis of covariance in Appendix A for I-SPY 2 Statistical Considerations). Experimental regimens will be evaluated for their efficacy relative to control within individual subtypes (see analysis of covariance in the (see Appendix A for I-SPY 2 Statistical Considerations). However, the utility of each treatment regimen will be evaluated only for the 10 biomarker signatures shown in Table 13.2 and not within individual subtypes.

Some of the 10 possible signatures may be inappropriate for a particular experimental regimen. A pharmaceutical company collaborator may request that its agent not be used for some subtypes. Should the Agents Working Group agree, the agent will be a candidate for the trial only if the subtypes considered form one of the signatures in Table 13.2. For example, an agent that targets HER2 may not be appropriate for participants with HER2– disease. Such an agent would have Signature #4 as its "base signature." It would be evaluated for Signatures #4, #8, and #9 only. Agents entering the trial without restrictions have Signature #1 as their base signature.

It is theoretically possible to restrict signatures to those that are highly prevalent. For example, a company might request that its agent be evaluated for Signature #1 only. A consequence would be that if the agent does poorly (relative to control) for HR+ tumors, it may be dropped from the trial for lack of efficacy even if it shows a benefit for HR– tumors. However, if signature #3 (HR–) had been allowed in addition to signature #1, the agent might continue in the trial but would be not used for participants who are HR+. Despite this theoretical possibility, we specifically disallow such restrictions. In particular, if an agent is open to being assigned to any participant subtype, it will be evaluated on the basis of all 10 possible signatures.

The "control base signature" is #1 (all participants) in the sense that throughout the trial, control will have a positive assignment probability for each biomarker type.

Any number of experimental regimens may be considered simultaneously in the trial, however, for practical purposes the maximum number of experimental regimens considered at any given time will be limited to 5. A smaller number is likely, especially early in the trial. Regimens may be added over time by the I-SPY 2 Agent Selection Committee (after review and approval by the Independent Agent Selection Committee). In deciding whether to add a regimen, the I-SPY 2 Agent Selection Committee will consider the trial's accrual rate and the number of regimens currently being considered for each of the eight biomarker subtypes. A tentative guideline is that no experimental regimen should be under active consideration (being assigned to participants) for more than 18 months, and preferably less. If the present and projected accrual and the current number of regimens in the trial mean that this goal will not be met, a new regimen should not be added.

13.3 Primary Endpoint and Probability of Success by Biomarker Signature

Participant assignment to one of the regimens in the trial will be randomized and adaptive. Namely, regimens that are performing better for the participant's biomarker type (assessed based on modeling described below) will have a greater assignment probability. A consequence is that participants in the trial are likely to receive better therapy and the better-performing regimens will proceed more rapidly through the trial. Regimens that perform sufficiently poorly will be dropped from the trial, as described below.

Continuously throughout the trial, each regimen's (Bayesian predictive) probability of being successful in a phase 3 confirmatory trial will be calculated for each biomarker signature [34]. These probabilities will be used in making trial decisions (recommendations to the trial's DSMB), as follows:

- A regimen will be dropped from the trial for futility should its predictive probability drop sufficiently low for all biomarker signatures. A minimum of 20 participants will be enrolled on a regimen before dropping a regimen.
- A regimen will graduate from the trial should one or more of its predictive probabilities reach a sufficiently high level. A minimum of 60 participants will be enrolled on a regimen before it can graduate. The exception is when assignment to an experimental regimen is restricted to patients with tumors that are either HER2+ or HER2– then the minimum number of patients assigned to the regimen is 35.
- If the maximum sample size of 120 participants assigned to a regimen (over all biomarker types) is reached, assignments to the regimen will end. The exception is when assignment of a regimen is to be restricted to patients with tumors that are either HER2+ or HER2– then the maximum total sample size for that regimen is 75.

Six months after a regimen graduates or when the maximal sample size is achieved, all participants are expected to have had surgery and the primary endpoint of pCR assessed. At that time the Bayesian predictive probabilities of a successful phase 3 trial for all biomarker signatures will be provided to the appropriate collaborating companies.

13.4 Design Algorithm Overview

The primary endpoint in the trial is pCR. We will use Bayesian logistic regression to model the relationship between six-month pCR rate and the predictor variables—including treatment and biomarkers HR, HER2, and MP statuses. This model will account for the control treatment effect (depending on biomarker subtype), baseline biomarker type, and for the possibility of an interaction between biomarker and experimental treatment. In addition, we also include terms that allow the interaction of biomarkers with each other. By including an interaction term for each experimental treatment and biomarkers, we can identify treatment effects for each potential signature. This model is

described in the attached Appendix A for I-SPY 2 Statistical Considerations—including the mathematical details.

We use an outcome-adaptive randomization trial design. The assignment of a participant to therapy will depend on all available data that will be updated on a regular basis. The goal is to learn as rapidly as possible which treatments are effective for which biomarker types [34]. Since pCR is assessed at surgery which will be approximately six months after treatment is initiated, waiting until each participant's outcome has been assessed is less than ideal. To enable using earlier information about the primary endpoint, we will employ a longitudinal model of outcome based on MRI measurements assessed at baseline, end of week 3 after starting paclitaxel, end of week 12 after starting paclitaxel, and end of doxorubicin/cyclophosphamide (weeks 28–30). These measurements are not perfectly predictive of pCR, but are correlated with it. Our longitudinal model will incorporate the empirical information about the predictability of pCR from MRI measurements over time and will be based on the longitudinal information from I-SPY 1 results as a prior distribution. The MRI measurements are not endpoints in themselves but serve as "auxiliary markers" for the primary endpoint of pCR [34].

The logistic model of pCR (see Appendix A for I-SPY 2 Statistical Considerations) will be used to assess the performance of experimental treatments relative to control and by biomarker type. Throughout the trial, decisions must be made for each experimental treatment to either "graduate" it and recommend progressing to phase 3, terminate it for futility, or continue it in the trial to accrue more information. Moreover, when an experimental therapy is continued in the trial, its assignment probabilities by biomarker type must be updated based on currently available results, including MRI measurements. This updating is based on the logistic model. Determining the course of action utilizes the predictive probability of future success in a phase 3 study. If the predictive probability of future success is sufficiently high, the regimen will "graduate" and its various signature predictive probabilities reported.

Information from I-SPY 1 will provide a basis for building the prior distribution for the control pCR rate, the baseline biomarker effects, and longitudinal model parameters. We will analyze the data from the I-SPY 1 clinical trial with the model described in Appendix A for I-SPY 2 Statistical Considerations, and obtain I-SPY 1 posterior distributions for the standard of care effect, baseline biomarker effects, and longitudinal model effects. For the current trial, we assume that prior distributions for the model parameters are normally distributed with means equal to the means obtained for the analysis of the I-SPY 1 data. The standard deviations of these distributions will be suitably inflated in recognition of the possible differences in the two trial populations.

13.5 Assessing Operating Characteristics of the Design via Simulation

The false-positive rate is the major scientific/inferential stumbling block in a study investigating the benefits of many treatment arms in the presence of many biomarker profiles [34]. False positives must be controlled. On the other hand, they cannot be controlled so tightly as to dramatically lower the true-positive rate. Since the design used in the trial will be completely prescribed in advance, we can address these issues and related design performance issues via simulation.

Simulations require inputs. We call each set of inputs a "scenario." This is a specification of the pCR rates for each regimen (including control) and for each of the eight biomarker subtypes.

We consider a variety of possible scenarios ranging from very pessimistic, in which the null hypothesis of no benefit holds for every regimen and every signature considered, to optimistic cases in which several of the regimens are truly effective, and for several signatures each. For each scenario, and following the design we build, we enter participants into a virtual trial and simulate outcomes for them. When the "trial" is over we record various summaries of the trial results, including the duration of the period in

which participants were randomized to each experimental regimen, whether the results were "positive" and for which regimens, numbers of participants within the various biomarker types who were assigned to each regimen, and the predictive probability that each regimen would be successful in a phase 3 study. We repeat this trial simulation procedure 1000 times to find the operating characteristics of the design.

The results of the simulation study are presented below. For each scenario, we report the average number of participants on the control and each agent, average time from the first participant to the last participant treated with each agent, and various probabilities of "graduation". As part of the simulation process, we have calibrated the decision cutoffs to obtain a 10% false-positive rate. The Technical Details section below describes in detail the statistical model and the decision rules implemented in the design.

We assume that each experimental regimen will be assigned a maximum of 120 participants. If assignment of a regimen is to be restricted to patients with tumors that are either HER2+ or HER2– then the maximum total sample size for that regimen is 75.

In the scenarios below when a treatment provides a benefit, the true pCR rate is bolded and has a log-odds-ratio of 1.5 compared to the standard treatment. The results for each scenario are presented in three tables. The first table, labeled "True pCR rates", provides the true pCR rates as well as the "true" signature for a therapy that provides benefit. The second table givens the average number of participants enrolled in each subtype for each arm and the total enrolled to each arm. The third table provides the "Graduation" probabilities for each signature. Since an arm may graduate for a subset of the population, we cannot simply report a single probability of graduating but rather categorize the probability of graduation as follows:

> P1 = Pr(Graduating for the true signature)—*e.g.*, we graduate a therapy for the HR+ signature when the therapy benefits only the HR+ participants.
>
> P2 = Pr(Graduating for a signature that contains all subtypes that benefit)—*e.g.*, for a therapy that benefits the HR+ participants, we graduate the therapy for the HR+ signature or the All signature.
>
> P3 = Pr(Graduating for a signature that contains only subtypes that benefit)—*e.g.*, for a therapy that benefits the HR+ participants we graduate the therapy for the HR+ or the (++) signature.
>
> P4 = Pr(Graduating for a signature that contains at least one subtype that benefits)—*e.g.*, for a therapy that benefits the HR+ participants, we graduate the therapy for any signature that contains a subtype also contained in the HR+ signature.
>
> P5 = Pr(Graduating for a signature that contains only subtypes that do NOT benefit)—*e.g.*, for a therapy that benefits the HR+ we graduate the HR– signature. This is a false positive conclusion.

How well the design does at graduating therapies depends on the increase in pCR rate as well as the size of the signature for which it provides an improvement. We consider a signature larger than another signature if it has a larger estimated prevalence. For example, the HER2– signature is larger than the HER2+ signature because it has a larger prevalence, even though the two signatures contain the same number of subtypes (Table 1). We begin with a few key points for select scenarios, then provide an overall interpretation of how the design performs.

In general, as the prevalence of the true signature increases so does the probability that the design graduates the treatment for the true signature or graduates it for any signature. The probability of graduating an arm for the true signature ranges from 0.76 for the triple negative signature (prevalence =

34%) to 0.86 for the All signature (prevalence = 100%) and the probability of graduating an arm for a signature that contains at least one subtype that benefits ranges from 0.87 for the triple negative signature to 0.99 for the All signature. In contrast, the prevalence of the true signature is inversely related to the average time an arm remains in the trial and the average sample size for an arm. That is, as the prevalence increase from 34% to 100%, the average time in the trial decreases from 16.3 months to 12.9 months and the average sample size for the experimental arm decreases from 83 to 74. In other words, treatments that benefit a larger portion of the population are more likely to graduate sooner and with fewer participants than treatments that benefit a smaller portion of the population. In the scenarios that have two treatments providing a benefit, we observed little reduction in the design's performance when the two treatments have the same true signature or signatures that overlapped in a few subtypes; see scenarios 7 and 9. In scenarios where E1 and E2 had true signatures that did not overlap a slight improvement was observed.

In Scenario 1, none of the experimental treatments provide an improvement over the standard treatment. The average number of participants enrolled per experimental treatment is 73.4 and the average time each therapy remains in the trial is about 17 months. The probability that we make an error and graduate an experimental treatment is approximately 10%. That is, the false-positive rate is approximately 10%.

In Scenario 2, E1 provides an improvement for all participants and as expected E1 graduates very quickly from the trial in about 13 months with an average of 74.2 participants treated. The design graduates E1 86% of the time for the true signature and 99% of the time graduates for a signature that contains only subtypes that benefit. The average sample size for E1 is nearly equal to all other treatments because the adaptive randomization assigns a larger portion of the participants to it, thus graduating it quickly whereas the other treatments remain in the study accruing participants for a longer duration. During the time that E1 is in the study it receives approximately half of the participants that enter the study.

In Scenario 3, the true signature is HER2+, which has an estimated prevalence of 37%. Since the prevalence for the true signature is low, this is a difficult scenario. The design graduates E1 for the HER2+ 76.6% of the time and graduates the arm for a signature that contains at least one subtype that benefits 87% of the time.

In Scenario 4 the true signature for E1 is HR+, which has an estimated prevalence of 49%. On average, E1 remains in the trial for 15.7 months and enrolls 79.8 participants. The design graduates E1 for the true HR+ signature 72% of the time and graduates E1 for a signature that contains at least one subtype that benefits 83% of the time. It is important to note that there a 12% chance of graduating E1 for a signature that contains only subtypes that do not benefit.

In Scenario 5 the true signature for E1 is triple negative, which has an estimated prevalence of 33%. On average, E1 remains in the trial for 16 months and enrolls 82.8 participants. The design graduates E1 for the true triple negative signature 74% of the time and graduates E1 for a signature that contains at least one subtype that benefits 82% of the time. It is important to note that there a 16% chance of graduating E1 for a signature that contains only subtypes that do not benefit.

In Scenarios 7–9 there is more than one treatment that provides a benefit for the participants. In Scenarios 7 and 9, the true signatures for E1 and E2 were the same or overlapped. If we have two treatments where the true signature is All, the probability of graduating for the true signature does not reduce when compared to a trial with only one treatment that has the true signature of All. However, the treatments do remain in the study for about 1 month longer than they would if there was no other treatment that provided a benefit.

Scenarios 10–13 allow for treatment to enter the study at times other than the start of the trial and have more than experimental treatment. In Scenario 10, E3 enters the study at the start of month 7 and true

signature for both E1 and E3 is All. By allowing E3 to enter the study at month 7 rather than the start of the study, the average number of participants enrolled to E3 is reduced and the amount of time E3 remains in the study is reduced. This is due to the fact the participants are randomized to fewer arms and thus more information can accrue in each arm in a shorter period of time. The probability of graduating E3 for the All signature is about 5% higher than it is for E1.

In Scenario 11 E1 enters the study at month 7 and E4 at month 9. The true signature for E2 is HER2+ and E4 is HER2–. This scenario is comparable to Scenario 8 in terms of the signatures the arms provide a benefit for, except that the treatments do not all start at the beginning of the study. The probability of graduating E1 for the HER2+ signature is reduced by about 10% when compared to Scenario 8. This is largely due to the addition of the fourth experimental arm. The performance for the arm that benefits the HER2– (E3) participants is much less because shortly after the arm enters the study, E1 and E2 exit the study.

Table 13.3 Scenario 1, True pCR Rates

	True pCR Rates			
Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	0.41	0.41	0.41
HR+HER2+MP-	0.17	0.17	0.17	0.17
HR+HER2-MP+	0.23	0.23	0.23	0.23
HR+HER2-MP-	0.037	0.037	0.037	0.037
HR-HER2+MP+	0.61	0.61	0.61	0.61
HR-HER2+MP-	0.56	0.56	0.56	0.56
HR-HER2-MP+	0.37	0.37	0.37	0.37
HR-HER2-MP-	0.24	0.24	0.24	0.24
True Signature		None	None	None

Hormone Receptor (HR); MammaPrint High 2 (MP+); MammaPrint High 1 (MP-); Investigational agent 1, 2, 3 (E1, E2, E3)

Table 13.4 Scenario 1, Average Number of Participants by Subtype

Cell	**S**	**E1**	**E2**	**E3**
HR+HER2+MP+	1.9	3	3	3
HR+HER2+MP-	7.6	11.7	11.7	11.7
HR+HER2-MP+	2.9	4.5	4.5	4.5
HR+HER2-MP-	10.9	16.9	16.9	16.9
HR-HER2+MP+	4.9	7.4	7.4	7.4
HR-HER2+MP-	3.4	5.1	5.1	5.1
HR-HER2-MP+	13.3	20.5	20.5	20.5
HR-HER2-MP-	2.8	4.3	4.3	4.3
Total	47.8	73.4	73.4	73.4

Table 13.5 Scenario 1, Graduation Probabilities (P)

Arm	**P1**	**P2**	**P3**	**P4**	**P5**	**Ave. Time in Trial**
E1	0	0	0	0	0.097	17.2
E2	0	0	0	0	0.097	17.2
E3	0	0	0	0	0.097	17.2

Scenario 2
Prevalence of true signature for E1 = 100%

	True pCR Rates			
Cell	**S**	**E1**	**E2**	**E3**
HR+HER2+MP+	0.41	**0.757**	0.41	0.41
HR+HER2+MP-	0.17	**0.479**	0.17	0.17
HR+HER2-MP+	0.23	**0.572**	0.23	0.23
HR+HER2-MP-	0.037	**0.147**	0.037	0.037
HR-HER2+MP+	0.61	**0.875**	0.61	0.61
HR-HER2+MP-	0.56	**0.85**	0.56	0.56
HR-HER2-MP+	0.37	**0.724**	0.37	0.37
HR-HER2-MP-	0.24	**0.59**	0.24	0.24
True Signature		All	None	None

	Average Number of Participants			
Cell	**S**	**E1**	**E2**	**E3**
HR+HER2+MP+	1.9	**3.1**	2.9	2.9
HR+HER2+MP-	7.6	**11.5**	11.5	11.5
HR+HER2-MP+	2.8	**4.4**	4.3	4.3
HR+HER2-MP-	10.7	**16.1**	16.9	16.9
HR-HER2+MP+	4.7	**7.7**	7.3	7.3
HR-HER2+MP-	3.4	**5**	5.2	5.2
HR-HER2-MP+	13	**22.1**	19.2	19.2
HR-HER2-MP-	2.9	**4.4**	4.3	4.3
Total	47	74.2	71.4	71.4

Graduation Probabilities

Arm	**P1**	**P2**	**P3**	**P4**	**P5**	**Ave. Time in Trial**
E1	0.86	0.86	0.993	0.993	0	12.9
E2	0	0	0	0	0.095	17.2
E3	0	0	0	0	0.095	17.2

Scenario 3
Prevalence of true signature for E1 = 37%

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	**0.757**	0.41	0.41
HR+HER2+MP-	0.17	**0.479**	0.17	0.17
HR+HER2-MP+	0.23	0.23	0.23	0.23
HR+HER2-MP-	0.037	0.037	0.037	0.037
HR-HER2+MP+	0.61	**0.875**	0.61	0.61
HR-HER2+MP-	0.56	**0.85**	0.56	0.56
HR-HER2-MP+	0.37	0.37	0.37	0.37
HR-HER2-MP-	0.24	0.24	0.24	0.24
True Signature		HER2+	None	None

Average Number of Participants

Cell	S	E1	E2	E3
HR+HER2+MP+	2	**3.9**	2.6	2.6
HR+HER2+MP-	7.7	**16.2**	9.6	9.6
HR+HER2-MP+	2.9	4.4	4.4	4.4
HR+HER2-MP-	10.9	17.7	16.6	16.6
HR-HER2+MP+	4.9	**9.7**	6.4	6.4
HR-HER2+MP-	3.4	**6.8**	4.4	4.4
HR-HER2-MP+	13.3	20	20.5	20.5
HR-HER2-MP-	2.9	4.5	4.3	4.3
Total	47.9	83.1	68.8	68.8

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.766	0.783	0.816	0.873	0.062	16.3
E2	0	0	0	0	0.096	17.3
E3	0	0	0	0	0.096	17.3

Scenario 4

Prevalence of true signature for E1 = 49%

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	**0.757**	0.41	0.41
HR+HER2+MP-	0.17	**0.479**	0.17	0.17
HR+HER2-MP+	0.23	**0.572**	0.23	0.23
HR+HER2-MP-	0.037	**0.147**	0.037	0.037
HR-HER2+MP+	0.61	0.61	0.61	0.61
HR-HER2+MP-	0.56	0.56	0.56	0.56
HR-HER2-MP+	0.37	0.37	0.37	0.37
HR-HER2-MP-	0.24	0.24	0.24	0.24
True Signature		HR+	None	None

Average Number of Participants

Cell	S	E1	E2	E3
HR+HER2+MP+	2	**3.6**	2.7	2.7
HR+HER2+MP-	7.6	**14.7**	10.3	10.3
HR+HER2-MP+	2.8	**5.1**	4.0	4.0
HR+HER2-MP-	10.9	**21.2**	14.8	14.8
HR-HER2+MP+	4.8	7.1	7.6	7.6
HR-HER2+MP-	3.4	5	5.2	5.2
HR-HER2-MP+	13.4	19.1	21.1	21.1
HR-HER2-MP-	2.7	4.2	4.4	4.4
Total	47.7	79.8	70.0	70.0

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.721	0.758	0.791	0.834	0.123	15.7
E2	0	0	0	0	0.10	17.3
E3	0	0	0	0	0.10	17.3

Scenario 5
Prevalence of true signature for E1 = 34%

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	0.41	0.41	0.41
HR+HER2+MP-	0.17	0.17	0.17	0.17
HR+HER2-MP+	0.23	0.23	0.23	0.23
HR+HER2-MP-	0.037	0.037	0.037	0.037
HR-HER2+MP+	0.61	0.61	0.61	0.61
HR-HER2+MP-	0.56	0.56	0.56	0.56
HR-HER2-MP+	0.37	**0.724**	0.37	0.37
HR-HER2-MP-	0.24	**0.59**	0.24	0.24
True Signature		(--) Triple Negative	None	None

Average Number of Participants

Cell	S	E1	E2	E3
HR+HER2+MP+	1.9	2.7	3.0	3.0
HR+HER2+MP-	7.7	10.2	12.5	12.5
HR+HER2-MP+	2.9	4.9	4.3	4.3
HR+HER2-MP-	11	17.6	16.5	16.5
HR-HER2+MP+	4.8	7.7	7.2	7.2
HR-HER2+MP-	3.4	5	5.3	5.3
HR-HER2-MP+	13.4	**28.8**	16.5	16.5
HR-HER2-MP-	2.8	**5.8**	4.0	4.0
Total	48	82.8	69.1	69.1

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.743	0.797	0.743	0.822	0.16	16
E2	0	0	0	0	0.088	17.2
E3	0	0	0	0	0.088	17.2

Scenario 6
Prevalence of true signature for E1 = 48%

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	**0.757**	0.41	0.41
HR+HER2+MP-	0.17	0.17	0.17	0.17
HR+HER2-MP+	0.23	**0.572**	0.23	0.23
HR+HER2-MP-	0.037	0.037	0.037	0.037
HR-HER2+MP+	0.61	**0.875**	0.61	0.61
HR-HER2+MP-	0.56	0.56	0.56	0.56
HR-HER2-MP+	0.37	**0.724**	0.37	0.37
HR-HER2-MP-	0.24	0.24	0.24	0.24
True Signature		MP2+	None	None

Average Number of Participants

Cell	S	E1	E2	E3
HR+HER2+MP+	2	**3.6**	2.7	2.7
HR+HER2+MP-	7.5	11	12.3	12.3
HR+HER2-MP+	2.9	**5.6**	3.8	3.8
HR+HER2-MP-	11.1	16.9	17.0	17.0
HR-HER2+MP+	4.8	**9.4**	6.4	6.4
HR-HER2+MP-	3.3	4.7	5.4	5.4
HR-HER2-MP+	13.5	**29.4**	16.1	16.1
HR-HER2-MP-	2.8	4.6	4.3	4.3
Total	47.9	85.1	67.8	67.8

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.847	0.855	0.847	0.924	0	15.8
E2	0	0	0	0	0.095	17.3
E3	0	0	0	0	0.095	17.3

Scenario 7
Prevalence of true signature for E1 = E2 = 100%, arm E2 enters the study at month 7.

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	**0.757**	**0.757**	0.41
HR+HER2+MP-	0.17	**0.479**	**0.479**	0.17
HR+HER2-MP+	0.23	**0.572**	**0.572**	0.23
HR+HER2-MP-	0.037	**0.147**	**0.147**	0.037
HR-HER2+MP+	0.61	**0.875**	**0.875**	0.61
HR-HER2+MP-	0.56	**0.85**	**0.85**	0.56
HR-HER2-MP+	0.37	**0.724**	**0.724**	0.37
HR-HER2-MP-	0.24	**0.59**	**0.59**	0.24
True Signature		All	All	None

Average Number of Participants

Cell	S	E1	E2	E3
HR+HER2+MP+	1.9	**2.9**	**2.9**	2.8
HR+HER2+MP-	7.5	**11.6**	**11.6**	10.9
HR+HER2-MP+	2.8	**4.4**	**4.4**	4.1
HR+HER2-MP-	10.7	**16.3**	**16.3**	16.1
HR-HER2+MP+	4.6	**7.3**	**7.3**	7.2
HR-HER2+MP-	3.2	**5.0**	**5.0**	5.1
HR-HER2-MP+	12.8	**20.8**	**20.8**	18.4
HR-HER2-MP-	2.7	**4.4**	**4.4**	4.1
Total	46.1	**72.6**	**72.6**	68.6

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.86	0.86	0.99	0.99	0	13.8
E2	0.86	0.86	0.99	0.99	0	13.8
E3	0	0	0	0	0.072	17.2

Scenario 8
Prevalence of true signature for E1 = 37% and E2 = 67%

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	**0.757**	0.41	0.41
HR+HER2+MP-	0.17	**0.479**	0.17	0.17
HR+HER2-MP+	0.23	0.23	**0.572**	0.23
HR+HER2-MP-	0.037	0.037	**0.147**	0.037
HR-HER2+MP+	0.61	**0.875**	0.61	0.61
HR-HER2+MP-	0.56	**0.85**	0.56	0.56
HR-HER2-MP+	0.37	0.37	**0.724**	0.37
HR-HER2-MP-	0.24	0.24	**0.59**	0.24
True Signature		HER2+	HER2-	None

Average Number of Participants

Cell	S	E1	E2	E3
HR+HER2+MP+	2	**3.9**	2.6	2.5
HR+HER2+MP-	7.6	**16.9**	9	9.6
HR+HER2-MP+	2.9	3.9	**5.4**	3.8
HR+HER2-MP-	10.8	16.3	**19.7**	14.7
HR-HER2+MP+	4.8	**9.6**	6.3	6.2
HR-HER2+MP-	3.5	**7.1**	3.8	4.5
HR-HER2-MP+	13.3	16.8	**27.3**	17.2
HR-HER2-MP-	2.9	4	**5.3**	3.8
Total	47.7	78.6	79.2	62.3

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.802	0.81	0.843	0.888	0.046	16.6
E2	0.759	0.781	0.893	0.93	0.089	15.2
E3	0	0	0	0	0.09	17.3

Scenario 9
Prevalence of true signature for E1 = 49% and E2 = 63%

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	**0.757**	0.41	0.41
HR+HER2+MP-	0.17	**0.479**	0.17	0.17
HR+HER2-MP+	0.23	**0.572**	**0.572**	0.23
HR+HER2-MP-	0.037	**0.147**	**0.147**	0.037
HR-HER2+MP+	0.61	0.61	0.61	0.61
HR-HER2+MP-	0.56	0.56	0.56	0.56
HR-HER2-MP+	0.37	0.37	**0.724**	0.37
HR-HER2-MP-	0.24	0.24	**0.59**	0.24
True Signature		HR+	HER2-	None

Average Number of Participants

Cell	S	E1	E2	E3
HR+HER2+MP+	1.9	**3.6**	2.7	2.5
HR+HER2+MP-	7.8	**15.5**	9.4	10.3
HR+HER2-MP+	2.8	**4.7**	**5**	3.6
HR+HER2-MP-	11	**19.6**	**17.6**	13.3
HR-HER2+MP+	4.8	7.3	7.4	7.5
HR-HER2+MP-	3.2	5.3	4.7	5.5
HR-HER2-MP+	13.3	16.5	**28.2**	16.7
HR-HER2-MP-	2.8	3.8	**5.4**	3.8
Total	47.6	76.2	80.4	63.2

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.67	0.695	0.773	0.802	0.105	16.2
E2	0.741	0.768	0.883	0.927	0.117	15.4
E3	0	0	0	0	0.09	17.4

Arms enter the study at times other than the start of the trial.

Scenario 10
Prevalence of true signature for E1 = E3 = 100%, arm E3 enters the study at month 7.

True pCR Rates

Cell	S	E1	E2	E3
HR+HER2+MP+	0.41	**0.757**	0.41	**0.757**
HR+HER2+MP-	0.17	**0.479**	0.17	**0.479**
HR+HER2-MP+	0.23	**0.572**	0.23	**0.572**
HR+HER2-MP-	0.037	**0.147**	0.037	**0.147**
HR-HER2+MP+	0.61	**0.875**	0.61	**0.875**
HR-HER2+MP-	0.56	**0.85**	0.56	**0.85**
HR-HER2-MP+	0.37	**0.724**	0.37	**0.724**
HR-HER2-MP-	0.24	**0.59**	0.24	**0.59**
True Signature		All	None	All

Average Number of Participants

Cell	S	E1	E3	E2
HR+HER2+MP+	2.2	**2.9**	2.9	**2.9**
HR+HER2+MP-	8.9	**11**	11.8	**12**
HR+HER2-MP+	3.3	**4.2**	4.4	**4.5**
HR+HER2-MP-	12.6	**15**	17.4	**17.7**
HR-HER2+MP+	5.6	**7.3**	7.6	**7.1**
HR-HER2+MP-	3.9	**4.9**	5.6	**4.9**
HR-HER2-MP+	15.3	**20.4**	19.6	**21**
HR-HER2-MP-	3.3	**4.2**	4.5	**4.4**
Total	55.1	70	73.9	74.6

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.876	0.876	0.995	0.995	0	12.1
E3	0	0	0	0	0.084	17.2
E2	0.918	0.918	0.998	0.998	0	10.3

Scenario 11
Prevalence of true signature for E1 = 37% and E3 = 67%. E3 enters the study at month 7 and E4 month 9.

True pCR Rates

Cell	S	E1	E2	E3	E4
HR+HER2+MP+	0.41	**0.757**	0.41	0.41	0.41
HR+HER2+MP-	0.17	**0.479**	0.17	0.17	0.17
HR+HER2-MP+	0.23	0.23	0.23	**0.572**	0.23
HR+HER2-MP-	0.037	0.037	0.037	**0.147**	0.037
HR-HER2+MP+	0.61	**0.875**	0.61	0.61	0.61
HR-HER2+MP-	0.56	**0.85**	0.56	0.56	0.56
HR-HER2-MP+	0.37	0.37	0.37	**0.724**	0.37
HR-HER2-MP-	0.24	0.24	0.24	**0.59**	0.24
True Signature		HER2+	None	HER2-	None

Average Number of Participants

Cell	S	E1	E2	E3	E4
HR+HER2+MP+	2.9	**3.1**	2.6	2.8	4
HR+HER2+MP-	11.6	**13.1**	10.1	10.3	15.6
HR+HER2-MP+	4.3	3.7	4.1	**5.5**	5.5
HR+HER2-MP-	16.7	14.2	15.5	**20.7**	20.4
HR-HER2+MP+	7.4	**8.1**	6.6	6.7	9.8
HR-HER2+MP-	5	**6.1**	4.6	4.3	6.8
HR-HER2-MP+	20.3	16.6	18.7	**27.4**	22.3
HR-HER2-MP-	4.4	3.8	4.2	**5.4**	4.8
Total	72.6	68.8	66.5	83	89.3

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.696	0.709	0.739	0.809	0.053	16.5
E3	0	0	0	0	0.103	17.2
E2	0.747	0.762	0.884	0.914	0.108	14.7
E4	0	0	0	0	0.138	16.8

Scenario 12
Prevalence of true signature for E1 = 37% and E3 = 67%. E3 enters the study at month 6, E4 at month 13, E5 at month 14.

True pCR Rates

Cell	S	E1	E2	E3	E4	E5
HR+HER2+MP+	0.41	**0.757**	0.41	0.41	**0.757**	0.41
HR+HER2+MP-	0.17	**0.479**	0.17	0.17	**0.479**	0.17
HR+HER2-MP+	0.23	**0.572**	**0.572**	0.23	**0.572**	0.23
HR+HER2-MP-	0.037	**0.147**	**0.147**	0.037	**0.147**	0.037
HR-HER2+MP+	0.61	0.61	0.61	0.61	**0.875**	0.61
HR-HER2+MP-	0.56	0.56	0.56	0.56	**0.85**	0.56
HR-HER2-MP+	0.37	0.37	**0.724**	0.37	**0.724**	0.37
HR-HER2-MP-	0.24	0.24	**0.59**	0.24	**0.59**	0.24
True Signature		HR+	HER2-	None	All	None

Average Number of Participants

Cell	S	E1	E2	E3	E4	E5
HR+HER2+MP+	3.5	**3.1**	2.3	2.4	**3**	4
HR+HER2+MP-	13.7	**12.6**	8.3	9.7	**12.2**	15.2
HR+HER2-MP+	5.3	**4.3**	**4.1**	3.6	**4.3**	5.7
HR+HER2-MP-	19.6	**17.6**	**14.9**	13.2	**16.1**	21.3
HR-HER2+MP+	8.6	6.3	6.3	6.8	**7.7**	9.9
HR-HER2+MP-	5.9	4.6	4	4.9	**5.1**	6.9
HR-HER2-MP+	23.6	16.7	**22.2**	17.4	**20.7**	23.1
HR-HER2-MP-	5.1	3.8	**4.7**	3.8	**4.1**	5
Total	85.4	69	66.7	61.7	73.3	91.2

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.584	0.607	0.681	0.72	0.107	15.7
E2	0.663	0.681	0.819	0.863	0.139	14.7
E3	0	0	0	0	0.091	17.2
E4	0.829	0.829	0.994	0.994	0	12.5
E5	0	0	0	0	0.150	16.5

Scenario 13
The following arms were not in the study at the beginning but joined at the following months. E3 at month 6, E4 at month 13, E5 at 17, E6 at 24, E7 at 30, E8 at 30

True pCR Rates

Cell	S	E1	E2	E3	E4	E5	E6	E7	E8
HR+HER2+MP+	0.41	**0.757**	0.41	0.41	**0.757**	0.41	0.41	0.41	**0.757**
HR+HER2+MP-	0.17	**0.479**	0.17	0.17	**0.479**	0.17	0.17	0.17	**0.479**
HR+HER2-MP+	0.23	**0.572**	0.23	**0.572**	**0.572**	0.23	0.23	0.23	0.23
HR+HER2-MP-	0.037	**0.147**	0.037	**0.147**	**0.147**	0.037	0.037	0.037	0.037
HR-HER2+MP+	0.61	0.61	0.61	0.61	**0.875**	0.61	0.61	0.61	**0.875**
HR-HER2+MP-	0.56	0.56	0.56	0.56	**0.85**	0.56	0.56	0.56	**0.85**
HR-HER2-MP+	0.37	0.37	0.37	**0.724**	**0.724**	0.37	**0.724**	0.37	0.37
HR-HER2-MP-	0.24	0.24	0.24	**0.59**	**0.59**	0.24	**0.59**	0.24	0.24
True Signature		HR+	None	HER2-	All	None	Trip-	Null	HER2+

Average Number of Participants

Cell	S	E1	E2	E3	E4	E5	E6	E7	E8
HR+HER2+MP+	4.9	**3.1**	2.5	2.3	**2.8**	2.5	2.6	2.2	**3.9**
HR+HER2+MP-	19.2	**13**	9.7	8.5	**11.3**	10.1	10.2	8.4	**15.3**
HR+HER2-MP+	7.3	**4.4**	3.6	**4.2**	**3.9**	3.5	4.6	4	4.5
HR+HER2-MP-	27.6	**17.7**	13.3	**15.2**	**14.6**	14	16.8	14.8	17.6
HR-HER2+MP+	11.9	6.4	7	6.5	**7.4**	5.9	7.1	5.4	**9**
HR-HER2+MP-	8.3	4.5	5	4.1	**5.1**	4.4	4.8	3.8	**6.4**
HR-HER2-MP+	33.4	16.7	17.6	**22.9**	**19.1**	14.6	**24.1**	16.6	18.1
HR-HER2-MP-	7.2	3.7	4	**4.7**	**4**	3.4	**5.1**	3.5	3.9
Total	119.8	69.4	62.7	68.4	68.3	58.3	75.4	58.7	78.7

Graduation Probabilities

Arm	P1	P2	P3	P4	P5	Ave. Time in Trial
E1	0.613	0.634	0.699	0.742	0.108	15.5
E2	0	0	0	0	0.099	17.2
E3	0.689	0.714	0.846	0.888	0.152	14.2
E4	0.834	0.834	0.994	0.994	0	12
E5	0	0	0	0	0.14	16.8
E6	0.774	0.826	0.774	0.851	0.248	13.5
E7	0	0	0	0	0.074	14.8
E8	0.665	0.684	0.704	0.733	0.13	14.1

14. ETHICAL AND REGULATORY CONSIDERATIONS

Regulatory documents are essential to clinical research. They serve to demonstrate the regulatory approval(s) and compliance of the Sponsor, Investigator, Monitor, and IRB with the current federal and state regulations and the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines.

Study sites selected for participation in I-SPY 2 TRIAL will be responsible for submitting essential regulatory documents to DCC. The collection of regulatory documents will take place in accordance with applicable ICH GCP guidelines, state and federal regulations. Regulatory documents must be maintained per all applicable institutional and federal regulations. Any and all questions related to regulatory document submission should be directed to the attention of DCC Regulatory as outlined in protocol §14.5. Please see the I-SPY 2 Manual of Operations, §*Essential Regulatory Document Collection Process*, for more detailed information and links for downloading required forms from the I-SPY 2 website.

All study-specific forms (Form FDA 1572 template, Financial Disclosure, Delegation of Responsibilities, Investigator's Brochure Acknowledgment) can be accessed via the I-SPY 2 website at http://www.ispytrials.org/.

The following documents comprise the essential regulatory document packet required for agent shipment authorization and study site activation.

14.1 Form FDA 1572

Prior to initiating this study at any site, the Principal Investigator will provide an original signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing all the investigators at the organization at each site that will participate in the protocol.

14.2 Other Required Documents

Signed and dated current (within two years) CV/Biosketch for the site Principal Investigator and all subinvestigators listed on Form FDA 1572.

Current professional licenses (where applicable) for the site Principal Investigator and all subinvestigators listed on Form FDA 1572.

Original signed and dated I-SPY 2 Financial Disclosure Form for the site Principal Investigator and all subinvestigators listed on Form FDA 1572.

Certification of Human Subjects Protection Training (NIH or institution-based training program certificate) for the site Principal Investigator and all subinvestigators listed on Form FDA 1572.

Delegation of Responsibilities Log signed by the site Principal Investigator which lists the names and responsibilities of all study staff, including all subinvestigators listed on Form FDA 1572.

Lab certifications*(*CLIA and CAP) and lab normal ranges for all labs listed on each site's Form FDA 1572.

Documentation of Federalwide Assurance (FWA). A print-out of the institutional FWA number may be accessed *via* the OHRP website as follows: http://ohrp.cit.nih.gov/search/fwasearch.aspx?styp=bsc (click the button for FWAs [FWA number]).

Investigator's Brochure Acknowledgment Form signed by the site Principal Investigator.

IRB approval for all QLHC-approved protocol versions, Informed Consent versions (Screening, Treatment, and Supplemental), Investigator's Brochure versions (if applicable) and recruitment materials.

14.3 IRB Approval

Prior to initiating the study and receiving the drug agent(s), the investigators at the organizations must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to QLHC according to sponsor Amendment Guidelines. QLHC-approved amended protocol must be approved by the IRB prior to implementation.

As investigational agents move in and out of the trial, protocol amendments will be issued; see §6 for more detail.

14.3.1 IRB Approval Timeline Guidelines

Participating institutions will be notified of the allowable timeframe permitted to get each amendment approved by their institutional IRB. For amendments that include new investigational agents in the trial, see §14.3.1.1.

14.3.1.1 Amendment Containing New Investigational Agent(s)

Major Modification (see §6 for definition): Participating institutions have 60 calendar days to submit and obtain IRB approval. If an institution's IRB approval letter is not received by the sponsor or their authorized designee ≤60 calendar days, accrual at that institution will be placed on hold until institutional IRB approval is obtained and approval letters have been received and processed by the sponsor or their authorized designee.

Minor Modification (see §6 for definition): Participating institutions have 30 calendar days to submit and obtain IRB approval. If an institution's IRB approval letters are not received by the sponsor or their authorized designee ≤30 calendar days, accrual at that institution will be placed on hold until institutional IRB approval is obtained and approval letters have been received and processed by the sponsor or their authorized designee.

14.4 Informed Consent

The I-SPY 2 TRIAL will be utilizing a two-step consent process. All potential study participants will be given a copy of the IRB-approved Screening Informed Consent to review. The clinical investigator and study coordinator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the screening phase, she will be asked to sign and date the consent form. Participants that complete the screening phase and are eligible for the treatment phase of the study will be given a copy of the IRB-approved Treatment Informed Consent to review. If the participant is randomized to an investigational agent, the appropriate Supplemental Informed Consent Form will be given to the participant at the same time as the Treatment Informed Consent. The investigator and study coordinator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the treatment phase of the study,

she will be asked to sign and date the Informed Consent document(s). The study agent(s) will not be released to a participant who has not signed the Treatment and appropriate Supplemental Consent Form documents. Participants who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants are provided the option to allow the use of blood samples and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes.

Prior to study initiation, the Informed Consent documents must be reviewed and approved by QLHC or their authorized designee, the Biomarkers Consortium Project Team, and the IRB at each organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by QLHC or their authorized designee and the Project Team, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents may be transmitted *via* email (preferred method) or facsimile with the exception of the following documents for which signed originals must be sent via traceable courier:

- Form FDA 1572
- Financial Disclosure Form

Please refer to the I-SPY 2 Manual of Operations for completion and submission guidelines.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Please refer to study Informed Consents.

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Appendix A
I-SPY 2 Statistical Considerations

Statistical Model for pCR with Adjustment for Time Trend

The probability distribution of pCR is based on a Bayesian logistic regression model. This model includes a component that adjusts for possible time trends in the underlying pCR rate of each patient at the time of her randomization.

The Core Logistic Regression Model

Let $y_i \in \{0,1\}$ be the indicator for the pCR response of patient i ($i = 1, \ldots, N$). Covariates $x_{1i}, x_{2i}, x_{3i} \in \{0,1\}$ represent the HR, HER2 and MP statuses of patient i (with 1 indicating positive and 0 negative for HR and HER2 and 'High1' and 'High2' for MP). Label A_i the treatment arm assigned to patient i.
The full model is

$$y_i \sim \text{Bernoulli}(p_i)$$

$$\text{logit}(p_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \theta_{A_i} + \gamma_{1,A_i} x_{1i} + \gamma_{2,A_i} x_{2i} + \gamma_{3,A_i} x_{3i}$$

The model's components are as follows:

- The $\beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i}$ terms capture the effect of being in a particular subgroup defined by (HR, HER2, MP) status.
- The $\gamma_{1,A_i} x_{1i}, \gamma_{2,A_i} x_{2i}, \gamma_{3,A_i} x_{3i}$ terms are the treatment effects within each of the (HR, HER2, MP) subgroups.
    - We set $\theta_0 = \gamma_{1,0} = \gamma_{2,0} = \gamma_{3,0} = 0$ to ensure parameter identifiability.
- The θ_{A_i} represent the effect of being on a particular treatment arm for all patients.

In addition to the core logistic regression structure of the above terms we include time trend parameters to capture the effect of possible drifting pCR rates over time. The time trend adjustment component of the model is described in a separate sub-section below.

For each of the coefficients Θ in the regression model, we assume independent normal prior distributions: $\Theta \sim N(\mu_\Theta, \sigma_\Theta^2) = N(0,1)$.

We also include the data from the control arm of the I-SPY 1 trial as historical prior information. However, the likelihood function evaluation corresponding to the I-SPY1 data is raised to the power of 0.2 to reflect a discounting or weak borrowing. In effect the historical results count the approximately 200 patients from I-SPY 1 as the equivalent of 40 I-SPY 2 patients.

Special Considerations in Fitting the Core Model

The above is a standard Bayesian logistic regression model that can be fit using Markov chain Monte Carlo (MCMC) methods. However, there are several issues that require careful consideration for selecting appropriate prior distributions.

There is some borrowing of information across treatment arms via the HR, HER, MP covariates. Over time, as several highly efficacious treatment agents may be in the trial and the high pCR rates observed on these arms can lead to inflated estimates of pCR rates on the control arm because the prior distribution for the treatment effects creates a shrinkage of the control to the treatment. This effect can then be exaggerated across the many arms in the trial. We refer to this as the "success bias." Having very diffuse priors on the treatment effect parameters – a $N(0,10)$ prior on the logit scale – would minimize the effects of these inflated estimates. However, when new treatment arms with very little pCR data are present in the trial, these diffuse priors can lead to inappropriate estimates of pCR response for these new arms. Using a less diffuse prior such as $N(0,1)$ on the logit scale prevents this. Ideally we would like to use a very diffuse prior distribution on the treatment effect parameters (the θ's and the γ's) for estimating the control-related parameters (the β's) but use relatively stronger and pessimistic prior distributions, $N(0,1)$, on the treatment effects for estimating the individual treatment effects.

We accomplish this dual goal by creating two models with shared parameters within the MCMC sampling. We run two parallel MCMC chains. In one version we use N(0,10) priors for all treatment effects – thus having the benefit of estimating the control arm without the success bias. These estimates for the pCR rates on the control arm are used simultaneously to then estimate all treatment effects using the $N(0,1)$ priors for all the parameters specified in the above model. We employ this dual model by creating "pseudo-parameters" θ' and γ' for each θ and γ in the model. These pseudo-parameters have $N(0,10)$ prior distributions. While drawing an MCMC sample of the βs (control effects) we use current sampled values of the pseudo-parameters in place of the θ and the γ parameter sample. These control parameters are then used to generate the treatment effects, θ and the γ, using the desired N(0,1) priors.

Adjusting for Time Trends

We explicitly incorporate terms in the model to account for time trends in pCR response. This is done by using a set of time-dependent offset terms in the logistic model. We partition time into bins of 90 day durations based on the time of randomization for a patient. At the time of any analysis, let t_i be the index of the time bin, relative to the day of the analysis being time 0, during which patient i was randomized. o if patient i was randomized between 0-90 days ago then $t_i = 1$; if patient i was randomized between 91 and 180 days ago, then $t_i = 2$, and so on. We then model time-trend parameters based on these different time bins—each time bin will have a parameter in the model for the log-odds ratio of pCR based on the time bin in which they were randomized. We use two sets of time-trend parameters, one for patients with HER2+ tumors and the other for those with HER2– tumors: $\{\delta_+(t), \delta_-(t)\}$ all indexed by time bins. So, e.g., a (HR+, HER2–, MP+) patient whose randomization time falls in the 9th bin, $t = 9$, would have a trend offset of $\delta_-(9)$.

We set $\delta_+(t) = \delta_-(t) = 0$ for $t = 1,2,3,4$ which means that for a one-year period from the time of the analysis the pCR rate is assumed to be constant. Beyond a year in the past, we model the $\{\delta(t)\}$ as a second-order Normal Dynamic Linear Model (NDLM). This enables us to fit a 'smooth' effect over time. It has the following structure:

$$\delta(1) = \delta(2) = \cdots = \delta(4) = 0$$

$$\delta(5) \sim N(\mu_0, \tau_0^2)$$

$$\delta(6) - \delta(5) \sim N(\mu_1, \tau_1^2)$$

$$\delta(t) - 2\delta(t-1) + \delta(t-2) \sim N(0, \tau^2) \text{ for } t > 6$$

$$\tau^2 \sim IG(\alpha, \beta)$$

For the priors in the model described above, the Normal priors are $N(\mu_0, \tau_0^2) = N(\mu_1, \tau_1^2)$= N(0,0.1) and $\alpha = 1, \beta = 0.001$ for the Gamma priors. After including the time trend component, the full logistic regression model is:

$$y_i \sim \text{Bernoulli}(p_i)$$

$$\text{logit}(p_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \theta_{t_i} + \gamma_{1,t_i} x_{1i} + \gamma_{2,t_i} x_{2i} + \gamma_{3,t_i} x_{3i} + \delta_-(t_i) I(x_{2i} = 0) + \delta_+(t_i) I(x_{2i} = 1)$$

(Where $I()$ is the indicator function.)

Imputation Model

If all the pCR data y_i were available, then this is a standard Bayesian logistic regression model that may be fit using Markov Chain Monte Carlo (MCMC) methods. When pCR data for some patients are not available we use imputation based on the patients for pCR status is known. We then use the imputed values in the logistic regression model. The model for the pCR response and the imputation model are interleaved: For every MCMC sample of the imputation model parameters, we impute the missing pCR values and use them to draw the next posterior sample from the above model. Thus, this procedure constitutes a multiple imputation scheme for the missing pCR values. Consequently, the uncertainty due to the missing values is appropriately reflected in the posterior estimates of the above model parameters.

Although the final pCR status might not be available for every patient, useful information about eventual pCR statuses for these patients may be available in the form of serial MRI assessments of tumor volume at three time points during the treatment period. For modeling missing pCR, we employ a simple imputation model that exploit MRI tumor volume measurements. We fit this separate imputation logistic regression model as described below to assess the probability of achieving pCR based on the available MRI information.The imputation model is used for modeling missing pCR results, while the previous model describes the modeling of the likelihood of pCR given baseline factors and treatment arm.

Define S_0, S_1, S_2, S_3 to be the tumor volumes at baseline, one month, three months and just prior to surgery. Then the tumor volume reduction at visit *k* is $r_k = \frac{(S_k - S_0)}{S_0}$ for $k = 1,2,3$. Negative values of r_k correspond to a decrease in tumor volume, with $r_k = -1.0$ indicating the tumor is not detectable by MRI at visit *k*. To formulate a multiple imputation model for pCR status in cases where pCR is not available, we discretize the range of r_k values into categories m_k, for m_k = 1, …, 13. The mapping between r_k and m_k is given in Table 1.

Table 1 : Categories for tumor volume reduction in comparison with the patient's baseline tumor volume

Category (m_k)	Fraction Increase in Volume (r_k)
1	≥ 0
2	$(-0.1, 0]$
3	$(-0.2, -0.1]$

4	$(-0.3, -0.2]$
5	$(-0.4, -0.3]$
6	$(-0.5, -0.4]$
7	$(-0.6, -0.5]$
8	$(-0.7, -0.6]$
9	$(-0.8, -0.7]$
10	$(-0.9, -0.8]$
11	$(-0.95, -0.9]$
12	$(-0.99, -0.95]$
13	< -0.99

Let m_{1i}, m_{2i}, m_{3i} respectively be the MRI tumor volume reduction categories for the three post-baseline visits for patient i.

We represent Patient i's treatment arm is A_i.

At any interim analysis, for a given patient i, it is possible that the pCR status or one or more of the tumor volumes are not be available. Specifically, for each time point $k = 1,2,3$, we use data from all patients for whom both the pCR response and MRI assessment r_t are available, and we fit this model:

$$\text{logit}(\pi_{k,i}) = \text{logit}(p_{x_i}) + \alpha_{k,j_i}$$

Where $\alpha_{k,j_i}$is the parameter corresponding to the category j_i, where j_i = 1, …, 13 (see Table 1), for patient i at time k and $p_{x_i}$is the parameter from the logistic regression for pCR corresponding to covariates $x_i = (x_{1i}, x_{2i}, x_{3i})$ of patient i. We impose the monotonicity constraint $\alpha_{k,l} \leq \alpha_{k,m}$ for $l < m$ to reflect the condition that greater tumor reduction cannot be less likely to lead to a pCR. For each of the coefficients α in the regression model, we assume independent normal prior distributions: $\alpha \sim N(\mu_\alpha, \sigma_\alpha^2) = N(0,1)$.

At each MCMC iteration, if the pCR status for patient j is missing, then it is imputed as

$$\hat{y_j} \sim \text{Bernoulli}(\pi_{K,j})$$

Where K is the latest time point for which we have a tumor volume measurement for patient j.

Distribution of Molecular Tumor Subtypes

The population incidence rates across the 8 disease subtypes defined by HR, HER2, and MP is modeled as a categorical distribution (i.e., multinomial distribution with 8 outcomes) over the subtypes indexed by {1,2,…,8}, with $Pr(C = c) = \phi_c$. We use a Dirichlet prior

$$(\phi_1, \phi_2, \dots, \phi_8) \sim Dirichlet(0.1, 0.1, \dots, 0.1)$$

which results in a posterior distribution

$$(\phi_1, \phi_2, \dots, \phi_8) | Data \sim Dirichlet(n_1 + 0.1, n_2 + 0.1, \dots, n_8 + 0.1)$$

where n_c is the number of patients observed in subclass c.

pCR Status Probabilities for Subtypes and Signatures

The response-adaptive treatment allocations that govern the trial conduct are specified in terms of the probability of pCR (by treatment arm) for each of the eight subtypes and each of the ten signatures. These probabilities are derived from the parameters of dose-response and prevalence models as follows.

Let $\pi_C(a)$ be the probability of pCR for treatment arm *a* in disease subtype *C*. If *C* represents (x_1, x_2, x_3) where $x_i \in \{0,1\}$ for the (HR,HER2,MP) value, then

$$\text{logit}(\pi_C(a)) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \theta_a + \gamma_{1,a} x_1 + \gamma_{2,a} x_2 + \gamma_{3,a} x_{3a}$$

Let $P_S(a)$ be the probability of pCR for treatment arm *a* for signature *S*. Then

$$P_S(a) = \frac{\sum_{C \in S} \pi_C(a) \cdot \phi_C}{\sum_{C \in S} \phi_C}$$

(The notation $C \in S$ means subtype *C* is included in signature *S*.)

Adaptive Treatment Allocation

For response-adaptive treatment allocation for a new patient, we update the posterior distribution based on the latest available data. For any subtype, *C*, and any experimental treatment arm, *A*, we compute the probability that the pCR rate for *A* is higher than that for the control arm [PrCtl(*C*,*A*)].
If the new patient is of subtype *c*, then she is assigned to control with 20% probability, and the allocation probability to any experimental arm, *a*, eligible for subtype *c* with probability proportional to PrCtl(*c*,*a*) (with the allocation probabilities being appropriately normalized over the eligible experimental arms to have sum 80%).

Predictive Probabilities Used for Determine Graduation and Futility Stopping

The criteria for graduation and early stopping for futility are based on the predictive probability of success for an experimental arm in the 10 specified signatures. The "predictive probability of success" is the Bayesian predictive probability that the arm in question will demonstrate statistical superiority over control in an equally randomized 2-arm Phase III trial with end point pCR rate with 150 patients/arm and 0.025 one-sided significance level.

A sufficiently high predictive probability of success for a signature qualifies an experimental arm to be eligible to graduate for that particular signature. If an experimental arm has a sufficiently low predictive probability of success for all signatures, it is stopped for futility.

Calculation of Posterior Probabilities

All posterior probabilities are based on all currently available information. The parameters of the pCR-status imputation models use Markov Chain Monte Carlo samples; and the subtype incidence probabilities are sampled directly from its posterior distribution.

Appendix B
Modified National Comprehensive Cancer Network (NCCN) Guidelines, Dose Modifications, and Management of Standard Therapy Toxicity

Dose Modifications for Standard Therapy: General Considerations

Standard dose reduction for paclitaxel is 25%, which refers to a decrease of 20 mg/m^2; dose reduction for paclitaxel may differ when treatment is given in combination with an investigational agent.
Standard dose reduction for doxorubicin/cyclophosphamide is 20%.

If paclitaxel is held for 3 weeks in a row OR a paclitaxel dose reduction below 60 mg/m^2 is required, stop all protocol therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant will remain on study for outcome assessment.

Missed doses of standard chemotherapy can be made up to complete the full regimen (*e.g.*, 12 cycles of paclitaxel and trastuzumab, if the participant is HER2+, and four cycles of AC). Whenever possible, standard therapy dose and schedule should be maintained.

Please refer to the tables below for standard therapy dose modifications (paclitaxel alone, paclitaxel + trastuzumab, and AC).

For dose modifications of the investigational agent, please see guidelines in each investigational agent's appendix.

Table 1. Paclitaxel Dose Modification

Event	Paclitaxel Dose Modification
Neutropenia	
≥1000/mm^3	No change to paclitaxel. • For ANC ≤1500/mm^3 consider the use of prophylactic myeloid growth factors (filgrastim), o Start on day 2 or 3 and use for 2–6 days according to participant need, at physician discretion, and to avoid dose reduction. o Growth factor should not be given on the same day as chemotherapy. o Pegfilgrastim may not be used with paclitaxel due to the weekly dosing in this study.
<1000/mm^3	Hold paclitaxel until ANC >1000/mm^3. Resume paclitaxel based on timing of recovery: • ≤1 week—no change to paclitaxel • >1 but <3 weeks—reduce paclitaxel dose by 25% for all subsequent cycles • ≥3 weeks—stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Neutropenic Fever	
ANC ≤1000/mm^3, fever ≥38.5°C	Hold paclitaxel until resolved (ANC >1000/mm^3, fever <38.5°C). Resume paclitaxel according to number of episodes:

Event	Paclitaxel Dose Modification
	• First episode: no change in paclitaxel • Second episode: 25% dose reduction of paclitaxel for all subsequent cycles • Third episode: stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Participants should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. GCSF may be used between days 2–6 according to participant need, at physician discretion, and to avoid dose reduction. Pegfilgrastim may not be used with paclitaxel due to the weekly dosing in this study.
Thrombocytopenia	
≥100,000/mm^3	No change to paclitaxel.
75–99,999/mm^3	Hold paclitaxel until ≥100,000/mm^3, resume paclitaxel based on timing of recovery: • ≤1 week—no change to paclitaxel. • >1 but <3 weeks—reduce paclitaxel dose by 25% for all subsequent cycles. • ≥3 weeks—stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
<75,000/mm^3	Hold paclitaxel until ≥100,000/mm^3. Resume paclitaxel with a 25% dose reduction for all subsequent cycles. If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Anemia	
All grades	No change in paclitaxel. For all anemia events related to paclitaxel regardless of grade, iron studies should be checked and iron should be replaced as indicated. • Red blood cell transfusions can be given at the investigators discretion as needed for symptom control.
Hepatic	
Grade 0 or 1	No change in paclitaxel.
≤ Grade 2	Grade 2 bilirubin: Hold paclitaxel until bilirubin resolves to ≤ grade 1. Resume paclitaxel based on time of recovery. • If bilirubin resolves to ≤ grade 1 in <2 weeks, resume paclitaxel at previous dose. • If bilirubin remains at grade 2 after holding two consecutive doses of paclitaxel (2 weeks), resume paclitaxel with a 25% reduction in dose for all subsequent doses. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the

Event	Paclitaxel Dose Modification
	discretion of the treating physician. Participant remains on study for outcome assessment. A rise in indirect bilirubin with a normal direct bilirubin believed to be attributable to Gilbert's disease does not require change in dose or agent hold. A note to file should be created. Grade 2 AST or ALT: Hold paclitaxel until AST/ALT resolve to ≤ grade 1. • If AST/ALT resolve to ≤ grade 1 in <3 weeks, resume paclitaxel at previous dose. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Grade 3	Grade 3 bilirubin (not due to Gilbert's disease): Stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. Grade 3 AST or ALT: Hold paclitaxel until AST/ALT resolve to ≤ grade 1. Resume paclitaxel at the previous dose. • If AST/ALT remains at grade 3 after holding two consecutive doses of paclitaxel, resume paclitaxel with a 25% dose reduction for all subsequent doses. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Grade 4	Grade 4 bilirubin, AST or ALT: Stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Nausea/Vomiting	
Grade 0–2	No change to paclitaxel.
≥Grade 3	Hold paclitaxel until resolved to ≤grade 1. • Resume paclitaxel at previous dose with modification of pre-medications. • For second episode ≥ grade 3 despite maximal supportive care: o Resume paclitaxel with a 25% dose reduction for all subsequent doses.
Mucositis	
Grade 0–2	No change to paclitaxel.
≥Grade 3	Hold paclitaxel until resolved to ≤grade 1. • Resume paclitaxel at the previous dose, with modification of premedications.

Event	Paclitaxel Dose Modification
	• For second episode ≥ grade 3 despite maximal supportive care: o Resume paclitaxel with a 25% dose reduction for all subsequent cycles.
Neurotoxicity	
Grade 0–2	No change to paclitaxel.
Grade 3	Hold paclitaxel until neuropathy improves to ≤ grade 2. • Resume paclitaxel with a 25% dose reduction for all subsequent cycles. If paclitaxel is held for 3 weeks in a row for neuropathy, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Grade 4	Stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
Anaphylaxis/Hypersensitivity	
Mild (*e.g.*, mild flushing, rash, pruritus)	Complete paclitaxel infusion. • No treatment required, but observe participant at least until symptoms have resolved.
Moderate (*e.g.*, moderate flushing, rash, mild dyspnea, chest discomfort)	Stop paclitaxel infusion. • Give intravenous diphenhydramine 20–25 mg and intravenous dexamethasone 10 mg. If symptoms resolve: • Resume paclitaxel infusion after recovery of symptoms at half the previous rate for 15 minutes. If no recurrence of symptoms, the planned rate may be resumed. If symptoms recur after paclitaxel re-challenge: • Stop paclitaxel infusion and stop all subsequent paclitaxel therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Severe (*e.g.*, hypotension requiring pressors, angioedema, respiratory distress requiring bronchodilators)	Stop paclitaxel infusion. • Administer diphenhydramine 25 mg and dexamethasone 10 mg IV. Add epinephrine or bronchodilators as needed per institutional guidelines. • Stop all subsequent paclitaxel therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Other Clinically Significant Toxicity Excluding Fatigue, Alopecia, and Leukopenia at Physician Discretion	
Grade 0 or 1	No change to paclitaxel.
Grade 2	Hold paclitaxel until resolved to ≤ grade 1. Resume paclitaxel at previous dose. • Increase supportive care measures if possible.
≥Grade 3	Hold paclitaxel and contact the DCC for further instruction (1-855-889-5170). If ≥grade 3toxicity recurs, • Stop paclitaxel and contact the DCC for further instruction (1-855-889-5170).

Table 2. Paclitaxel and Trastuzumab Dose Modification

Event	Paclitaxel + Trastuzumab Dose Modification
Neutropenia	
≥1000/mm^3	No change to paclitaxel and trastuzumab. • For ANC ≤1500/mm^3 consider the use of prophylactic myeloid growth factors (filgrastim), o Start on day 2 or 3 and use for 2–6 days according to participant need, at physician discretion, and to avoid dose reduction. o Growth factor should not be given on the same day as chemotherapy. o Pegfilgrastim may not be used with paclitaxel due to the weekly dosing in this study.
<1000/mm^3	Hold paclitaxel until ANC >1000/mm^3 but continue trastuzumab. Resume paclitaxel based on timing of recovery: • ≤1 week—no change to paclitaxel and trastuzumab. • >1 but <3 weeks—reduce paclitaxel dose by 25% for all subsequent cycles. No change to trastuzumab • ≥3 weeks—stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Neutropenic Fever	
ANC ≤1000/mm^3, fever ≥38.5°C	Hold paclitaxel until resolved (ANC >1000/mm^3, fever <38.5°C) but continue trastuzumab. Resume paclitaxel according to number of episodes: • First episode: no change in paclitaxel and trastuzumab • Second episode: 25% dose reduction of paclitaxel for all subsequent cycles. No change to trastuzumab. • Third episode: stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. **If paclitaxel is held for 3 weeks in a row,** stop paclitaxel and trastuzumab. Participants should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. GCSF may be used between days 2–6 according to participant need, at physician discretion, and to avoid dose reduction. Pegfilgrastim may not be used with paclitaxel due to the weekly dosing in this study.
Thrombocytopenia	
≥100,000/mm^3	No change to paclitaxel and trastuzumab.
75–99,999/mm^3	Hold paclitaxel until ≥100,000/mm^3 but continue trastuzumab. Resume paclitaxel based on timing of recovery: • ≤1 week—no change to paclitaxel and trastuzumab. • >1 but <3 weeks—reduce paclitaxel dose by 25% for all subsequent cycles. No change to trastuzumab. • ≥3 weeks—stop paclitaxel and trastuzumab. Participant should proceed

Event	Paclitaxel + Trastuzumab Dose Modification
	with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
<75,000/mm^3	Hold paclitaxel until ≥100,000/mm^3 but continue trastuzumab. • Resume paclitaxel with a 25% dose reduction for all subsequent cycles. No change to trastuzumab If paclitaxel is held for 3 weeks in a row, stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Anemia	
All grades	No change in paclitaxel and trastuzumab. For all anemia events related to paclitaxel regardless of grade, iron studies should be checked and iron should be replaced as indicated. • Red blood cell transfusions can be given at the investigators discretion as needed for symptom control.
Hepatic	
Grade 0 or 1	No change in paclitaxel and trastuzumab.
≤ Grade 2	Grade 2 bilirubin: Hold paclitaxel until bilirubin resolves to ≤ grade 1 but continue trastuzumab. Resume paclitaxel based on timing of recovery: • If bilirubin resolves to ≤ grade 1 in <2 weeks, resume paclitaxel at previous dose. • If bilirubin remains at grade 2 after holding two consecutive doses of paclitaxel (2 weeks), resume paclitaxel with a 25% reduction in dose for all subsequent doses. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. A rise in indirect bilirubin with a normal direct bilirubin believed to be attributable to Gilbert's disease does not require change in dose or an agent hold. A note to file should be created. Grade 2 AST or ALT: Hold paclitaxel until AST/ALT resolve to ≤ grade 1, but continue trastuzumab. • If AST/ALT resolve to ≤ grade 1 in <3 weeks, resume paclitaxel at previous dose. No change to trastuzumab. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Grade 3	Grade 3 bilirubin (not due to Gilbert's disease): Stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. Grade 3 AST or ALT:

Event	Paclitaxel + Trastuzumab Dose Modification
	Hold paclitaxel until AST/ALT resolve to ≤ grade 1but continue trastuzumab. Resume paclitaxel at the previous dose. • If AST/ALT remains at grade 3 after holding two consecutive doses (2 weeks) of paclitaxel, resume paclitaxel with a 25% dose reduction for all subsequent doses. No change to trastuzumab • If paclitaxel is held for 3 weeks in a row, stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Grade 4	Grade 4 bilirubin, AST or ALT: Stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Nausea/Vomiting	
Grade 0–2	No change to paclitaxel and trastuzumab.
≥ Grade 3	Hold paclitaxel until resolved to ≤grade 1 but continue trastuzumab. • For first episode, resume paclitaxel at previous dose with modification of premedications. No change to trastuzumab. • For second episode of ≥grade 3 despite maximal supportive care: o Resume paclitaxel with a 25% dose reduction for all subsequent doses. No change to trastuzumab.
Mucositis	
Grade 0–2	No change to paclitaxel and trastuzumab.
≥ Grade 3	Hold paclitaxel until resolved to ≤grade 1 but continue trastuzumab. • For first episode, resume paclitaxel at the previous dose, with modification of pre-medications. No change to trastuzumab. • For second episode ≥grade 3 despite maximal supportive care, o Resume paclitaxel with a 25% dose reduction for all subsequent cycles. No change to trastuzumab.
Neurotoxicity	
Grade 0–2	No change in paclitaxel and trastuzumab.
Grade 3	Hold paclitaxel until neuropathy improves to ≤grade 2 but continue trastuzumab. • Resume paclitaxel with a 25% dose reduction for all subsequent cycles. No change to trastuzumab. If paclitaxel is held for 3 weeks in a row for neuropathy, stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Grade 4	Stop paclitaxel and trastuzumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
Cardiac	
Asymptomatic decline in LVEF LVEF has declined 16 or more percentage points from baseline	Stop trastuzumab for 4 weeks but continue paclitaxel. • Reassess LVEF at 4 weeks: o If LVEF has recovered to baseline or the absolute decrease from baseline is <15 percentage points, resume trastuzumab at previous

Event	Paclitaxel + Trastuzumab Dose Modification
OR LVEF has declined 10 to 15 percentage points from baseline AND is below the lower limit of normal	dose. o If LVEF has not either recovered to baseline or the absolute decrease from baseline is >15 percentage points, stop trastuzumab and paclitaxel therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment of the treating physician. Participant remains on study for outcome assessment.
Symptomatic cardiac dysfunction	Stop trastuzumab and paclitaxel therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Anaphylaxis/Hypersensitivity	
Mild (*e.g.*, mild flushing, rash, pruritus)	Complete paclitaxel infusion. • No treatment required, but observe participant at least until symptoms have resolved.
Moderate (*e.g.*, moderate flushing, rash, mild dyspnea, chest discomfort)	For paclitaxel: • Stop paclitaxel infusion. • Give intravenous diphenhydramine 20–25 mg and intravenous dexamethasone 10 mg. • Resume paclitaxel infusion after recovery of symptoms at half the previous rate for 15 minutes. If no recurrence of symptoms, the planned rate may be resumed. For trastuzumab: • Stop trastuzumab infusion. • Treat symptomatically according to institutional guidelines. • Resume trastuzumab infusion at a slower rate according to institutional guidelines after reaction has resolved. If no recurrence of symptoms, the planned rate may be resumed. If symptoms recur after paclitaxel or trastuzumab re-challenge: • Stop paclitaxel or trastuzumab infusion and stop all subsequent paclitaxel and trastuzumab therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Severe (*e.g.*, hypotension requiring pressors, angioedema, respiratory distress requiring bronchodilators)	Stop paclitaxel or trastuzumab. • Administer diphenhydramine 25 mg and dexamethasone 10 mg IV. Add epinephrine or bronchodilators as needed per institutional guidelines. • Stop all subsequent paclitaxel and trastuzumab therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Other Clinically Significant Toxicity Excluding Fatigue, Alopecia, and Leukopenia at Physician Discretion	
Grade 0 or 1	No change to paclitaxel and trastuzumab.
Grade 2	Hold paclitaxel until resolved to ≤ grade 1 but continue trastuzumab. Resume paclitaxel at previous dose. • Increase supportive care measures if possible.

Event	Paclitaxel + Trastuzumab Dose Modification
≥ Grade 3	Hold paclitaxel and contact the DCC for further instruction (1-855-889-5170). If toxicity deemed related to trastuzumab, at physician discretion: • Hold trastuzumab. • Contact the DCC for further instructions (1-855-889-5170).

Table 3. Doxorubicin/Cyclophosphamide (AC) Dose Modification

Event	Doxorubicin/Cyclophosphamide (AC) Dose Modification
Neutropenia	
≥1000/mm^3	No change to AC.
<1000/mm^3	Hold until ANC >1000/mm^3, resume AC based on timing of recovery: • ≤1 week—no change to AC. • >1 but <3 weeks—reduce AC dose by 20% for subsequent cycles. • ≥3 weeks—stop AC. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Neutropenic Fever	
ANC ≤1000/mm^3, fever ≥ 38.5°C	Hold until resolved (ANC >1000/mm^3, fever <38.5°C), resume according to number of episodes: • First episode: no change to AC. • Second episode: reduce AC dose by 20% for all subsequent cycles. • Third episode: stop AC. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Thrombocytopenia	
≥ 100,000/mm^3	No change to AC.
75–99,999/mm^3	Hold until ≥ 100,000/mm^3, resume based on timing of recovery: • ≤1 week—no change to AC. • >1 week but <3 weeks—reduce AC dose by 20% for all subsequent cycles. • ≥3 weeks —stop AC. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
<75,000/mm^3	Hold until ≥ 100,000/mm^3. • Resume AC a with 20% dose reduction for all subsequent cycles. If AC is held for 3 weeks in a row, stop AC. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Anemia	
All grades	No change to AC.
Hepatic	
Grade 0 or 1	No change to AC.
≥ Grade 2	Hold AC until ≤ grade 1. • Resume AC at previous dose.

Event	Doxorubicin/Cyclophosphamide (AC) Dose Modification
	If AC is held for 3 weeks in a row, stop AC. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Nausea/Vomiting	
Grade 0–2	No change to AC.
≥ Grade 3	Hold AC until resolved to ≤ grade 1. • Resume AC with a 20% dose reduction for all subsequent cycles.
Mucositis	
Grade 0–2	No change to AC.
≥ Grade 3	Hold AC until resolved to ≤ grade 1. • Resume AC with a 20% dose reduction for all subsequent cycles.
Cardiac	
Grade 0–2	No change to AC.
≥ Grade 3	Discontinue AC if: • A participant has symptoms of CHF and a diagnosis of CHF is confirmed; • A participant has a myocardial infarction; • 15% absolute decline in LVEF from baseline or >10% decline in LVEF from baseline to below LLN. For any other cardiac toxicity > grade 3, hold AC and contact DCC for instructions (1-855-889-5170). **NOTE:** PACs or PVCs without cardiac dysfunction (*e.g.*, acute dysrhythmias) during and shortly after doxorubicin infusion are NOT an indication to permanently stop doxorubicin. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Other Clinically Significant Toxicity Excluding Fatigue, Alopecia, and Leukopenia at Physician Discretion	
Grade 0 or 1	No change to AC.
Grade 2	Hold AC until resolved to ≤ grade 1. Resume AC at previous dose. • Increase supportive care measures, if possible.
≥ Grade 3	Hold AC and contact DCC for instructions (1-855-889-5170). If ≥ grade 3 toxicity recurs, stop AC. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.

Abbreviations: AC: anthracycline (doxorubicin + cyclophosphamide); ANC: absolute neutrophil count; CHF: congestive heart failure; LLN: lower limit of normal; LVEF: left ventricular ejection fraction; PAC: premature atrial complex; PVC: premature ventricular complex.

Toxicity Management of Paclitaxel, Paclitaxel + Trastuzumab, Doxorubicin/Cyclophosphamide

See attached NCCN guidelines and Sparano *et al.*, N Engl J Med. 358: 1663–1671, 2008.

National Comprehensive Cancer Network®

Chemotherapy Order Template™

Breast Cancer

AC (DOXOrubicin/Cyclophosphamide) Every 21 Days → PACLItaxel Every 21 Days

AC (DOXOrubicin/Cyclophosphamide) Every 21 Days Course

INDICATION:
Adjuvant

REFERENCES:

1. NCCN Clinical Practice Guidelines in Oncology™ Breast Cancer. V.2.2008.
2. Mamounas EP, et al. *J Clin Oncol.* 2005;23(16):3686-96.[9]

NCCN SUPPORTIVE CARE:
1. *Emetic Risk:* Day 1 High
2. *Fever Neutropenia Risk:* Intermediate

CHEMOTHERAPY REGIMEN
21-day cycle for 4 cycles

- **DOXOrubicin** 60 mg/m^2 IV Push on Day 1
- **Cyclophosphamide** 600 mg/m^2 IV over 30 minutes on Day 1
- Oral hydration is strongly encouraged with cyclophosphamide; poorly hydrated patients may need supplemental IV hydration. Patients should attain combined oral and IV hydration of 2 – 3 L/day on day of chemotherapy.
  See example of recommended supplemental IV hydration below.

This course is 4 cycles of AC (DOXOrubicin/cyclophosphamide) Every 21 Days.
PACLItaxel Every 21 Days is initiated following completion of this course.
Please see Order Template BRS5b for PACLItaxel Every 21 Days course.

SUPPORTIVE CARE

Antiemetic therapy (See www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf)

- Aprepitant 125 mg PO or fosaprepitant 115 IV Day 1, aprepitant 80 mg PO Days 2 – 3
  AND
- Dexamethasone 12 mg PO/IV Day 1, then 8 mg PO/IV Days 2 – 4
  AND
- 5-HT3 antagonist:
  Ondansetron 16 – 24 mg PO or 8 – 12 mg (maximum 32 mg/day) IV Day 1
  OR
  Granisetron 2 mg PO daily or 1 mg PO BID or 0.01 mg/kg (maximum 1 mg) IV daily Day 1
  OR
  Dolasetron 100 mg PO or 1.8 mg/kg IV or 100 mg IV Day 1
  OR
  Palonosetron 0.25 mg IV Day 1
  AND
- ± Lorazepam 0.5 – 2 mg PO/IV or sublingual every 4 or every 6 hours Days 1 – 4

PRN for breakthrough: Patients should be given at least one medication in a different category than that given above to have as needed for breakthrough. Please consult the NCCN Clinical Practice Guidelines in Oncology™ Antiemesis for appropriate antiemetic therapy.

Template continued on page 2

Myeloid growth factor therapy (see www.nccn.org/professionals/physician_gls/PDF/myeloid_growth.pdf)

CSFs not generally recommended as primary prophylaxis based on FN risk of chemotherapy regimen. For more information on prophylaxis of FN, refer to NCCN Clinical Practice Guidelines in Oncology™ Myeloid Growth Factors and Appendix C to the NCCN Chemotherapy Order Templates

Other Supportive Therapy

- For cyclophosphamide: *Example of recommended supplemental IV hydration*: Sodium chloride 0.9% infused IV at a rate of 1.5 – 3 mL/kg/hour for a total of 500 mL on day of chemotherapy.

MONITORING AND HOLD PARAMETERS

- CBC with differential should be assessed routinely for potential dose evaluation.
- For DOXOrubicin:
  - DOXOrubicin is an anthracycline. Cumulative anthracycline dosage should be monitored.
  - Ejection fraction should be assessed prior to initiation of anthracycline treatment and as clinically indicated.
  - Liver function should be assessed prior to each cycle for potential dose evaluation.
- For cyclophosphamide: Renal function should be assessed prior to each cycle for potential dose evaluation.

SAFETY PARAMETERS AND SPECIAL INSTRUCTIONS

- For DOXOrubicin: **DOXOrubicin is a vesicant.**
- For aprepitant and fosaprepitant: Refer to Appendix D for specific information regarding associated drug interactions.

National Comprehensive Cancer Network®

BRS13a

Chemotherapy Order Template™

Breast Cancer

Dose-Dense AC (DOXOrubicin/Cyclophosphamide) →Dose-Dense PACLItaxel

Dose-Dense AC (DOXOrubicin/ Cyclophosphamide) Course

INDICATION:
Adjuvant

REFERENCES:

1. NCCN Clinical Practice Guidelines in Oncology™ Breast Cancer. V.1.2009.
2. Citron ML, et al. *J Clin Oncol.* 2003; 21(8):1431-9.[a]

NCCN SUPPORTIVE CARE:
1. *Emetic Risk:* Day 1 High
2. *Fever Neutropenia Risk:* High

CHEMOTHERAPY REGIMEN

14-day cycle for 4 cycles

- **DOXOrubicin** 60 mg/m^2 IV Push on Day 1
- See *Safety Parameters and Special Instructions* for information on slow IV Push administration.
- **Cyclophosphamide** 600 mg/m^2 IV over 30 minutes on Day 1
- Oral hydration is strongly encouraged with cyclophosphamide; poorly hydrated patients may need supplemental IV hydration. Patients should attain combined oral and IV hydration of 2 – 3 L/day on day of chemotherapy.
  See *Other Supportive Therapy* for example of recommended hydration.

This course is 4 cycles of dose-dense AC (DOXOrubicin/cyclophosphamide).
Dose-dense PACLItaxel is initiated following completion of this course.
Please see Order Template BRS13b for dose-dense PACLItaxel course.

SUPPORTIVE CARE

Antiemetic therapy (See www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf)

Days 1 – 4

- Aprepitant 125 mg PO or fosaprepitant 115 mg IV Day 1, aprepitant 80 mg PO Days 2 – 3
  AND
- Dexamethasone 12 mg PO/IV Days 1 – 4
  AND
- 5-HT3 antagonist (recommended on days of highly emetogenic chemotherapy administration) :
  Palonosetron 0.25 mg IV Day 1
  OR
  Dolasetron 100 mg PO or 1.8 mg/kg IV or 100 mg IV Day 1
  OR
  Granisetron 2 mg PO daily or 1 mg PO BID or 0.01 mg/kg (maximum 1 mg) IV daily Day 1 or transdermal patch containing 34.3 mg granisetron applied 24 – 48 hours prior to first dose of chemotherapy (patch supplies 5 days of therapeutic drug starting 24 hours after application)
  OR
  Ondansetron 16 – 24 mg PO or 8 – 12 mg (maximum 32 mg/day) IV Day 1
  AND
- ± Lorazepam 0.5 – 2 mg PO/IV or sublingual every 4 or every 6 hours as needed Days 1 – 4
  AND
- ± H_2 blocker or proton pump inhibitor

Template continued on page 2

07/09/2009

PRN for breakthrough: Patients should be given at least one medication in a different category than that given above to have as needed for breakthrough. Please consult the NCCN Clinical Practice Guidelines in Oncology™ Antiemesis for appropriate antiemetic therapy.

Myeloid growth factor therapy (See www.nccn.org/professionals/physician_gls/PDF/myeloid_growth.pdf)

- **Filgrastim (Category 1*)**
  5 mcg/kg/day subcutaneously daily recommended to start 24 – 72 hours after completion of chemotherapy and to continue until post-nadir ANC recovery to normal or near-normal levels by laboratory standards. Dose is rounded to the nearest vial size by institution-defined weight limits. Same-day administration is not recommended.
  OR
- **Pegfilgrastim (Category 1*)**
  6 mg subcutaneously recommended to be given 24 – 72 hours after completion of chemotherapy for one dose only. There are insufficient data to support dose and schedule of weekly regimens or schedules less than 2 weeks and these cannot be recommended. Same-day administration is not recommended.
  OR
- **Sargramostim (Category 2B*)**
  250 mcg/m^2/day subcutaneously daily recommended to start 24– 72 hours after completion of chemotherapy and to continue until post-nadir ANC recovery to normal or near-normal levels by laboratory standards. Dose is rounded to the nearest vial size by institution-defined weight limits. Same-day administration is not recommended.

Other Supportive Therapy

- For cyclophosphamide: *Example of recommended hydration*: Sodium chloride 0.9% infused IV at a rate of 1.5 – 3 mL/kg/hour for a total of 500 mL on day of chemotherapy.

MONITORING AND HOLD PARAMETERS

- CBC with differential should be assessed routinely for potential dose evaluation.
- For DOXOrubicin:
  - DOXOrubicin is an anthracycline. Cumulative anthracycline dosage should be monitored.
  - Ejection fraction should be assessed prior to initiation of treatment and as clinically indicated.
  - Liver function should be assessed routinely for potential dose evaluation.
- For cyclophosphamide: Renal function should be assessed prior to each cycle for potential dose evaluation.

Template continued on page 3

SAFETY PARAMETERS AND SPECIAL INSTRUCTIONS

- For DOXOrubicin:
  - **DOXOrubicin is a vesicant.**
  - This agent is administered IV Push. The preferred IV Push method for a vesicant is administration through the side port of a freely flowing IV; alternatively, the drug can be administered via direct IV push.
- For aprepitant and fosaprepitant: Refer to Appendix D for specific information regarding associated drug interactions.

*The NCCN Guidelines Steering Committee has devised a set of Categories of Consensus. These annotations contain two dimensions: the strength of the evidence behind the recommendation and the degree of consensus about its inclusion.

- **Category 1:** There is uniform NCCN consensus, based on high-level evidence, that the recommendation is appropriate.
- **Category 2A:** There is uniform NCCN consensus, based on lower-level evidence including clinical experience, that the recommendation is appropriate.
- **Category 2B:** There is nonuniform NCCN consensus (but no major disagreement), based on lower-level evidence including clinical experience, that the recommendation is appropriate.
- **Category 3:** There is major NCCN disagreement, regardless of the level of evidence, that the recommendation is appropriate.

National Comprehensive Cancer Network®

BRS16a

Chemotherapy Order Template™

Breast Cancer

AC (DOXOrubicin/Cyclophosphamide) Every 21 Days → T (PACLItaxel) Every 21 Days + Trastuzumab

AC (DOXOrubicin/Cyclophosphamide) Every 21 Days Course

INDICATION:	REFERENCES:	NCCN SUPPORTIVE CARE:
Adjuvant	1. NCCN Clinical Practice Guidelines in Oncology™ Breast Cancer. V.2.2008. 2. Romond EH, et al. *N Engl J Med.* 2005;353(16):1673-84.[a]	1. *Emetic Risk:* Day 1 High 2. *Fever Neutropenia Risk:* Intermediate

CHEMOTHERAPY REGIMEN

21-day cycle for 4 cycles

- **DOXOrubicin** 60 mg/m^2 IV Push on Day 1
- **Cyclophosphamide** 600 mg/m^2 IV over 30 minutes on Day 1
- Oral hydration is strongly encouraged with cyclophosphamide; poorly hydrated patients may need supplemental IV hydration. Patients should attain combined oral and IV hydration of 2 – 3 L/day on day of chemotherapy.
  See example of recommended supplemental IV hydration below.

This course is 4 cycles of AC (DOXOrubicin/cyclophosphamide) Every 21 Days.
T (PACLItaxel) Every 21 Days and trastuzumab course is initiated following completion of this course.
Please see Order Template BRS16b for T (PACLItaxel) Every 21 Days + trastuzumab course.

SUPPORTIVE CARE

Antiemetic therapy (See www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf)

- Aprepitant 125 mg PO or fosaprepitant 115 mg IV Day 1, aprepitant 80 mg PO Days 2 – 3
  AND
- Dexamethasone 12 mg PO/IV Day 1, then 8 mg PO/IV Days 2 – 4
  AND
- 5HT3 antagonist:
  Ondansetron 16 - 24 mg PO or 8 – 12 mg (maximum 32 mg/day) IV Day 1
  OR
  Granisetron 2 mg PO daily or 1 mg PO BID or 0.01 mg/kg (maximum 1 mg) IV daily Day 1
  OR
  Dolasetron 100 mg PO or 1.8 mg/kg IV or 100 mg IV Day 1
  OR
  Palonosetron 0.25 mg IV Day 1
  AND
- ± Lorazepam 0.5 - 2 mg PO/IV or sublingual every 4 or every 6 hours Days 1 – 4

PRN for breakthrough: Patients should be given at least one medication in a different category than that given above to have as needed for breakthrough. Please consult the NCCN Clinical Practice Guidelines in Oncology™ Antiemesis for appropriate antiemetic therapy.

Template continued on page 2

Myeloid growth factor therapy (See www.nccn.org/professionals/physician_gls/PDF/myeloid_growth.pdf)

CSFs not generally recommended for primary prophylaxis based on FN risk of chemotherapy regimen. For more information on prophylaxis of FN, refer to NCCN Clinical Practice Guidelines in Oncology™ Myeloid Growth Factors and Appendix C to the NCCN Chemotherapy Order Templates.

Other Supportive Therapy

- For cyclophosphamide: *Example of recommended supplemental IV hydration*: Sodium chloride 0.9% infused IV at a rate of 1.5 – 3 mL/kg/hour for a total of 500 mL on day of chemotherapy.

MONITORING AND HOLD PARAMETERS

- CBC with differential should be assessed routinely for potential dose evaluation.
- For DOXOrubicin:
  - DOXOrubicin is an anthracycline. Cumulative anthracycline dosage should be monitored.
  - Ejection fraction should be assessed prior to initiation of treatment and as clinically indicated.
  - Liver function should be assessed prior to each cycle for potential dose evaluation.
- For cyclophosphamide: Renal function should be assessed prior to each cycle for potential dose modification.

SAFETY PARAMETERS AND SPECIAL INSTRUCTIONS

- For DOXOrubicin: **DOXOrubicin is a vesicant.**
- For aprepitant and fosaprepitant: Refer to Appendix D for specific information regarding associated drug interactions.

National Comprehensive Cancer Network®

BRS5b

Chemotherapy Order Template™

Breast Cancer

AC (DOXOrubicin/Cyclophosphamide) Every 21 Days → PACLItaxel Every 21 Days

PACLItaxel Every 21 Days Course

INDICATION:
Adjuvant

REFERENCES:

1. NCCN Clinical Practice Guidelines in Oncology™ Breast Cancer. V.2.2008.
2. Mamounas EP, et al. *J Clin Oncol.* 2005;23(16):3686-96.[g]

NCCN SUPPORTIVE CARE:
1. *Emetic Risk:* Day 1 Low
2. *Fever Neutropenia Risk:* Refer to NCCN Clinical Practice Guidelines in Oncology™ Myeloid Growth Factors. V.1.2008.

CHEMOTHERAPY REGIMEN
21-day cycle for 4 cycles

- **PACLItaxel** 175 – 225 mg/m^2 IV over 3 hours on Day 1

This course is 4 cycles of PACLItaxel Every 21 Days.
This course is initiated following completion of the AC (DOXOrubicin/ cyclophosphamide) Every 21 Days course.
Please see Order Template BRS5a for the AC (DOXOrubicin/cyclophosphamide) Every 21 Days course.

SUPPORTIVE CARE

Premedications

PACLItaxel requires premedication for hypersensitivity:

- **H2 antagonist:**
  Famotidine 20 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  OR
  Ranitidine 50 mg IV or 150 mg PO 30 – 60 minutes pre-PACLItaxel
  OR
  Cimetidine 300 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  AND
- **H1 antagonist:**
  Diphenhydramine 12.5 – 50 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  AND
- **Dexamethasone:**
  Dexamethasone 20 mg PO approximately 12 and 6 hours pre-PACLItaxel
  OR
  Dexamethasone 20 mg IV 30 minutes pre-PACLItaxel

Template continued on page 2

Antiemetic therapy (See www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf)

Day 1
No additional dexamethasone needed on Day 1 if dexamethasone already given for hypersensitivity.

- Dexamethasone 12 mg PO/IV Day 1
  OR
- Prochlorperazine 10 mg PO/IV every 4 or every 6 hours Day 1
  OR
- Metoclopramide 10 – 40 mg PO/IV every 4 or every 6 hours ± Diphenhydramine 25 – 50 mg PO/IV every 4 or every 6 hours Day 1
  AND
- ± Lorazepam 0.5 – 2 mg PO/IV every 4 or every 6 hours Day 1

PRN for breakthrough: Patients should be given at least one medication in a different category than that given above to have as needed for breakthrough. Please consult the NCCN Clinical Practice Guidelines in Oncology™ Antiemesis for appropriate antiemetic therapy.

MONITORING AND HOLD PARAMETERS

- CBC with differential should be assessed routinely for potential dose evaluation.
- For PACLItaxel:
  - Liver function should be assessed prior to each cycle for dose potential evaluation.
  - Hypersensitivity reaction may occur with infusion. Monitor for and treat hypersensitivity reactions as per institutional standard.
  - Signs and symptoms of neurotoxicity should be assessed prior to each cycle. Modifications of chemotherapy may be warranted.

SAFETY PARAMETERS AND SPECIAL INSTRUCTIONS

- For PACLItaxel:
  - PACLItaxel is an irritant.
  - PACLItaxel should be prepared either in glass or non-PVC containers and administered through non-PVC tubing and an in-line filter of not greater than 0.22 microns.

National Comprehensive Cancer Network®

Chemotherapy Order Template™

Breast Cancer

Dose-Dense AC (DOXOrubicin/Cyclophosphamide) → Dose-Dense PACLItaxel

Dose-Dense PACLItaxel Course

INDICATION:
Adjuvant

REFERENCES:

1. NCCN Clinical Practice Guidelines in Oncology™ Breast Cancer. V.1.2009.
2. Citron ML, et al. *J Clin Oncol.* 2003; 21(8):1431-9.[8]

NCCN SUPPORTIVE CARE:
1. *Emetic Risk:* Day 1 Low
2. *Fever Neutropenia Risk:* High

CHEMOTHERAPY REGIMEN

14-day cycle for 4 cycles

- **PACLItaxel** 175 mg/m^2 IV over 3 hours on Day 1

This course is 4 cycles of dose-dense PACLItaxel.
This course is initiated following completion of the dose-dense AC (DOXOrubicin/cyclophosphamide) course.
Please see Order Template BRS13a for the dose-dense AC (DOXOrubicin/ cyclophosphamide) course.

SUPPORTIVE CARE

Premedications

PACLItaxel requires premedication for hypersensitivity:

- **H_2 antagonist:**
  Famotidine 20 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  OR
  Ranitidine 50 mg IV or 150 mg PO 30 – 60 minutes pre-PACLItaxel
  OR
  Cimetidine 300 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  AND
- **H_1 antagonist**:
  Diphenhydramine 12.5 – 50 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  AND
- **Dexamethasone:**
  Dexamethasone 20 mg PO approximately 12 and 6 hours pre-PACLItaxel
  OR
  Dexamethasone 20 mg IV 30 minutes pre-PACLItaxel

Template continued on page 2

National Comprehensive Cancer Network®

Chemotherapy Order Template™

Breast Cancer

Dose-Dense AC (DOXOrubicin/Cyclophosphamide) → Dose-Dense PACLItaxel

Dose-Dense PACLItaxel Course

Antiemetic therapy (See www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf)

Day 1
No additional dexamethasone needed on Day 1 if dexamethasone already given for hypersensitivity.

- Dexamethasone 12 mg PO/IV Day 1
  OR
- Prochlorperazine 10 mg PO/IV every 4 or every 6 hours Day 1
  OR
- Metoclopramide 10 – 40 mg PO/IV every 4 or every 6 hours Day 1
  AND
- ± Lorazepam 0.5 – 2 mg PO/IV every 4 or every 6 hours as needed Day 1
  AND
- ± H_2 blocker or proton pump inhibitor

PRN for breakthrough: Patients should be given at least one medication in a different category than that given above to have as needed for breakthrough. Please consult the NCCN Clinical Practice Guidelines in Oncology™ Antiemesis for appropriate antiemetic therapy.

Myeloid growth factor therapy (See www.nccn.org/professionals/physician_gls/PDF/myeloid_growth.pdf)

- **Filgrastim (Category 1*)**
  5 mcg/kg/day subcutaneously daily recommended to start 24 – 72 hours after completion of chemotherapy and to continue until post-nadir ANC recovery to normal or near-normal levels by laboratory standards. Dose is rounded to the nearest vial size by institution-defined weight limits. Same-day administration is not recommended.
  OR
- **Pegfilgrastim (Category 1*)**
  6 mg subcutaneously recommended to be given 24 – 72 hours after completion of chemotherapy for one dose only. There are insufficient data to support dose and schedule of weekly regimens or schedules less than 2 weeks and these cannot be recommended. Same-day administration is not recommended.
  OR
- **Sargramostim (Category 2B*)**
  250 mcg/m^2/day subcutaneously daily recommended to start 24 – 72 hours after completion of chemotherapy and to continue until post-nadir ANC recovery to normal or near-normal levels by laboratory standards. Dose is rounded to the nearest vial size by institution-defined weight limits. Same-day administration is not recommended.

MONITORING AND HOLD PARAMETERS

- CBC with differential should be assessed routinely for potential dose evaluation.
- For PACLItaxel:
  - Liver function should be assessed routinely for potential dose evaluation.
  - Hypersensitivity reaction may occur with infusion. Monitor for and treat hypersensitivity reactions per institutional standard.
  - Signs and symptoms of neurotoxicity should be assessed prior to each dose. Modifications of chemotherapy may be warranted.

Template continued on page 3

SAFETY PARAMETERS AND SPECIAL INSTRUCTIONS

- For PACLItaxel:
  - PACLItaxel is an irritant.
  - PACLItaxel should be prepared either in glass or non-PVC containers and administered through non-PVC tubing and an in-line filter of not greater than 0.22 microns.

*The NCCN Guidelines Steering Committee has devised a set of Categories of Consensus. These annotations contain two dimensions: the strength of the evidence behind the recommendation and the degree of consensus about its inclusion.

- **Category 1:** There is uniform NCCN consensus, based on high-level evidence, that the recommendation is appropriate.
- **Category 2A:** There is uniform NCCN consensus, based on lower-level evidence including clinical experience, that the recommendation is appropriate.
- **Category 2B**: There is nonuniform NCCN consensus (but no major disagreement), based on lower-level evidence including clinical experience, that the recommendation is appropriate.
- **Category 3:** There is major NCCN disagreement, regardless of the level of evidence, that the recommendation is appropriate.

National Comprehensive Cancer Network®

Chemotherapy Order Template™

Breast Cancer

AC (DOXOrubicin/Cyclophosphamide) Every 21 Days → T (PACLItaxel) Every 21 Days + Trastuzumab

T (PACLItaxel) Every 21 Days + Trastuzumab Course

INDICATION:
Adjuvant

REFERENCES:

1. NCCN Clinical Practice Guidelines in Oncology™ Breast Cancer. V.1.2009.
2. Romond EH, et al. *N Engl J Med.* 2005;353(16):1673-84.[8]

NCCN SUPPORTIVE CARE:
1. *Emetic Risk:* Day 1 Low; Trastuzumab Minimal
2. *Fever Neutropenia Risk:* Refer to NCCN Clinical Practice Guidelines in Oncology™ Myeloid Growth Factors. V.1.2009.

CHEMOTHERAPY REGIMEN

21-day cycle for 4 cycles

- **PACLItaxel** 175 mg/m^2 IV over 3 hours on Day 1

Weekly to complete 52 weeks total of trastuzumab

- **Trastuzumab**
  - 4 mg/kg IV over 90 minutes on Day 1 of Week 1 followed by
  - 2 mg/kg IV over 30 minutes weekly beginning with Week 2

OR

Weekly to complete 12 weeks total of trastuzumab

- **Trastuzumab**
  - 4 mg/kg IV over 90 minutes on Day 1 of Week 1 followed by
  - 2 mg/kg IV over 30 minutes weekly beginning with Week 2

Followed by

21-day cycle to complete 52 weeks total of trastuzumab

- **Trastuzumab**
  - 6 mg/kg IV over 30 – 90 minutes every 21 days beginning Week 13

This course is 4 cycles of T (PACLItaxel) Every 21 Days and 52 weeks of trastuzumab.
This course is initiated following completion of the AC (DOXOrubicin/cyclophosphamide) Every 21 Days course.
Please see Order Template BRS16a for the AC (DOXOrubicin/cyclophosphamide) Every 21 Days course.

Template continued on page 2

SUPPORTIVE CARE

Premedications

PACLItaxel requires premedication for hypersensitivity:

- **H_2 antagonist**:
  Famotidine 20 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  OR
  Ranitidine 50 mg IV or 150 mg PO 30 – 60 minutes pre-PACLItaxel
  OR
  Cimetidine 300 mg IV/PO 30 – 60 minutes pre-PACLItaxel
  AND
- **H_1 antagonist**:
  Diphenhydramine 12.5 – 50 mg IV/PO 30 – 60 minutes pre- PACLItaxel
  AND
- **Dexamethasone**:
  Dexamethasone 20 mg PO approximately 12 and 6 hours pre-PACLItaxel
  OR
  Dexamethasone 20 mg IV 30 minutes pre-PACLItaxel

Antiemetic therapy (See www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf)df

Day 1
No additional dexamethasone needed on Day 1 if dexamethasone already given for hypersensitivity.

- Dexamethasone 12 mg PO/IV Day 1
  OR
- Prochlorperazine 10 mg PO/IV every 4 or every 6 hours Day 1
  OR
- Metoclopramide 10 – 40 mg PO/IV every 4 or every 6 hours Day 1
  AND
- ± Lorazepam 0.5 – 2 mg PO/IV every 4 or every 6 hours as needed Day 1
  AND
- ± H_2 blocker or proton pump inhibitor

PRN for breakthrough: Patients should be given at least one medication in a different category than what given above to have as needed for breakthrough. Please consult the NCCN Clinical Practice Guidelines in Oncology™ Antiemesis for appropriate antiemetic therapy.

Days of trastuzumab:

PRN for breakthrough: Although this is a minimally emetic chemotherapy regimen, all patients should be provided with antiemetic therapy for breakthrough emesis. Please consult the NCCN Clinical Practice Guidelines in Oncology™ Antiemesis for appropriate antiemetic therapy.

Template continued on page 3

MONITORING AND HOLD PARAMETERS

- CBC with differential should be assessed routinely for potential dose evaluation.
- For PACLItaxel:
  - Liver function should be assessed prior to each cycle for potential dose evaluation.
  - Hypersensitivity reaction may occur with infusion. Monitor for and treat hypersensitivity reactions per institutional standard.
  - Signs and symptoms of neurotoxicity should be assessed prior to each dose. Modifications of chemotherapy may be warranted.
- For trastuzumab:
  - Hypersensitivity reaction may occur with infusion. Monitor for and treat hypersensitivity reactions per institutional standard.
  - Ejection fraction should be assessed prior to initiation of treatment and as clinically indicated.

SAFETY PARAMETERS AND SPECIAL INSTRUCTIONS

- For PACLItaxel:
  - PACLItaxel is an irritant.
  - PACLItaxel should be prepared either in glass or non-PVC containers and administered through non-PVC tubing and an in-line filter of not greater than 0.22 microns.

Appendix C
Overview of Investigational Agents and Biomarkers

1. Overview of Investigational Agents Study Status

Table 1.1 Investigational Agents Approved, Pending Activation for Randomization

There are no Investigational Agents approved, pending activation for randomization at this time.

Table 1.2 Investigational Agents Approved, Activated for Randomization

Agent	Target	HER2+ / HR+	HER2+ / HR–	HER2– / HR+	HER2– / HR–
Pembrolizumab	PD-1 inhibitor	No	No	Yes	Yes

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Table 1.3 Investigational Agents Graduated or Dropped, No Longer Activated for Randomization

Agent	Target	HER2+ / HR+	HER2+ / HR–	HER2– / HR+	HER2– / HR–

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2. Summary of All Additional Eligibility Criteria Required for All Investigational Agents

Table 2 Additional Investigational Agents Eligibility Criteria

Investigational Agents Inclusion Criteria

Inclusion criteria:

- Creatinine clearance >**40** mL/min per 24-hour urine collection or calculated according to the Cockcroft-Gault formula (either formula can be used dependent on the reported units):

CrCl (mL/min) =	$\frac{(140 - \text{age}) \times \text{actual body weight (kg)}}{72 \times \text{serum creatinine (mg/dL)}}$	(× 0.85 for females)
	$\frac{(140 - \text{age}) \times \text{actual body weight (kg)}}{0.8136 \times \text{serum creatinine (µmol/L)}}$	(× 0.85 for females)

- Urinary protein quantitative value of ≤30 mg/dL in urinalysis or ≤1+ on dipstick. (If criteria cannot be met, 24-hour urine collection can be done to calculate total protein excretion. If a 24 hour total urinary protein excretion is <1000 mg, the participant may be included.)
- Diastolic blood pressure (DBP) <100 mm Hg and systolic blood pressure (SBP) <160 mm Hg on at least one of three sequential blood pressure determinations performed during their clinic visit, or within the week during the screening process. (At the discretion of the investigator, a participant with DBP ≥90 mm Hg but <100 mm Hg or with SBP ≥140 but <160 mm Hg may be excluded based on prior history of poorly controlled hypertension or lack of compliance with management.)
- PTT or APTT ≤1.5 × ULN per institutional laboratory range and INR ≤1.5.
- HgbA1C ≤8%. Participants with diabetes who meet the HbgA1C criteria are eligible. Participants currently on metformin OR another oral hypoglycemic agent as their medication to manage their insulin resistance are eligible and should stay on their current treatment. Insulin-dependent diabetics are eligible for study participation.
- QTcF ≤470 msec on EKG (test results can be used if done within 30 days of entry to screening phase)
- No evidence of clinically significant bradycardia (HR <50 bpm), or history of clinically significant bradyarrhythmias such as sick sinus syndrome, 2nd degree AV block (Mobitz Type 2), or participants taking digoxin
- No history of venous (DVT), arterial, thromboembolism or pulmonary thromboembolism within 12 months prior to screening.
- No history of stroke (cerebrovascular accident) or TIA within 12 months prior to screening
- No history of hypertensive crisis or hypertensive encephalopathy within six months prior to screening.
- No history of clinically significant bleeding within six months prior to screening
- No treatment with immune modulators such as systemic cyclosporine or tacrolimus within 30 days prior to treatment.
- No major surgery within **28 days** prior to enrollment in the screening phase or with a persistent open wound.
- No minor surgical procedures, within **three days** prior to randomization. (Placement of tunneled central venous access device acceptable).
- No serious non-healing wound, ulcer (including gastrointestinal) or bone fracture.
- No therapeutic anti-coagulation (*i.e.*, warfarin or LMWH).
- No history of abdominal fistula, GI perforation, or intra-abdominal abscess within 6 months prior to screening.
- No active chronic gastrointestinal disorder with diarrhea as a major symptom in the past two years (*e.g.*, Crohn's disease, malabsorption, or grade >2 diarrhea of any etiology at baseline).
- No history of uncontrolled seizures
- Serum potassium, magnesium, and calcium levels within the laboratory's reference range
- No ventricular tachycardia or a supraventricular tachycardia that requires treatment with a Class Ia antiarrhythmic drug (eg, quinidine, procainamide, disopyramide) or Class III antiarrhythmic drug (eg, sotalol, amiodarone, dofetilide). Use of other antiarrhythmic drugs is permitted.
- No use of medications that have been linked to the occurrence of torsades de pointes (see Table 8 for the list

of such medications at the end of the appendix M) • No second- or third-degree atrioventricular (AV) block unless treated with a permanent pacemaker • No complete left bundle branch block (LBBB) • No history of long QT Syndrome or a family member with this condition

NOTE: In addition to the eligibility criteria in section 4.1.2 of the main protocol, participants must also meet all additional eligibility criteria described above (Table 2) in order to be eligible for the treatment phase of I-SPY 2. Each investigational agent-specific eligibility criteria can also be found in §2.2 of each investigational agent-specific appendix.

3. Dose and Administration Schedules for Investigational Agents

3.1 Dose and Administration Schedules for Investigational Agents Approved, Activated for Randomization

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Table 3.1.2 Paclitaxel (q1w × 12 weeks), and Pembrolizumab (q3w × 12 weeks) [a]; Followed by AC (q2w or q3w)

Agent	Dose	Route	Cycle[b]
Paclitaxel	80 mg/m^2	IV	1–12
Pembrolizumab[a]	**200 mg**	**IV**	**1,4,7,10**
Doxorubicin	60 mg/m^2	IV	13–16
Cyclophosphamide	600 mg/m^2	IV	13–16

[a] The pembrolizumab infusion should precede paclitaxel treatment and be administered over 30 minutes (-5 min/+10 min). A 30-minute waiting period between pembrolizumab and paclitaxel dosing is recommended.

[b]**Note that each cycle for paclitaxel combinations = 1 week, each cycle for AC = 2 or 3 weeks.**

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3.2 Dose and Administration Schedules for Approved Investigational Agents Pending Activation for Randomization

There are no Investigational Agents approved, pending activation for randomization at this time.

3.3 Dose and Administration Schedules for Investigational Agents Graduated or Dropped, No Longer Active for Randomization

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Appendix O
Pembrolizumab

I-SPY 2 Investigational Agent Information

INVESTIGATIONAL AGENT INFORMATION SUMMARY

General Information:

Agent Class:	PD-1 Inhibitor
Structural Class:	Human monoclonal antibody (IgG4)
Manufacturer:	Merck
Agent Chaperone	Rita Nanda (University of Chicago)
Agent Co-Chaperone:	Minetta Liu (Mayo Rochester)

Pharmaceutical Information:

Dosage Form:	Aqueous solution for intravenous infusion
Physical Description:	Aqueous solution in single use vial
Strengths to be used in trial:	200 mg fixed dose
Packaging Unit	Supplied in 100 mg/4 mL
Storage Conditions:	Vials should be stored at (2 – 8 °C).

Administration Information:

Route:	Intravenous
Standard Regimen:	200mg fixed dose
Agent Preparation:	See section 3.5
Pre-medication:	See section 2.1.1
Administration:	Treatment will be administered on an outpatient basis. See section 2.1 for further details.

Concomitant Medications: There are no known agents known to interact adversely with concomitantly administered pembrolizumab.

Refer to §2.6 for side-effect management and dose reduction plans.

The above is intended as a summary only; please see the complete appendix for additional investigational agent information.

2. RATIONALE FOR TESTING

1.1 Biological Actions

Pembrolizumab (previously known as SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [1]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [2; 3; 4; 5; 6]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [7; 8]. The structure of murine PD-1 has been resolved [9]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [7; 10; 11; 12]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [13; 14]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells [15; 16]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [17] The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [18; 19; 20; 13]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues [13]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL) [21]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a human programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. [47]. It is approved for use in the US and is currently under review in other geographic regions.

1.1.1 *In Vitro* and Mechanistic Studies

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T-cells and leads ultimately to tumor rejection, either as a mono-therapy or in combination with other treatment modalities. Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated anti-tumor responses as a mono-therapy in multiple models of squamous cell carcinoma, pancreatic carcinoma, MEL and colorectal carcinoma. Blockade of the PD-1 pathway effectively promoted CD8+ T-cell infiltration into the tumor and the presence of IFN-γ, granzyme B and perforin, indicating that the mechanism of action involved local infiltration and activation of effector T-cell function in vivo [22; 23; 24; 25; 26; 27]. Experiments have confirmed the in vivo efficacy of PD-1 blockade as a mono-therapy as well as in combination with chemotherapy in syngeneic mouse tumor models (see the Investigator's Brochure [IB]).

1.2 Efficacy Studies

1.2.2 Animal Studies

In *in vivo* efficacy studies in mice, due to the lack of cross-reactivity of MK-3475 and PD-1 from non-primate species, a commercially available anti-mouse PD-1 analog antibody (clone J43) was used. J43's affinity for recombinant mouse PD-1 is 0.64 nM, and is a relatively weak PD-1 binder, with ~22 fold lower affinity than MK-3475. It is also inhibits recombinant murine PD-1 ligands PD-L1 and PD-L2 with IC50 values of 8.2 nM and 2.9 nM, respectively. The ligand blocking capacity of J43 is less potent than that of MK-3475 (~14 fold for PDL1 and ~1.5 fold for PD-L2, respectively).

The anti-PD-1 antibody was evaluated in monotherapy as well as in combination with 5-fluorouracil (5-FU) and gemcitabine. In anti-PD-1 antibody (J43) monotherapy experiments, MC38 colon adenocarcinoma tumor cells were implanted in syngeneic hosts and staged at 50 – 80 mm^3 before initiating dosing. Mice received either the anti-mouse PD-1 antibody or an isotype control antibody intraperitoneally. Dosing was 2 mg/kg or 10 mg/kg every 3 to 4 days for a total of five treatments. Efficacy was evaluated by monitoring tumor volumes and long-term survival for each experimental group. Tumor growth was significantly inhibited in most mice that received the anti-mouse PD-1 antibody at 10 mg/kg. Additionally, 50% of mice that received the anti-PD-1 antibody at 10 mg/kg experienced complete tumor regression, resulting in long-term tumor free survival. Additionally, 20% of mice that received the 2 mg/kg dose demonstrated complete rejection of the tumor. In contrast, none of the mice treated with the control antibody demonstrated complete tumor rejection.

The anti-PD-1 antibody was also evaluated in combination with 5-FU. Mice received the anti-mouse PD-1 antibody or the isotype antibody control at 10 mg/kg, +/- the addition of 5-FU at 40 mg/kg administered concurrently. The MC38 colon adenocarcinoma tumor model was then staged to 100 – 120 mm^3 before administering treatment q3X5. The combination treatment of anti-PD-1 antibody with 5-FU demonstrated the greatest efficacy, with a 60% complete tumor regression rate compared to the anti-PD-1 antibody treatment, which resulted in a 20% complete tumor regression rate. None of the mice treated with the control antibody +/- 5-FU experienced complete regression.

The anti-PD-1 antibody was also evaluated in combination with gemcitabine in mouse MC38 colon adenocarcinoma tumor models. Mice received either the anti-mouse PD-1 antibody or an isotype control antibody at 10 mg/kg at q3X7, +/- concurrent gemcitabine at 10 mg/kg q3X3 (concurrent administration with the first three treatments of the anti-mouse PD-1 antibody or control). The combined treatment of the

anti-PD-1 antibody and gemcitabine demonstrated the greatest efficacy compared to any monotherapy, with an 80% complete tumor regression rate.

1.2.3 Human Studies

Efficacy data are available for a total of 655 P001 melanoma subjects treated with pembrolizumab and 540 P002 melanoma subjects treated with either pembrolizumab or chemotherapy. The data cutoff dates are 18-Apr-2014 for P001 melanoma subjects and 12-May-2014 for P002 subjects.

Through a series of amendments, P001 evolved into 4 Phase II-like melanoma substudies, known as Parts B1, B2, B3, and D. The 4 parts include IPI-refractory and IPI-naïve melanoma, as well as 3 different pembrolizumab dose regimens (2 mg/kg Q3W,10 mg/kg Q3W, and 10 mg/kg Q2W). The efficacy analysis was based upon the All Patients as Treated (APaT) population—all subjects who received at least 1 dose of study treatment and may or may not have had measurable disease at baseline per independent central review. For subjects without measurable disease at baseline, a partial response was not possible (even if there was a reduction in tumor burden) per RECIST 1.1, and non–complete response (CR)/non–progressive disease (PD) was equivalent to stable disease (SD) in subjects with measurable disease at baseline. Overall there were 44 complete responses and 159 partial responses. The ORR was 31% (95% CI: 28% to 35%). Disease Conrtol Rate (DCR) was achieved in 51% of all subjects. See investigator brochure for response rates broken down by cohort.

P002 is a partially blinded, randomized, Phase II pivotal study of pembrolizumab 2 mg/kg Q3W and 10 mg/kg Q3W versus investigator-choice (standard-of-care) chemotherapy in a 1:1:1 ratio in subjects with IPI-refractory metastatic melanoma. There are 2 coprimary efficacy endpoints for this study: progression-free survival (PFS) and overall survival (OS). A secondary efficacy endpoint is ORR.

The data cutoff date for efficacy events included in the efficacy analysis was 12-May-2014. The analysis was based upon the Intention-to-Treat (ITT) population – subjects were included in the treatment group to which they were randomized. The PFS analysis is available for 361 subjects who received pembrolizumab and 179 subjects who received chemotherapy (control arm). The hazard ratio was 0.57 and 0.50 in the pembrolizumab 2 mg/kg Q3W arm and 10 mg/kg Q3W arm over the control arm, respectively, favoring the pembrolizumab arms for PFS (the one-sided p-value was <0.0001 in both comparisons). This met prespecified criteria for a positive study.

The PFS rate at Month 6 was 34.3% (95% CI: 27.4 to 41.3%) and 37.7% (95% CI: 30.6% to 44.8%) for pembrolizumab 2 mg/kg and 10 mg/kg, respectively, versus 15.6% (95% CI: 10.5% to 21.5%) for the control arm. The median PFS was 2.9 months in both pembrolizumab arms and 2.7 months in the control arm. The PFS data show that administration of pembrolizumab (both dose regimens) resulted in a clinically meaningful improvement in PFS versus treatment with chemotherapy. The ORR per RECIST 1.1 by IRO is2 1% in the pembrolizumab 2 mg/kg arm, 25% in the 10 mg/kg arm, and 4% in the chemotherapy arm (p<0.0001 for each pembrolizumab dose versus chemotherapy).

A preliminary analysis of OS indicated that the hazard ratio was 0.88 in the pembrolizumab 2 mg/kg Q3W arm over the control arm and 0.78 in the pembrolizumab 10 mg/kg Q3W arm over the control arm. The one-sided p-value was 0.229 and 0.066 in 2 mg/kg Q3W and 10 mg/kg Q3W over the control arm, respectively, both favoring pembrolizumab. The prespecified final analysis of OS will be performed after 370 deaths have occurred.

The overall response rates for pembrolizumab treatment in P001 and P002 compared favorably to historical response rates for available treatments for melanoma, particularly in subjects who had progressed after multiple prior therapies.

1.3 Ongoing/Planned NCI Or Industry Testing

Clinical data supporting pembrolizumab use for the treatment of mTNBC.

In the first report of clinical activity of an immune checkpoint inhibitor in TNBC, a Merck-sponsored multi-center, non-randomized Phase Ib trial (KN 012) showed that single agent pembrolizumab given at 10 mg/kg IV Q2W is a well-tolerated and effective treatment with significant therapeutic activity in a subset of heavily pre-treated subjects with mTNBC.

Methods: PD-L1 expression in ⩾1% tumor cells or in stroma (i.e., PD-L1 positive mTNBC) was required for study entry. PD-L1 tumor status was determined by immunohistochemical analysis of archival tumor specimens using the Merck proprietary 22C3 antibody (Qualtek assay). Primary objectives of this study were to determine the safety, tolerability, and antitumor activity of pembrolizumab in subjects with PD-L1 positive mTNBC. Secondary objectives included assessments of progression-free survival (PFS), overall survival (OS), and duration or response (DOR). Adverse events (AEs) reported in any subject receiving at least 1 dose of study treatment were monitored and graded using National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI CTCAE v. 4.0). Tumor imaging was obtained every 8 weeks and evaluated by both investigator and a central imaging vendor to assess clinical responses as defined by RECIST 1.1.

Results: A total of 32 female subjects with a median age of 50.5 years (range 29-72 years) and PD-L1 positive mTNBC were enrolled in the study (the prevalence of PD-L1 tumor positivity in mTNBC was 58%, as determined by the Qualtek assay). Most of these subjects had received and progressed on multiple lines of therapy for advanced disease (47% of subjects had received ⩾ 3 prior treatments in the metastatic setting). According to data through 06-Nov-2014, five subjects (15.6%) experienced at least one drug-related serious adverse event (SAE); each of four subjects experienced one of the following: Grade 3 anemia, headache, aseptic meningitis or pyrexia, and a fifth subject experienced Grade 5 disseminated intravascular coagulation (DIC) with thrombocytopenia and decreased blood fibrinogen. Of the 27 subjects with centrally confirmed measurable disease, one subject (3.7%) had a complete response (CR), 4 subjects (14.8%) had a confirmed partial response (PR), 25.9% had stable disease (SD), and 44.4% had progressive disease (PD) based on RECIST 1.1 as assessed by central imaging vendor. As of 06-Nov-2014, the median duration of response had not been reached (range 15 to 40+ weeks), and three subjects (1 CR; 2 PR) were still on treatment after at least 11 months.

Conclusions: This is the first report of clinical activity of an immune checkpoint inhibitor in TNBC. The preliminary results from this study suggest that single agent MK-3475 is a well-tolerated and effective treatment with significant therapeutic activity in a subset of heavily pre-treated subjects with recurrent/metastatic triple-negative breast cancer.

For additional information on pembrolizumab in other tumor types, please refer to the most current version of the Investigator Brochure.

1.4 In Vitro and Animal Studies

For the most recent toxicity/safety and PK/PD pre-clinical data, please refer to the Investigator Brochure.

1.5.2 Human Studies

The pharmacokinetic profile of pembrolizumab has low clearance and limited volume of distribution, which is typical for therapeutic antibodies. Exposure to pembrolizumab is approximately linear in the dose range of 1-10 mg/kg, the range considered clinically relevant. Furthermore, MK-3475 has a low potential of eliciting the formation of anti-drug antibodies. The PK profile of pembrolizumab was investigated in Part A of the ongoing study P001. Results have been obtained following a single dose at 1, 3, and 10 mg/kg pembrolizumab to17 subjects with solid tumors in Cycle 1 (Part A and A-1). The observed PK profile of pembrolizumab was typical of those observed for other IgG mAbs with a half-life (t1/2) of approximately 2 to 3 weeks. There was no indication of dose dependency of t1/2 in the 3 dose groups. A dose-related increase in exposure was observed from 1 to 10 mg/kg. The long t1/2 supports a dosing interval of every 2 weeks or every 3 weeks.

As of the data cut-off of the most current IB, the occurrence of treatment emergent anti-drug antibodies (ADA) has been observed in 1 patient, less than 1% of the patients screened for immunogenicity assessment. This indicates a low potential of pembrolizumab to elicit the formation of ADA, and further, no impact on pembrolizumab exposure has been observed.

1.6 Rationale for Dose Selection

An open-label Phase I trial (KEYNOTE-001) is being conducted to evaluate the safety and clinical activity of single agent pembrolizumab (MK-3475). The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab (MK-3475) showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified.

In KEYNOTE-001, two randomized cohort evaluations of melanoma subjects receiving pembrolizumab (MK-3475) at a dose of 2 mg/kg versus 10 mg/kg Q3W have been completed, and one randomized cohort evaluating of 10 mg/kg Q3W versus 10 mg/kg Q2W has also been completed. The clinical efficacy and safety data demonstrate a lack of clinically important differences in efficacy response or safety profile at these doses. For example, in Cohort B2, advanced melanoma subjects who had received prior ipilimumab therapy were randomized to receive pembrolizumab (MK-3475) at 2 mg/kg versus 10 mg/kg Q3W. The ORR was 26% (21/81) in the 2 mg/kg group and 26% (25/79) in the 10 mg/kg group (FAS). The proportion of subjects with drug-related AE, grade 3-5 drug-related AE, serious drug-related AE, death or discontinuation due to an AE was comparable between groups or lower in the 10 mg/kg group. In Cohort B3, advanced melanoma subjects (irrespective of prior ipilimumab therapy) were randomized to receive pembrolizumab (MK-3475) at 10 mg/kg Q2W versus 10 mg/kg Q3W. The ORR was 30.9% (38/123) in the 10mg/kg Q2W group and 24.8% (30/121) in the 10 mg/kg Q3W group (APaT). The proportion of subjects with drug-related AE, grade 3-5 drug-related AE, serious drug-related AE, death or discontinuation due to an AE was comparable between groups.

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q3W dosing schedule. Because Q3W dosing is more convenient for patients, Q3W dosing will be further studied.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumors is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumor burden or indication on distribution behavior of pembrolizumab (as assessed by the population PK model)

and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumor type.

The choice of the 200 mg Q3W fixed dosing regimen is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will also be simpler and more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will reduce complexity in the logistical chain at treatment facilities and reduce wastage.

2. INVESTIGATIONAL STUDY AGENT ADMINISTRATION IN THE I-SPY 2 TRIAL

Intervention will be administered on an outpatient basis. Reported clinical AEs and potential risks are described in §3.2.

2.1 Dose Regimen and Dose Groups

The dose schedule for pembrolizumab is:

Table 1. Paclitaxel (q1w × 12 weeks), and pembrolizumab (q3w × 12 weeks) [a]; Followed by AC (q2w or q3w)

Agent	Dose	Route	Cycle[b]
Paclitaxel	80 mg/m^2	IV	1–12
Pembrolizumab[a]	**200 mg**	**IV**	**1,4,7,10**
Doxorubicin	60 mg/m^2	IV	13–16
Cyclophosphamide	600 mg/m^2	IV	13–16

[a] The pembrolizumab infusion should precede paclitaxel treatment and be administered over 30 minutes (-5 min/+10 min). A 30-minute waiting period between pembrolizumab and paclitaxel dosing is recommended.

[b] Note that each cycle for paclitaxel combinations = 1 week, each cycle for AC = 2 or 3 weeks.

2.1.1 Paclitaxel Premedication Regimen

Uniform steroid premedication is required for the paclitaxel arm. Adherence to the below is mandatory for all patients:

- **Administer dexamethasone 10-20mg (IV or PO) and diphenhydramine HCl 25 to 50 mg (IV or PO) once thirty (30) minutes prior to the first dose of paclitaxel.**
  - Steroid administration the night before and/or morning of dosing is not permitted.
  - Other premedication agents (ex. Famotidine) are allowable at the clinician's discretion.
  - On days that pembrolizumab and paclitaxel are given together the steroids should follow the completion of the pembrolizumab infusion.
- **Administer dexamethasone 10 mg (IV or PO) and diphenhydramine HCl 25 to 50 mg (IV or PO) once thirty (30) minutes prior to the second dose of paclitaxel.**

- **For participants who do not experience an infusion related hypersensitivity reaction with either the first or second doses of paclitaxel, discontinue subsequent steroid premedication**.
    - Other non-steroidal premedication agents may be continued at the clinician's discretion (ex. diphenhydramine HCl, Famotidine, etc).
- For participants who do experience an infusion hypersensitivity reaction with any dose of paclitaxel, steroid premedication should be continued at a maximum of dexamethasone 10-20mg (IV or PO) and diphenhydramine HCl 25 to 50 mg (IV or PO) once thirty (30) minutes prior to paclitaxel dosing for the study's duration.

2.2 Additional Eligibility Criteria

There are no additional eligibility criteria for assignment to pembrolizumab.

Participants must meet all investigational agent-specific criteria as described in Appendix C §2 and the main protocol §4.1.2 in order to be eligible for the treatment phase of I-SPY 2.

2.3 Contraindications

- None known at this time.

2.4 Concomitant Medications

The medications listed below are not allowed during the period of pembrolizumab administration:

- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine.
    - Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g. Flu - Mist®) are live attenuated vaccines, and are not allowed.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an adverse event of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.
    - Inhaled steroids are allowed for management of asthma.
    - Use of prophylactic corticosteroids to avoid or treat acute allergic reactions is permitted.

2.5 Clinical Evaluation and Procedures

Laboratory evaluations for general safety monitoring are described in protocol §8.1─ 8.3; additional evaluations/procedures necessary for this agent include:

- No live vaccines within 30 days prior to first treatment with pembrolizumab. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g. Flu - Mist®) are live attenuated vaccines, and are not allowed.
- TSH blood test
    - Prior to C1D1
    - If clinically indicated during treatment
- Collection of 3 unstained slides from the diagnostic core biopsy (FFPE core). See I-SPY 2 MOP for further instructions.

2.6 Dose Modifications and Management of Toxicity

Guidelines for dose modification and management of pembrolizumab-related events, provided in Table 4. Dose adjustments are to be made according to the organ system showing the greatest degree of toxicity. Toxicity will be graded for severity using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Treatment delay of ≥3 weeks due to toxicity will lead to stopping all protocol therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant will remain on study for outcome assessment.

Pembrolizumab will be continued every three weeks throughout the paclitaxel chemotherapy course, unless specified otherwise in Table 4 below. Missed doses of pembrolizumab will not be made up. Dose reductions of pembrolizumab are not permitted. Missed doses of standard chemotherapy can be made up to complete the full regimen

For purposes of this trial, 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 subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated
Please refer to the I-SPY 2 MOP for instructions on reporting overdose.

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 4 below.
For details on immune-related AEs and infusion AEs, please refer to Section 3.2.

Table 2. Paclitaxel + Pembrolizumab Dose Modification

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
Neutropenia		
≥1000/mm^3	No change to paclitaxel. • For ANC ≤1500/mm^3, consider the use of prophylactic myeloid growth factors (filgrastim), o Start on day 2 or 3 and use according to participant need, at physician discretion, and to avoid dose reduction. o Growth factor should not be given on the same day as chemotherapy. o Pegfilgrastim may not be used with paclitaxel due to the weekly dosing in this study.	No Change to pembrolizumab
<1000/mm^3	Hold paclitaxel until ANC≥1000/mm^3. Consider adding prophylactic G-CSF for subsequent cycles. Resume paclitaxel based on timing of recovery: • ≤1 week: No change to paclitaxel • ≥1 but <3 weeks:	No change to pembrolizumab.

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
	○ Dose reduce paclitaxel by 25% for all subsequent cycles. • ≥3 weeks: Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. G-CSF may be used between days 2–6 according to participant need, at physician discretion, and to avoid dose reduction. Pegfilgrastim may not be used with paclitaxel due to the weekly dosing used in this study.	
Neutropenic Fever		
ANC≤1000/mm^3, fever≥38.5°C	Hold paclitaxel until resolved (ANC>1000/mm^3, fever <38.5°C, and resolution of any signs of infection). Resume paclitaxel according to number of episodes: • First episode: no change to paclitaxel. Consider adding prophylactic GCSF for subsequent cycles. • Second episode: Reduce paclitaxel by 25% for all subsequent doses. • Third episode: Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant will remain on study for outcome assessment. • If paclitaxel is held for 3 weeks in a row, permanently discontinue. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. G-CSF may be used between days 2–6 according to participant need, at physician discretion, and to avoid dose reduction. Pegfilgrastim may not be used with paclitaxel due to the weekly dosing in this study.	Hold pembrolizumab until resolved (ANC>1000/mm^3, fever <38.5°C, and resolution of any signs of infection). Resume pembrolizumab at previous dose.

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
Thrombocytopenia		
≥100,000/mm^3	No change to paclitaxel and pembrolizumab.	No change to pembrolizumab
75–99,999/mm^3	Hold paclitaxel until ≥100,000/mm^3, resume paclitaxel based on timing of recovery: • ≤1 week—no change to paclitaxel. • >1 but <3 weeks— o First Episode: Reduce paclitaxel dose by 25% for all subsequent cycles. o Second Episode: stop paclitaxel and pembrolizumab. Participant remains on study for outcome assessment. • 3 weeks—stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	No change to pembrolizumab
<75,000/mm^3	Hold paclitaxel until ≥100,000/mm^3. Resume paclitaxel according to the number of episodes: • First Episode: Reduce paclitaxel by 25% for all subsequent cycles. • Second Episode: stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	Hold pembrolizumab until ≥100,000/mm^3. Resume pembrolizumab at previous dose
Anemia		
All grades	For all anemia events related to paclitaxel regardless of grade, iron studies should be checked and iron should be replaced as indicated. • Red blood cell transfusions can be given at the investigators discretion	No change to pembrolizumab

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
	as needed for symptom control.	
Hepatic		
Grade 1	No change to paclitaxel.	No change to pembrolizumab
Grade 2	Grade 2 AST, ALT or bilirubin (in the absence of Gilbert's disease): Hold paclitaxel until AST or ALT or bilirubin resolves to ≤ Grade 1. If resolved within 2 weeks, resume paclitaxel based on number of episodes: • First episode: No change to paclitaxel. • Second episode: Reduce paclitaxel by 25% for all subsequent doses. • Third episode: Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant will remain on study for outcome assessment. If paclitaxel or pembrolizumab are held for 3 weeks in a row, stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment. A rise in indirect bilirubin with a normal direct bilirubin believed to be attributable to Gilbert's disease does not require change in dose or agent hold. A note to file should be created.	Grade 2 AST, ALT or bilirubin (in the absence of Gilbert's disease): Hold pembrolizumab until AST or ALT or bilirubin resolves to ≤ Grade 1. Resume pembrolizumab at previous dose. • Treat with IV or oral corticosteroids. When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
≥ Grade 3	≥ Grade 3 AST or ALT or bilirubin: Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	Discontinue pembrolizumab • For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours. When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
AST or ALT ⩾ 3 × ULN with signs and symptoms consistent with hepatitis OR AST or ALT ≥ 3 × ULN and total bilirubin > 2 × ULN and/or INR > 1.5 and (ALP) < 2X ULN	Stop paclitaxel,and pembrolizumab therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment ***IF these events occur they must be reported as an SAE, Please see section 2.6.1 for additional guidance on appropriate management and monitoring***	Stop paclitaxel,and pembrolizumab therapy. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment ***IF these events occur they must be reported as an SAE, Please see section 2.6.1 for additional guidance on appropriate management and monitoring***
Nausea/Vomiting/Anorexia		
Grade 1–2	No change to paclitaxel.	No change to pembrolizumab
≥ Grade 3	Hold paclitaxel until resolved to ≤grade 1. Resume paclitaxel based on number of episodes: • First Episode: Resume paclitaxel at previous dose with modification of premedications. • Second episode despite maximal supportive care: Resume paclitaxel with a 25% dose reduction for all subsequent doses. • Third Episode: Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	Hold pembrolizumab until resolved to ≤grade 1. Resume pembrolizumab at previous dose
Diarrhea/Colitis Participants should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).		
Grade 1	No change to paclitaxel.	No change to pembrolizumab
Grade 2 and 3	No change to paclitaxel. • Participants who experience 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. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis. o For **Grade 2**	Hold pembrolizumab* until diarrhea resolves to ≤ Grade, 1. • Participants who experience 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. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
	diarrhea/colitis that persists greater than 3 days, administer oral corticosteroids. o For **Grade 3 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids. o When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.	o For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids. o For **Grade 3 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids. o When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. If pembrolizumab is held for 3 weeks in a row, stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Grade 4	Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Mucositis/Stomatitis		
Grade 0–2	No change to paclitaxel.	No change to pembrolizumab
≥ Grade 3	Hold paclitaxel until symptoms have resolved to ≤grade 1 Resume paclitaxel at the previous dose with modification of premedications. • Consideration should be given for the addition of G-CSF. For persistent toxicity ≥grade 3 despite maximal supportive care: • Dose reduce paclitaxel by 25% by one dose level for all subsequent doses.	Hold pembrolizumab until symptoms have resolved to ≤grade 1. ▪ Resume pembrolizumab at the previous dose
Neurotoxicity		
Grade 0–2	No change to paclitaxel.	No change to pembrolizumab.
Grade 3	Hold paclitaxel until neuropathy improves to ≤grade 2.	Hold pembrolizumab until neuropathy improves to ≤grade 2.

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
	• Resume paclitaxel with a 25% dose reduction for all subsequent cycles. If paclitaxel and pembrolizumab are held for 3 weeks in a row for neuropathy, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	▪ Resume pembrolizumab at previous dose
Grade 4	Stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment	Discontinue pembrolizumab
Anaphylaxis/Hypersensitivity		
Mild (*e.g.*, mild flushing, rash, pruritis)	Mild symptoms (grade 1: *e.g.*, transient flushing, rash or fever): • Complete paclitaxel infusion • No treatment required, but observe participant at least until symptoms have resolved.	Mild symptoms (grade 1: *e.g.*, transient flushing, rash or fever): • Complete pembrolizumab infusion No treatment required, but observe participant at least until symptoms have resolved.
Moderate (*e.g.*, moderate flushing, rash, mild dyspnea, chest discomfort)	Moderate symptoms (grade 2: *e.g.*, rash, flushing, urticaria, dyspnea, chest discomfort): For paclitaxel: • Hold paclitaxel infusion. • Give intravenous diphenhydramine 20–25 mg and intravenous dexamethasone 10 mg. • Resume paclitaxel infusion after recovery of symptoms at half the previous rate for 15 minutes. If no recurrence of symptoms, the planned rate may be resumed. If symptoms recur after re-challenge: • Stop infusion and stop all subsequent paclitaxel and pembrolizumab treatment. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	Moderate symptoms (grade 2: *e.g.*, rash, flushing, urticaria, dyspnea, chest discomfort): For pembrolizumab: • Stop pembrolizumab infusion. • Give IV dexamethasone 10 mg and diphenhydramine HCl 25 to 50 mg • If symptoms resolve within one 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 subject should be premedicated for the next scheduled dose. If symptoms recur after re-challenge: • Stop infusion and stop all subsequent paclitaxel and pembrolizumab treatment. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
Severe (*e.g*., hypotension requiring pressers, angioedema, respiratory distress requiring bronchodilators)	Severe or life-threatening symptoms (grade 3 or 4: *e.g.*, hypotension, angioedema, respiratory distress or anaphylaxis): • Stop paclitaxel infusion. • Administer diphenhydramine 25 mg and dexamethasone 10 mg iv. Add epinephrine or bronchodilators as needed per institutional guidelines. • Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.	Severe or life-threatening symptoms (grade 3 or 4: *e.g.*, hypotension, angioedema, respiratory distress or anaphylaxis): • Stop pembrolizumab infusion. • Administer diphenhydramine 25 mg and dexamethasone 10 mg iv. Add epinephrine or bronchodilators as needed per institutional guidelines. • Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment.
Rash		
Grade 1 and 2	No change to paclitaxel. ▪ **Grade 2:** Symptomatic treatment should be given such as topical glucocorticosteroids (e.g., betamethasone 0.1% cream or hydrocortisone 1%) or urea-containing creams in combination with oral anti-pruritics(e.g., diphenhydramine HCl or hydroxyzine HCl). o Treatment with oral steroids is at physician's discretion for Grade 2 events.	No change to pembrolizumab. ▪ **Grade 2:** Symptomatic treatment should be given such as topical glucocorticosteroids (e.g., betamethasone 0.1% cream or hydrocortisone 1%) or urea-containing creams in combination with oral anti-pruritics(e.g., diphenhydramine HCl or hydroxyzine HCl). o Treatment with oral steroids is at physician's discretion for Grade 2 events.
Grade 3	No change to paclitaxel	Hold pembrolizumab until improves to ≤grade 1. – Treatment with oral steroids is recommended, starting with 1 mg/kg prednisone or equivalent once per day or dexamethasone 4 mg four times orally daily. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. – Consider Dermatology Consultation and biopsy for confirmation of diagnosis. If pembrolizumab is held for 3 weeks in a row for, stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
		surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
≥ Grade 4	Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment ▪ Initiate steroids at 1 to 2 mg/kg prednisone or equivalent. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Dermatology consultation and consideration of biopsy and clinical dermatology photograph.	
Pneumonitis		
Grade 1	No change.	
Grade 2	Hold paclitaxel until pneumonitis improves to ≤grade 1. Resume based on number of episodes: • First Episode: No change to paclitaxel • Second Episode: Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4weeks If paclitaxel and pembrolizumab are held for 3 weeks in a row for, stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment	Hold pembrolizumab until pneumonitis improves to ≤grade 1. Resume based on number of episodes: • First Episode: No change to pembrolizumab • Second Episode: Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment Treat with systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4weeks If paclitaxel and pembrolizumab are held for 3 weeks in a row for, stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
≥ Grade 3	Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment Treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed. Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.	

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
Type 1 diabetes mellitus (if new onset) or ≥ Grade 3 Hyperglycemia		
≥ Grade 3 hyperglycemia or new onset of TIDM	No change to paclitaxel	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure until clinically and metabolically stable. If pembrolizumab is held for 3 weeks in a row, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
Hypophysitis		
Grade 1	No change to paclitaxel.	No change to pembrolizumab.
≥ Grade 2	No change to paclitaxel	Hold pembrolizumab* until hypophysitis resolves to ≤grade 1. If pembrolizumab is held for 3 weeks in a row, stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment • For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered. • For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
Hyperthyroidism		
Grade 1 and 2	No change to paclitaxel.	No change to pembrolizumab • **Grade 2** hyperthyroidism events (and **Grade 3-4** hypothyroidism): o In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy. o In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
Grade 3	No change to paclitaxel	Hold pembrolizumab* until hyperthyroidism resolves to ≤grade 1. ▪ Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered. If paclitaxel and pembrolizumab are held for 3 weeks in a row for neuropathy, stop paclitaxel. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
Grade 4	Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered	
Renal Failure or Nephritis		
Grade 1	No change to paclitaxel	No change to pembrolizumab
Grade 2	No change to paclitaxel	Hold pembrolizumab* until resolves to ≤grade 1. • Treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4

Event	Paclitaxel Dose Modification	Pembrolizumab Dose Modification if combined treatment week (1,4,7, 10)
		weeks. If pembrolizumab is held for 3 weeks in a row stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment
≥ Grade 3	Stop paclitaxel and pembrolizumab. Participant should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Participant remains on study for outcome assessment Treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.	
Other Clinically Significant Toxicity Excluding Fatigue, Alopecia, and Leukopenia at Physician Discretion		
Grade 0 or 1	No change to paclitaxel	No change to pembrolizumab
Grade 2	Hold paclitaxel until resolved to ≤ grade 1. • Resume paclitaxel at previous dose.	Hold pembrolizumab until resolved to ≤ grade 1. • Resume pembrolizumab at previous dose.
≥ Grade 3	Stop paclitaxel and pembrolizumab and contact the DCC for further instruction (1-855-889-5170).	

Abbreviations: ANC: absolute neutrophil count; IV: intravenous injection
Grades refer to CTCAE version 4
*All dose reductions will be based on blood counts obtained on a planned day of chemotherapy. Nadir counts will not be measured routinely.

The following dose levels will be utilized for the purpose of dose modifications for toxicity:

Table 3. Dose Adjustments for Paclitaxel

Dose Adjustment	Paclitaxel Dose, mg/m^2
Standard dose	80
25% reduction	60

[a]Dose to be given only if a dose reduction is required.

Table 4. Dose Adjustments for Pembrolizumab

Dose Adjustment[a]	Pembrolizumab Dose, mg
Standard dose	200 mg

[a] No dose reductions are permitted

2.6.1 Liver Monitoring and Assessments for Hy's Law Events

Hy's Law/severe potential drug induced liver injury (DILI) should be suspected in patients who meet ALL 4 criteria listed below. **Study drugs should be permanently discontinued** and patients are to be followed according to the recommendations in Table 5. These events must be reported as soon as possible to the Sponsor as a SAE.

1. AST or ALT increases to ≥ 3 × ULN
2. Total bilirubin increases to > 2 × ULN and/or INR > 1.5
3. Alkaline phosphatase (ALP) value does not reach 2 × ULN
4. No alternative cause explains the combination of the above laboratory abnormalities; important alternative causes include, but are not limited to:
    - Hepatobiliary tract disease
    - Viral hepatitis (eg, hepatitis A/B/C/D/E, Epstein-Barr virus, cytomegalovirus, herpes simplex virus, varicella, toxoplasmosis, parvovirus)
    - Congestive heart failure, hypotension, or any cause of hypoxia to the liver causing ischemia
    - Exposure to hepatotoxic agents/drugs or hepatotoxins, including herbal and dietary supplements, plants, and mushrooms
    - Alcoholic hepatitis
    - Non-alcoholic steatohepatitis (NASH)
    - Autoimmune hepatitis
    - Wilson's disease and hemochromatosis
    - Alpha-one antitrypsin deficiency

Table 5. Safety Monitoring After Events Potentially Meeting Hy's Law Criteria or Suggesting Potential Drug-Induced Liver Injury

Results	Frequency for Repeating Liver (AST, ALT, Bilirubin [Total and Direct]) and INR Tests
After the initial liver test abnormality	Within 24 hours
If AST or ALT ≥ 3 × ULN, and total bilirubin > 2 × ULN or INR > 1.5	Every 24 hours until laboratory abnormalities improve
If ALT or AST ≥ 3 × ULN and total bilirubin and/or INR are normal OR total bilirubin is < 2xULN and /or INR < 1.5	Every 48 to 72 hours until laboratory abnormalities improve
If the liver test abnormalities improve AND the subject is asymptomatic	Frequency may decrease

3. INVESTIGATIONAL AGENT PHARMACEUTICAL INFORMATION

3.1 Investigational Study Agent (IND # 105,139, IND Sponsor: QLHC)

Pembrolizumab (MK-3475) is a sterile, non-pyrogenic aqueous solution supplied in single-use glass vial containing 100 mg/4 mL of pembrolizumab. The product is preservative-free and essentially free of extraneous particulates.

Confidential pharmaceutical information for investigational study agents supplied by pharmaceutical partners is available through an FDA IND cross-reference letter.

3.2 Reported Clinical AEs and Potential Risks

As of the data cutoff dates for the August 31, 2015 investigator brochure (18-Apr-2014 for P001 melanoma subjects, 29-Aug-2014 for P001 non-small cell lung cancer (NSCLC) subjects, 12-May-2014 for P002, and 30-Nov-2014 for all other protocols), pembrolizumab monotherapy and combination therapy have been administered to 6294 subjects with hematologic malignancies and solid tumors, in a total of 18 ongoing, Phase I, II, and III clinical trials sponsored by Merck.

In the pembrolizumab monotherapy trials (P001/P002, P012, P013, and P028, plus the P011monotherapy arm), the overall incidence of AEs ranged from 83.0% (73 of 88 subjects in P012) to 100% (10 of 10 subjects in P011). The most commonly reported AEs included fatigue, diarrhoea, decreased appetite, nausea, and anaemia. The incidence of drug –related AEs (DRAEs) ranged from 39.8% (35 of 88 subjects in P013) to 80.0% (8 of 10 subects in P011). The most commonly reported DRAEs across all studies were nausea, fatigue, and diarrhoea. The incidence of Grade 3-5 DRAEs across studies ranged from 6.8% (6 of 88 in P013) to 12.0% (187 of 1562 subjects) in P001/P002. The most commonly reported Grade 3-5 DRAEs were anemia, alanine aminotransferase increased, and aspartate aminotransferase increased. Most subjects who experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 1.9% (8 of 430 subjects in P028) to 12.3% (192 of 1562 subjects in P001/P002). The majority of AEs leading to discontinuation were not considered drug related. Discontinuations due to a DRAE were infrequent and ranged from 0% (no subjects in P011) to 4.5% (4 of 88 subjects in P013). The most commonly reported DRAEs leading to discontinuation were pneumonitis, alanine aminotransferase increased, and aspartate aminotransferase increased.

The central function of the PD-1/PD-L1 pathway is to maintain immune tolerance to the fetal allograft and its important role in maintaining pregnancy has been recently emphasized in the literature. The evidence from experimental results of the blockade of PD-L1 signaling in murine models indicates that there is a theoretical risk associated with the administration of MK-3475 to women of child-bearing potential. As a result, pembrolizumab should not be administered to pregnant women or lactating women who are breast-feeding.

Reference Safety Information is included in the current investigator brochure (Section 5) and should be used for the determination of expectedness for regulatory reporting.

Adverse Events of Special Interest

Adverse Events of Special Interest (AEOSI), including immune-mediated AEs, are being investigated. Please refer the investigational brochure for the complete list of AEOSI and their incidence.

Immune-Related Adverse Events

An irAE is defined as a clinically significant AE of any organ that is associated with study drug exposure, is of unknown etiology, and is consistent with an immune-related mechanism. Based on the IB dated August 31, 2015 AEOSI data for melanoma and lung subjects demonstrates that irAEs were reported in 16.1% of subjects (251 of 1562) overall; AEOSI were considered by the Investigators to be drug related in 14.3% of subjects (223 of 1562). The majority of AEOSI were Grade 1 or 2 in severity. Overall, serious AEOSI occurred in 4.2% of subjects at 2 mg/kg Q3W, 3.7% of subjects at 10 mg/kg Q3W, and

3.9% of subjects at 10 mg/kg Q2W. There was one AEOSI (pneumonitis) related to death in 10 mg/kg Q3W arm, in a subject with NSCLC. The rate of discontinuation due to AEOSI was low (2.6%).

The most commonly reported immune-related adverse events across the dose schedules are hypothyroidism (7.2%), pneumonitis (2.9%), hyperthyroidism (2.2%), colitis (1.3%) and skin AEOSI (1.3% including all terms). Please refer to the most recent IB for all immune related events Based on the mechanism of action of MK-3475 and similar immunomodulatory agents, the Sponsor is interested in potential irAEs, and encourages appropriate investigation of signs and symptoms suggestive of these. Consultation with the appropriate medical specialist should be considered when investigating a possible irAE. These events can occur after the first dose to several months after the last dose of treatment. Mild irAEs are usually treated symptomatically and do not require dosing delays or discontinuation. Higher grade and persistent lower grade irAEs typically necessitate withholding or discontinuing treatment and administration of systemic steroids or other immunosuppressive agents (such as tumor necrosis factor blockers), when systemic steroids are not effective. Early recognition of irAEs and initiation of treatment are critical to reduce the risk of complications, since the majority of irAEs are reversible with the use of steroids and other immune suppressants

Infusion Reactions:
Based on the IB dated August 31, 2015, infusion reactions have been reported with pembrolizumab at a rate of 2.5%; these were generally Grade 1 and 2 and the majority were considered related by the Investigator. One event of Grade 4 anaphylaxis has been reported. Infusion reactions may present as allergic reaction, serum sickness, infusion reaction, cytokine release syndrome, or anaphylaxis. Mild infusion reactions can generally be treated with interruption of the infusion and medical intervention including IV fluids, antihistamines, nonsteroidal anti-inflammatory drugs, acetaminophen, and narcotics as needed. More severe or life thereatening reactions may require pressors, corticosteroids, and epinephrine. Pembrolizumab therapy should not be redosed in these more severe cases.

3.3 Investigational Agent Availability

Pembrolizumab is manufactured by Merck. The agent product is packaged in vial form. Dose strengths of 100 mg/ 4 mL are to be used in this trial. The investigator or designee will record the lot number, expiration date and the amount of study medication dispensed to each participant.

Pembrolizumab is provided under a CTPA between Merck and the QLHC.

3.4 Investigational Agent Distribution

Shipment of investigational agents to a participating site will not be approved until documentation of IRB approval of the sponsor-approved protocol and consent is available, and the collection of all essential documents is complete.

Investigational agents may be requested by the investigator (or their authorized designees) at each organization. Investigational agents will be shipped directly to the institution or site where the agent will be prepared and administered. The transfer of agents between institutions is not permitted (unless prior approval from the sponsor is obtained). Agents are requested by completing the Investigational Agent Request Form (to include complete shipping contact information) and submitting the form to the sponsor-designated DCC, see I-SPY 2 MOP for additional details

Once then QLHC or their designee establishes that the requesting site is authorized to receive investigational agents, the order will be forwarded to the manufacturer, who will ship the investigational

agent directly to the study site. Instructions for ordering investigational agents are available in the I-SPY 2 MOP.

3.5 Investigational Agent Preparation and Handling

Pembrolizumab must be prepared by a dedicated pharmacist or qualified research staff member. Efforts should be made to ensure that the study supplies used, preparation procedure, and conditions are consistent for each dose of pembrolizumab that is administered.

The pembrolizumab drug product must be diluted prior to IV administration following the preparation guidelines provided in the I-SPY 2 MOP. The diluted solution must be used within 4 hours of preparation. The appropriate drug administration instructions per the preparation guidelines must be carefully followed prior to use. Refer to the I-SPY 2 MOP for detailed pembrolizumab preparation guidelines.

3.5.1 Steps for Infusion Dose Preparation

Pembrolizumab vials should be stored protected from light at 2 °C to 8 °C (36 °F to 46 °F) for the 100 mg/4 mL vial. Vials are for single use only. In order to ensure the safety of the clinical study participant, it is important that the investigational product has been stored, dispensed, prepared, administered and/or destroyed in accordance with the instructions provided.

Study drug preparation should be performed under standard aseptic conditions following chemotherapeutic precautions and since no anti-microbial preservative is present in the solutions.

In general, the following concentration range guidelines should be used when preparing pembrolizumab for infusion. The final concentration of the pembrolizumab infusion should be within 1 mg/mL and 10 mg/mL. The infusion bag must be filled to at least 30% of its capacity (the infusion volume to bag capacity ratio should not be less than 0.3).

To calculate the volume of pembrolizumab and saline solution:

1. Volume of pembrolizumab (mL) = required dose amount (mg) / 25 (mg/mL)

2. Volume of normal saline = total infusion volume – volume of pembrolizumab from above

Gently invert the bag 10 – 15 times.

Ensure that the entire contents of the solution are administered. Do not co-administer drugs through the same line. Discard any unused solution.

The pembrolizumab infusion should be administered at room temperature over the course of 30 minutes via an infusion pump, with a window of -5 and +10 minutes. Pembrolizumab solutions may be stored at room temperature for up to a cumulative four hours. This includes the duration of the infusion. Alternatively, IV bags of prepared solutions may be stored for up to 20 hours under refrigeration at 2 °C to 8 °C (36 °F to 46 °F). Do not freeze the solution.

3.6 Investigational Agent Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all investigational agents. The investigator is required to maintain adequate records of receipt, dispensing, and final disposition of study agent. On the receipt, record from whom study agent was received and to whom study agent was shipped, date, quantity, and batch or lot

number. On the dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

3.7 Investigational Agent Packaging and Labeling

Pembrolizumab is packaged and labeled by Merck Sharpe & Dohme, Corp.. according to their established procedures as well as in accordance with current ICH, GCP, FDA, and national requirements. Labels are printed and attached to the study agent bottle or other packaging container prior to shipping to the site. Each is labeled with a single panel label that will include, but is not limited to, the following information:

- Blank spaces to write the study number and investigator name
- IND caution statement
- Agent identification
- Lot number
- Storage conditions
- Dosing instructions
- Blank spaces to write the participant's identification number, initials, and date dispensed
- Caution statement indicating that the agent is for clinical trial use only

Each label must remain affixed to the vial.

3.8 Investigational Agent Storage

Pembrolizumab solution for infusion vials should be stored at 2 °C to 8 °C (36 °F to 46 °F) and stored in the original boxes in order to be protected from light. Do not shake or freeze vials. Do not use drug product vials if discoloration is observed or if particulate matter other than translucent to white proteinaceous particles is observed.

Additionally, IV bags of the prepared infusion may be stored under refrigeration at 2 °C to 8 °C (36 °F to 46 °F) for up to 20 hours. If refrigerated, allow the IV bags to come to room temperature prior to use.

3.9 Investigational Agent Destruction/Disposal

Once agent accountability is performed, the participating sites should use local/institutional procedures for disposal of returned/unused study agent and bottles/containers. Copies of all certificates of destruction of any unused study agent must be provided to DCC. **Prior to destruction,** the pharmacist should contact the assigned study monitor.

Unused pembrolizumab shall be returned to the designated facility. For U.S. sites, unused drug shall be returned to Fisher Clinical Services. For all non-U.S. Sites, please contact DCC for instructions.

References

Supplement 1
I-SPY 2 Registry Study for Low-risk Subjects

Background:
In the original I-SPY 1 TRIAL, 7% of participants (11 participants) had tumors that were ER+, HER2−, and low risk as defined by the 70-gene prognosis signature. The average age of these participants was 53. The average initial tumor size was 5 cm and all were clinically node-negative. None of the participants achieved a pCR. Of the nine participants evaluable for RCB, one participant had an RCB of 1; the other eight had RCB scores of 2 or 3. To date, there have been no recurrences or deaths in this small cohort of participants (median RFS of 3.9 years) despite the fact that many had lymph node-positive disease and high RCB after neoadjuvant chemotherapy.

Between March 2010 and June 2011, 30 of the first 149 (20%) participants screened for I-SPY 2 were categorized as ER+, HER2−, and MammaPrint low risk. The higher incidence of ineligible low-risk participants in I-SPY 2 as compared to I-SPY 1 is likely because the required minimum tumor size for study entry was decreased from 3.0 cm in I-SPY 1 to 2.5 cm in the current study. These participants are not eligible for entry into the treatment phase of I-SPY 2 due to their favorable biological tumor characteristics and predicted low response to cytotoxic therapy.

Of the first 12 ineligible low-risk participants identified in the screening phase of I-SPY 2, all but one had clinically node-positive disease, and tumor sizes ranged from 3 cm to 11 cm, suggesting at least preliminarily that participants being screened for I-SPY 2 may have more clinically advanced disease than those in I-SPY 1. Interestingly, treatment plans for these 12 women varied greatly depending on physician and participant preferences, as well as institutional practices. Four proceeded to upfront surgery, two received neoadjuvant endocrine therapy, and four received neoadjuvant chemotherapy. These data reflect the current lack of an accepted standard for treatment of clinically high-risk but molecularly low-risk participants.

In an effort to capture practice patterns and clinicopathologic outcomes in this important cohort of participants who are ineligible for the treatment phase of I-SPY 2 due to their assumed good prognosis, the I-SPY 2 clinical investigator team in collaboration with the participating institutions has developed a registry study for participants with ER+, HER2−, and MammaPrint low risk tumors. Subjects who participate in this registry study will consent to the collection of all treatment and response data as well as 10-year follow-up for clinical outcome. For participants who receive neoadjuvant systemic therapy, a routine post-treatment breast MRI will be performed in order to enrich the existing ISPY biomarker database with molecularly low-risk tumor specimens.

Objectives:
To establish a registry containing the treatment, clinical and pathologic outcomes, and extended (15-year) follow-up data of participants enrolled in the screening phase of I-SPY 2 with MammaPrint low risk, ER+, HER2− breast cancer.
To expand the I-SPY biomarker database through the collection of MRI volume and tumor tissue in participants with MammaPrint low risk, ER+, HER2− breast cancer during or after neoadjuvant treatment and at the time of surgical resection.

Eligibility:
Histologically confirmed invasive ER+, HER2− breast cancer
Low-risk gene signature by MammaPrint analysis
No clinical or imaging evidence of distant metastases; however, regional Stage IV, where supraclavicular lymph nodes are the only sites of metastasis, is allowed

Study Plan:
Subjects who do not meet criteria for participation in the I-SPY 2 main treatment protocol due to determination of low-risk status will be consented to participate in this registry study, which will track the treatment administered to each participant, but does not mandate any specific treatment. Treatment will be at the discretion of the treating physician and participant. The following treatment plans reflect the current practice patterns observed:

Proceed to definitive surgical resection of the breast cancer and receive adjuvant therapy as dictated by pathology and participant-provider preference
A 12–24 week course of neoadjuvant endocrine therapy, followed by surgical resection and adjuvant therapy as dictated by pathology (PEPI score), and participant-provider preference
Tamoxifen with or without ovarian suppression for pre- or perimenopausal participants
Aromatase inhibitor for postmenopausal participants
A standard course of neoadjuvant chemotherapy, followed by surgical resection and adjuvant therapy as dictated by participant-provider preference. If an anthracycline- and taxane-containing chemotherapy regimen is selected, the I-SPY 2 standard regimen of weekly paclitaxel × 12 followed by four cycles of AC every two to three weeks is encouraged (but not required).

Schedule of Assessments

For participants proceeding to upfront surgical resection

Tissue will be collected at the time of surgery per the I-SPY 2 main protocol standard procedures.
Pathologic findings and follow-up data including adjuvant treatment received will be collected every six months after surgery for years one–five and annually for years six–15.

Table 1a: Schedule of Assessments for Participants Undergoing Upfront Surgical Resection

	Surgery	**q6mo (years 1–5) and yearly (years 6–15)**
Surgical specimen	x	
Follow-up		x

For participants receiving neoadjuvant systemic therapy

The following study assessments will be performed (see Table 1b):
Preoperative breast MRI per I-SPY 2 main study protocol. For participants who did not obtain a baseline I-SPY 2 study MRI, the preoperative MRI is not required.
Treatment summary form that outlines the neoadjuvant systemic therapy received
Surgical specimen
Follow-up every six months after surgery for years one–five and annually for years six–15.

Table 1b: Schedule of Assessments for Participants Receiving Neoadjuvant Systemic Therapy

	3 weeks	**Prior to surgery[a]**	**Surgery**	**q6mo (years 1–5) and yearly (years 6–15)**
Preoperative MRI		x[b,c]		
Treatment Summary Form		x		

	3 weeks	Prior to surgery[a]	Surgery	q6mo (years 1–5) and yearly (years 6–15)
Surgical Specimen			x	
Follow-up				x

[a]After completion of neoadjuvant systemic therapy
[b]For participants who did not obtain a baseline ISPY2 study MRI, the pre-operative MRI is not required.
[c]Renal function must be adequate

Oncotype Dx recurrence score data will be collected in all participants for whom it is available.

Toxicity Management and Dose Modifications
All toxicity management and dose modifications will be performed at the discretion of the treating physician per standard of care guidelines.

Specimen Management
All study samples (biopsies, blood work, MRIs) will be collected, processed, handled, and shipped per the I-SPY 2 main study protocol. Please see §10 in main protocol. There are limited procedures in this registry protocol as outlined above under "Schedule of Assessments".

Post-surgical Follow-up
Participants will be followed for survival and recurrence for a total of 15 years after the date of surgical resection. Follow-up will be performed every six months for years one–five and annually for years six–15. All follow-up assessments and procedures will be performed as standard of care.

Data Monitoring:
Participant data will be collected using protocol-specific CRFs. The completed forms will be stored at each participating institution until TRANSCEND is updated and able to support this data. Table 2 outlines the required CRFs:

Table 2: Case Report Forms

Form	Submission Schedule
On-Study CRF	Complete within 1 week of participant deciding to join the Low Risk Registry Trial
Oncotype Dx Form	Complete within 6 weeks of surgery
Pre-surgery Treatment Form	Prior to surgery
Preoperative MRI Form	Within 1–2 weeks of MRI
Post-surgery Summary	Within 1 week after diagnostic pathology report is available
Post-surgery Treatment Form	Within 2 months of surgery
Follow-up Form	Every 6 months (years 1–5) or annually (years 6–15) from date of initial surgery and at death
Lost to Follow-up Form	Completed after 2 years of no contact with participant or participant's doctors
No Longer Lost to Follow-up Form	To complete if necessary
Off-study Form	Completed if participant elects to withdraw consent, discontinue from study, completes the study, or dies.

Statistical Plan:
The frequency of each treatment type (upfront surgery, neoadjuvant endocrine therapy, neoadjuvant chemotherapy) will be determined. Descriptive statistics will be performed to characterize the participants

and outcomes in each treatment group. K-M curves for RFS and OS will be determined for each treatment group and compared to those of participants enrolled in the treatment phase of the main I-SPY 2 protocol.

Supplement 2
Quality of Life Measurement within the I-SPY 2 Study

Background and Rationale

Introduction of novel agents into standard adjuvant or neoadjuvant chemotherapy regimens may significantly alter the expected toxicity profiles of these regimens. The toxicities of these agents may not be well characterized at the time of trial participation and may lead to both short and long term changes in quality of life (QOL) for the participant. The ISPY 2 trial provides a unique opportunity to study short and long term QOL in participant receiving neoadjuvant therapy with novel agents. Participants receiving neoadjuvant treatments including chemotherapy, biological therapy, or hormonal therapy face additional decisions and stressors that may impact QOL. For example, a participant who receives an aggressive course of chemotherapy before surgery but still has significant residual disease at the time of surgery may experience more severe anxiety, depression, and fear of recurrence than a participant who receives the same treatment in the adjuvant setting where one does not get any information or feedback about how well the treatment worked. Because the trial design informs participants in the screening process whether their tumor has a low-risk *vs.* high-risk profile, the trial allows the opportunity to study the impact of providing such prognostic information shortly after diagnosis. The trial also allows the opportunity to evaluate QOL differences in participants who undergo breast-conserving surgery (BCS) compared to participants who undergo mastectomy either because they did not achieve sufficient tumor response to allow BCS or who chose mastectomy despite BCS being an option.

In order to better understand a variety of QOL domains, we will incorporate a series of QOL assessments at multiple time points throughout the trial. All participants consenting to participate in ISPY 2, including those participants who are screened and found to be ineligible for the chemotherapy and novel agent treatments, will be asked to participate in this QOL component of the clinical trial.

Quality of Life Measures

1. European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) and Breast 23 (EORTC QLQ BR23)

The EORTC QLQ-C30 consists of 30 items with responses on a Likert scales ranging between one to four (28 items) and one to seven (2 items). Higher scores indicate worse quality of life outcomes. The recall period for each question is one week. The questionnaire consists of five “functioning” scales: physical functioning (five items), role functioning (two items), emotional functioning (four items), cognitive functioning (two items) and social functioning (two items). The questionnaire also includes one three-item symptom scale measuring fatigue; two two-item symptom scales measuring pain and nausea and vomiting; six single-item symptom scales measuring dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial impact; and an overall global health status/QOL scale (two items). The EORTC-QLQ-C30 is a commonly used quality of life instrument available in 79 languages, able to be administered on paper or by computer and taken by self or an interviewer with reasonable correlation of results. ‘Validity’ of the QLQ-C30 is available from a diverse range of populations and clinical settings including its original validation with lung cancer participants [1]. Scale reliability for all functioning scales with the exception of role functioning has demonstrated a Cronbach's alpha coefficient of ≥ 0.70. The EORTC-QLQ-C30 had also been validated in other tumor types and has been utilized in a number of recent trials of chemotherapy for locally advanced high risk breast cancer [2, 3]. The EORTC BR23 is a

validated 23 item module focusing on symptoms and domains of greatest relevance to breast cancer and its treatment. Responses to each item are on a 1–4 Likert scale.

2. PROMIS Quality of Life Questions/Instruments

Participant-Reported Outcomes Measurement Information System (PROMIS) represents an initiative supported by the NCI to develop and further validate a series of questions and/or statements that can be used either as groups or single items to investigate particular QOL domains. Many of the individual items have been drawn from "legacy instruments" such as the EORTC or FACT (Functional Assessment of Cancer Therapy) and have been studied for validity, reproducibility, and internal consistency in the normal and healthy US population. Validation in cancer populations is ongoing and a major priority of the NCI. The items can be used individually or in the context of domain-specific "short forms". For complete details of the development, selection process, and planned validation for PROMIS items, see references[4, 5] or the PROMIS website (http://www.nihpromis.org).

In the context of the ISPY 2 QOL substudy, a total of 50 individual items will cover the domains of physical function (four items), anxiety (eight items), depression (eight items), fatigue (eight items), applied cognition (eight items), social roles (four items), and female sexual function (10 items). To avoid excessive participant burden of questionnaire completion, not all domains will be asked at every time point.

3. Distress Thermometer

The NCCN Distress Management panel has recommended screening all participants with cancer regularly for psychological distress as part of routine care [6]. The single item Distress Thermometer (DT) has compared favorably with longer measures used to screen for distress. In a trial of 380 participants with either leukemia or a variety of solid tumors including a large number of breast cancer participants, receiver operating characteristic (ROC) curve analyses of DT scores yielded area under the curve estimates relative to the Hospital Anxiety and Depression Scale (HADS) cutoff score (0.80) and the Brief Symptom Inventory (BSI)-18 cutoff scores (0.78) indicative of good overall accuracy [7]. ROC analyses also showed that a DT cutoff score of 4 had optimal sensitivity and specificity relative to both the HADS and BSI-18 cutoff scores. This item takes less than one minute to complete.

4. Fear of Recurrence Scale

Fear of cancer recurrence has been defined as the worry that the cancer will return or progress either in the same site in the body or elsewhere in the body [8, 9]. In participants' minds, fear of recurrence can encompass numerous possible catastrophic outcomes associated with progression and/or recurrence: death and dying, more disfiguring and painful treatments, worsening symptoms, loss of autonomy and control, and leaving behind children or other family members. Fear of recurrence is correlated with increased presence of intrusive thoughts and avoidance behaviors, increased vigilance and attention to physical symptoms, and inhibition of goal-directed behavior or difficulty planning for the future.

The Fear of Recurrence Scale developed by Kornblith, consists of five items, measuring a participant's beliefs and anxieties concerning their disease recurring, relevant to cancer survivor populations, as well as those with early stage disease, and participants who are being followed after treatment completion. Each item is scored 1 to 5 on a Likert scale, from 'strongly agree' to 'strongly disagree'. The Fear of Recurrence Scale has been used in multiple cancer survivors studies, including adult leukemia survivors [10], endometrial and breast cancer survivors [11], and ovarian cancer survivors [12]. The Fear of Recurrence Scale meets the basic criterion of internal consistency: the alpha coefficient was 0.75 in an adult leukemia survivor study, (n=203) and 0.78 for the breast and endometrial cancer survivor study. The

five-item Fear of Recurrence Scale takes approximately one to two minutes to complete. The statements (items) are as follows:

1. Because cancer is unpredictable, I feel I cannot plan for the future
2. My cancer will probably come back in five years or I will probably have a relapse in the next five years.
3. My fear of having my cancer coming back gets in the way of my enjoying life.
4. I am afraid of my cancer coming back.
5. I am certain I have been cured of cancer.

Since several of the items in this scale assume that the participant has already completed treatment for cancer (or at least has had the cancer removed surgically), they are not appropriate for participants undergoing neoadjuvant chemotherapy. Therefore only the first two items of this scale will be administered before surgery, and the final three items will be added at the post-op visits.

Specific Aims of the QOL Measurement Study

Primary Aim

To evaluate QOL based on changes in the EORTC QLQ 30 and EORTC BR23 longitudinally from before surgery and through two years post operatively in participants receiving neoadjuvant chemotherapy, biological therapies, and/or other treatments for LABC. We will analyze the following as predictors of changes in QOL:

- MammaPrint results: high- *vs.* low-risk MammaPrint profile
- Breast conserving surgery *vs.* mastectomy
- Receipt of standard chemotherapy and or/hormonal therapy *vs.* standard chemotherapy and/or hormonal therapy and a novel agent
- RCB: pCR and minimal residual disease (RCB 0 or 1) *vs.* significant residual disease (RCB 2 or 3)
- Receipt of hormonal therapy after surgery

Secondary Aims

To evaluate changes in distress using the DT longitudinally from before surgery and through two years post operatively in participants receiving neoadjuvant chemotherapy, biological therapies, and/or other treatments for locally advanced breast cancer. We will analyze the following as predictors of changes in distress:

- MammaPrint results: high- *vs.* low-risk MammaPrint profile
- Breast conserving surgery *vs.* mastectomy
- Receipt of standard chemotherapy and/or hormonal therapy *vs.* standard chemotherapy and/or hormonal therapy and a novel agent
- RCB: pCR and minimal residual disease (RCB 0 or 1) *vs.* significant residual disease (RCB 2 or 3)
- Receipt of hormonal therapy after surgery

To evaluate fear of recurrence longitudinally from before surgery and up through two years post-operatively using a validated five-item Fear of Recurrence scale in participants receiving neoadjuvant chemotherapy, biological therapies, and/or other treatments for LABC. We will analyze the following as predictors of Fear of Recurrence:

- MammaPrint results: high *vs.* low risk MammaPrint profile
- BCS *vs.* mastectomy

- Receipt of standard chemotherapy and/or hormonal therapy *vs.* standard chemotherapy and/or hormonal therapy and a novel agent
- RCB: pCR and minimal residual disease (RCB 0 or 1) *vs.* significant residual disease (RCB 2 or 3)
- Receipt of hormonal therapy after surgery

Exploratory Aim

To compare QOL scores between the EORTC QLQ30 and BR23 and the PROMIS items in categories where there is some overlap of content including physical function, anxiety, depression, sexual function, and cognitive function.

Methods/Procedures

Participants will be first approached to consent to and complete QOL questionnaires at the time they sign consent to be screened for the trial. This battery of questionnaires will serve as their baseline. Table 1 details the sections of the QOL questionnaire battery that will be completed at various time points. All participants screened for ISPY 2 will be asked to complete baseline QOL questionnaires after they have signed the screening consent. At the time of enrollment into the trial when they are assigned a treatment group, participants will receive a packet of dated questionnaires for completion at various time points. Participants who are found to be ineligible for the study based on having a tumor with a MammaPrint low-risk profile will also be invited to participate in ongoing QOL assessments. A separate plan for survey administration and collection will apply to participants in the low-risk group who do not follow up regularly at the screening ISPY 2 center for ongoing breast cancer care.

Participants eligible to participate in the main ISPY 2 study will be asked to bring the completed questionnaires in to their clinic visits at the intervals shown in Table 1. Participants who have not filled out the questionnaires at the time of their clinic visit will be provided another copy of the dated questionnaire, and they will be asked to complete it during their infusion time while receiving neoadjuvant treatment or after the clinic visit if they are in the post-operative phase. Participants with a low-risk profile who participate in the Registry study and receive ongoing care at the screening ISPY 2 site will have questionnaires administered in clinic visits if possible at the time points indicated in Table 1. A participant whose clinic visit is within two weeks of the specified time point will be asked to bring the completed questionnaire to the clinic visit. If the appointment is not within two weeks of the specified time point, the participant will be asked to complete the questionnaires at home and return by mail in a pre-paid envelope. Participants in the low-risk group who do not follow up regularly at the screening ISPY 2 center for ongoing breast cancer care will receive packets of dated questionnaires with prepaid return envelopes at the time they are informed of their low-risk profile and when they are offered participation in the Low-risk Registry study.

To assure compliance with questionnaire completion in all participants for the post-operative follow up from month 1 through month 24, and in participants in the low-risk group who do not follow up regularly at the screening ISPY 2 center for ongoing breast cancer care, a reminder letter will be mailed one week prior to the due date reminding participants that QOL questionnaires should be completed and returned. Another reminder letter will be mailed if the QOL questionnaires are not received two weeks after the due date.

Table 1. Schedule of Survey Subgroup Administration

Measure	TIMEPOINTS							
	Baseline (screening)	**C1D1[a]**	**Week 13[b]**	**Pre-Op**	**1 Month Post-op**	**6 Month Post-op**	**12 Month Post-op**	**24 Month Post-op**

Measure	TIMEPOINTS							
	Baseline (screening)	**C1D1[a]**	**Week 13[b]**	**Pre-Op**	**1 Month Post-op**	**6 Month Post-op**	**12 Month Post-op**	**24 Month Post-op**
EORTC 30 and 23	X		X	X	X	X	X	X
PROMIS Physical Function	X		X	X	X	X	X	X
PROMIS Anxiety	X	X	X	X	X	X	X	X
PROMIS Depression	X		X	X	X	X	X	X
PROMIS Fatigue	X		X	X	X	X	X	X
PROMIS Social Roles	X		X	X	X	X	X	X
PROMIS Cognition	X			X	X	X	X	X
PROMIS Sexual Function	X			X	X	X	X	X
Distress Thermometer	X	X	X	X	X	X	X	X
Fear of Recurrence[c]	X	X		X	X	X	X	X

[a]C1D1 (Chemo start for high risk, treatment start for low risk)
[b]Beginning of AC or week 13 for low risk
[c]Modified version (items 1 and 2) administered at all time points before surgery

Statistical Considerations/Methods

Primary endpoint: EORTC QLQ-C30 and EORTC BR23

Data from these instruments will be standardized to scales of 0–100 for each function and symptom using methods described in the EORTC Scoring Manual. Mean scores (and their 95% confidence intervals) for each function and each symptom will be plotted against time for the different participant subgroups (*e.g.*, high-*vs.* low-risk MammaPrint profile). Statistical comparisons between different groups will be made using mixed effect models, linear if the plots look linear and nonlinear if the plots show nonlinear scores over time. Models will have fixed effects for subgroup and time with random effects for participant and random error. We will also test whether covariates such as age and tumor characteristics (*e.g.*, size and grade) act as significant modifiers of score results. These comparisons will be carried out in R using the nlme program that can model either linear or nonlinear functions over time.

Secondary endpoints: DT and Fear of Recurrence Scale

Mean and median scores for each participant subgroup will be plotted against time since treatment initiation. Mixed effect models will be used to test for differences between subgroups.

PROMIS: Results for questions from PROMIS will be correlated with those from the EORTC function and symptom scales. We will also examine differences between participant subgroups using PROMIS questions and compare them with differences using QLQ-C30 and BR23 using effect size to determine whether one is more sensitive than the other in detecting differences over time.

Statistical Power

It is not possible to determine power for the mixed effect model without data on within- and between-participant variability over time. However, we can estimate power based on a two-sample t-test for

comparing means of two different groups at a single time point or mean differences from baseline to a fixed time point (*i.e.*, mean at time 1 minus mean at time 0). A study evaluating QOL before and up to 18 months after breast cancer diagnosis and treatment in Iranian women provides data on means and standard deviations for the EORTC QLQ-C30 subscales [13]. These are shown in Table 2 along with the minimum detectable differences based on expected ISPY 2 trial data.

From the time of initiation of this QOL protocol, the ISPY 2 trial is expected to enroll at least another 600 participants. Based on the rates of screened participants to date, approximately 20% of participants have not been eligible to participate in the main study due to having a MammaPrint low-risk tumor profile. All low-risk participants will be invited to participate in the Registry study and this QOL component. If 80% of the screened low-risk participants agree to participate in the Registry and QOL study, we would expect data from 100 MammaPrint low and 500 MammaPrint high risk participants. Based on data from the ISPY1 clinical trial, we expect that the number of participants undergoing mastectomy *vs.* BCS will favor mastectomy (123 participants had mastectomy *vs.* 92 participants with BCS in SPY1). Similarly, based on ISPY1 data, the ratio of participants with RCB 0 or 1 *vs.* RBC 2 or 3 should be approximately 3:5 (74 participants with RCB 0 or 1 *vs.* 127 participants with RCB 2 or 3 in ISPY1). Assuming a similar balance in ISPY 2, there will be more than 100 participants in each of these subgroups (lumpectomy, mastectomy, RCB 0/1, RCB 2/3); therefore, the power calculation to detect differences between groups will be valid for the larger sample sizes in each of these groups.

Table 2. Minimal Detectable Differences with Sample Size of n=100 in Each of Two Groups

QLQ-C30	**Baseline Values**		**Minimal Detectable Diff**		
Functioning*	**Mean**	**SD**	**% Diff**	**Abs Diff**	**Mean2**
Physical functioning	68.7	24.9	14%	9.9	58.8
Role functioning	69.7	27.1	15%	10.7	59.0
Emotional functioning	59.4	23.5	16%	9.3	50.1
Cognitive functioning	79.4	20.1	10%	8.0	71.4
Social functioning	85	18	8%	7.1	77.9
Global quality of life	59.2	31.8	21%	12.6	46.6
Symptoms+					
Fatigue	17	19.4	45%	7.7	24.7
Nausea	1.7	7.3	170%	2.9	4.6
Pain	4.5	9.7	85%	3.8	8.3
Dyspnea	5.7	14.3	99%	5.7	11.4
Sleep problems	27.5	30.2	44%	12.0	39.5
Appetite loss	20.9	29.2	55%	11.6	32.5
Constipation	2.3	9.8	169%	3.9	6.2
Diarrhea	0.33	3.3	396%	1.3	1.6
Financial difficulties	17.7	25.8	58%	10.2	27.9
*Scores range from 0 to 100 with higher scores representing higher level of functioning or quality of life					
+Scores range from 0 to 100 with higher scores representing higher level of symptoms					
QLQ-BR23					
Symptoms+					
Body image	86.2	18.8	9%	7.4	78.8
Sexual functioning	82.3	22.4	11%	8.9	73.4
Sexual enjoyment	51.6	25.3	19%	10.0	41.6
Future perspective	29.9	29.5	39%	11.7	18.2
Symptoms+		0			
Arm	7.7	13.3	68%	5.3	13.0
Breast	15.6	15	38%	5.9	21.5
Systematic TX side effects	15.7	11.8	30%	4.7	20.4

*Scores range from 0 to 100 with higher scores representing higher level of functioning or quality of life
+Scores range from 0 to 100 with higher scores representing higher level of symptoms

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Quality of Life Survey

EORTC 30

During the past week:	Not at all	A Little	Quite a bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7

Very poor Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7

Very poor Excellent

EORTC BR23

During the past week	Not at All	A Little	Quite a Bit	Very Much
Did you have a dry mouth?	1	2	3	4
Did food and drink taste different than usual?	1	2	3	4
Were your eyes painful, irritated or watery?	1	2	3	4
Have you lost any hair?	1	2	3	4
Answer this question only if you had any hair loss: Were you upset by the loss of your hair?	1	2	3	4
Did you feel ill or unwell?	1	2	3	4
Did you have hot flashes?	1	2	3	4
Did you have headaches?	1	2	3	4
Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
Have you been feeling less feminine as a result of your disease or treatment?	1	2	3	4
Did you find it difficult to look at yourself naked?	1	2	3	4
Have you been dissatisfied with your body?	1	2	3	4
Were you worried about your health in the future?	1	2	3	4

During the past 4 weeks:	Not at All	A Little	Quite a Bit	Very Much
To what extent were you interested in sex?	1	2	3	4
To what extent were you sexually active? (with or without intercourse)?	1	2	3	4
To what extent was sex enjoyable for you?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
Did you have any pain in your arm or shoulder?	1	2	3	4
Did you have a swollen arm or hand?	1	2	3	4
Was it difficult to raise your arm or to move it sideways?	1	2	3	4
Have you had any pain in the area of your affected breast?	1	2	3	4
Was the area of your affected breast swollen?	1	2	3	4
Was the area of your affected breast oversensitive?	1	2	3	4
Have you had skin problems on or in the area of your affected breast (e.g., itchy, dry, flaky)?	1	2	3	4

Fear of Recurrence Scale (Only items 1 and 2 will be used preoperatively)

1. Because cancer is unpredictable, I feel I cannot plan for the future
2. My cancer will probably come back in 5 years or I will probably have a relapse in the next 5 years.
3. My fear of having my cancer coming back gets in the way of my enjoying life.
4. I am afraid of my cancer coming back.
5. I am certain I have been cured of cancer.

PROMIS Physical Functioning					
During the past week:	**Without any difficulty**	**With a little difficulty**	**With some difficulty**	**With much difficulty**	**Not at all**
Are you able to do chores such as vacuuming or yard work?	1	2	3	4	5
Are you able to go up and down stairs at a normal pace?	1	2	3	4	5
Are you able to go for a walk of at least 15 minutes?	1	2	3	4	5
Are you able to run errands and shop?	1	2	5	4	5

PROMIS Anxiety					
During the past week:	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
I felt fearful	1	2	3	4	5
I found it hard to focus on anything other than my anxiety	1	2	3	4	5
My worries overwhelmed me	1	2	3	4	5
I felt nervous	1	2	3	4	5
I felt like I needed help for my anxiety	1	2	3	4	5
I had sudden feelings of panic	1	2	3	4	5
I felt indecisive	1	2	3	4	5
I had difficulty calming down	1	2	3	4	5

PROMIS Depression					
During the past week	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
I felt worthless	1	2	3	4	5
I felt helpless	1	2	3	4	5
I felt depressed	1	2	3	4	5
I felt hopeless	1	2	3	4	5
I felt unhappy	1	2	3	4	5
I felt that I had nothing to look forward to	1	2	3	4	5
I withdrew from other people	1	2	3	4	5
I found that things in my life were overwhelming	1	2	3	4	5
I felt worthless	1	2	3	4	5

PROMIS Fatigue					
During the past week	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
I feel fatigued	1	2	3	4	5
How much were you bothered by your fatigue on average?	1	2	3	4	5
To what degree did your fatigue interfere with your physical functioning?	1	2	3	4	5
To what degree did you have to push your get things done because of your fatigue?	1	2	3	4	5
Did fatigue make you less effective at home?	1	2	3	4	5
How exhausted were you on average?	1	2	3	4	5
How often did you feel tired even when you hadn't done anything?	1	2	3	4	5
How often were you too tired to think	1	2	3	4	5

PROMIS Fatigue					
During the past week	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
clearly?					
I feel fatigued	1	2	3	4	5

PROMIS Applied Cognition					
	Never	**Rarely (once)**	**Sometimes (2 or 3 times)**	**Often (about once a day)**	**Always (several times a day)**
My thinking has been slow	1	2	3	4	5
It has seemed like my brain was not working as well as usual	1	2	3	4	5
I have had to work harder than usual to keep track of what I was doing	1	2	3	4	5
I have had trouble shifting back and forth between different activities that require thinking	1	2	3	4	5
I have had trouble concentrating	1	2	3	4	5
I have had to work really hard to pay attention or I would make a mistake	1	2	3	4	5
I have had trouble forming thoughts	1	2	3	4	5
My problems with memory, concentration, or making mental mistakes have interfered with the quality of my life	1	2	3	4	5
My thinking has been slow	1	2	3	4	5

PROMIS Social Roles					
During the past week (last 7 days)	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
I am satisfied with my ability to meet the needs of those who depend on me					
I am satisfied with how much work I can do (include work at home)					
I am satisfied with my ability to do household chores/tasks					
I am satisfied with the amount of time I spend performing my daily routines					

PROMIS Female Sexual Functioning						
During the past 30 days (4 weeks)		**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Always**
How interested have you been in sexual activity?		1	2	3	4	5
During the past 30 days (4 weeks)		**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
How often have you felt like you wanted to have sex?		1	2	3	4	5

PROMIS Female Sexual Functioning						
	No sexual activity	**Almost always or always**	**Most times (more than half the time)**	**Sometimes (about half the time)**	**A few times (less than half the time)**	**Almost never or never**
How often did you become lubricated ("wet") during sexual activity or intercourse?	0	5	4	3	2	1
	Have not tried to get lubricated in the past 30 days	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very**
How difficult has it been for your vagina to get lubricated ("wet") when you wanted it to?	0	5	4	3	2	1
	Have not had any sexual activity in the past 30 days	**Very comfort-able**	**Comfort-able**	**Uncom-fortable**	**Very uncomfort-able**	
How would you describe the comfort of your vagina during sexual activity?	0	1	2	3	4	
	Have not had any sexual activity in the past 30 days	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
How often have you had difficulty with sexual activity because of discomfort or pain in your vagina?	0	1	2	3	4	5
How often have you stopped sexual activity because of discomfort or pain in your vagina?	0	1	2	3	4	5
	Have not tried to have an orgasm/ climax in the past 30 days	**Excellent**	**Very good**	**Good**	**Fair**	**Poor**
How would you rate your ability to have a satisfying orgasm/climax?	0	5	4	3	2	1
	Have not had sexual activity in the past 30 days	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very Much**
When you have had sexual activity, how much have you enjoyed it?	0	1	2	3	4	5

PROMIS Female Sexual Functioning						
	Have not had sexual activity in the past 30 days	**Not at all**	**A little bit**	**Somewhat**	**Quite a bit**	**Very**
When you have had sexual activity, how satisfying has it been?	0	1	2	3	4	5

Supplement 3
SURMOUNT study within the I-SPY 2 Study

SURMOUNT: Surveillance Markers of Utility for Recurrence after Neoadujvant Therapy for Breast Cancer

1.0 Background

Adjuvant chemotherapy has been clearly established to improve cure rates in early stage breast cancer, but is not universally effective. Despite this therapy, many women subsequently relapse over years to decades following diagnosis. Neoadjuvant chemotherapy is increasingly used in the primary treatment of non-metastatic breast cancer because of its ability to downstage patients for improved surgical outcomes and provides an in vivo assessment of tumor chemosensitivity. Several measures, including pathological response (pCR) and residual cancer burden (RCB) provide proximate surrogate endpoints that reflect later outcomes [1]. While those with pCR or RCB 0/1 have excellent prognosis overall, less than 50% of patients attain this status, and those without such response have a high likelihood of recurrence. Patients with Her2+ or triple negative breast cancer who do not achieve pCR (approximately 40% and 65% of patients, respectively) have a particularly poor prognosis, with greater than 50% of patients demonstrating distant relapse within 5 years [2].

1.1. DTCs and CTCs as Prognostic Markers in Early Breast Cancer

Currently, there is no useful test or biomarker that can be used after standard (neo)adjuvant therapy to monitor patients and accurately identify those who will recur before the appearance of overt metastatic disease. Radiologic scanning and circulating tumor markers, such as CA27-29 and CA15-3, are not effective and currently not recommended by the American Society of Clinical Oncology for use in the surveillance period[3]. This lack of ability to monitor for residual or recurrent cells at a point where intervention could be successful leads to enormous distress for patients [4-6] and limits the ability to fully utilize the arsenal of effective agents to prevent subsequently incurable metastatic disease. Thus, there is critical need for accurate and effective biomarkers for recurrence in the surveillance period.

New and emerging technologies make it possible to sensitively detect circulating tumor cells (CTCs) in peripheral blood and/or disseminated tumor cells (DTCs) in bone marrow of patients with cancer. Residual cancer cells in the bone marrow have proven clinical relevance for relapse based on the analysis of thousands of breast cancer patients[10,11]. Accordingly, at present bone marrow DTCs represent the best biological surrogate for the population of cells that ultimately gives rise to recurrence. Early evidence for the prognostic significance of DTCs in breast cancer patients emerged from a pooled analysis involving 4,703 patients with stage 1, 2, or 3 breast cancer in whom DTCs were identified in 31% of patients[12]. This study demonstrated that the presence of DTCs in bone marrow predicts poor breast cancer-specific survival, poor overall survival, and a high risk of local and loco-regional recurrence[12-14]. In fact, in multivariate analysis, bone marrow status was the strongest independent predictor of disease-free and overall survival. Additional studies have revealed that the presence and persistence of DTCs in bone marrow after adjuvant therapy predicts poor prognosis[15-18].

However, since serial bone marrow biopsy is a painful and challenging clinical endpoint to incorporate into therapeutic trials and routine clinical care, attention has turned to circulating tumor cells (CTCs) as an alternative[19-21]. CTCs have proven prognostic relevance in metastatic breast cancer[19,22] and their clinical value for monitoring therapy is a topic of intensive investigation[23,24]. Indeed, the CellSearch™ system has been approved by the FDA for this purpose and CTCs detected by this method have proven prognostic relevance in metastatic breast cancer[19,22]. For example, in pioneering work Cristofanilli et al. demonstrated that the presence of ≥5 CTC per 7.5 mL blood detected using the CellSearch™ system was associated with poor prognosis in metastatic breast cancer patients[19]. Unfavorable CTC counts (≥ 5

CTC/7.5 mL blood) that persisted after the first cycle of chemotherapy also predicted poor clinical outcome[22]. Numerous studies in the metastatic setting have demonstrated the presence of CTCs and correlated their presence with response to therapy and outcome[19,25,26].

More recently, studies have focused on the identification of CTCs and DTCs in women with early breast cancer in an effort to identify those at high risk of relapse[27,28]. However, the rate of detection of CTCs using EPCAM-based capture technologies in these studies has been low, typically on the order of 10-15%, reflecting the rarity of these cells in low-risk populations with favorable prognosis[11]. Slade et al. demonstrated that a significant proportion of primary breast cancer patients with poor prognosis (>3 positive nodes) have detectable CTCs[31]. Recently, clearcut data have emerged demonstrating the relevance of CTCs in primary breast cancer patients for predicting early relapse (<5 years after initial diagnosis)[32, 33]. The largest of these studies is the multicenter German SUCCESS trial. This trial is the largest adjuvant breast cancer study to date to monitor CTCs and it found that the presence of CTCs detected by CellSearch™ prior to systemic treatment is an independent predictor for recurrence-free and overall survival for women within 5 years of diagnosis[33]. This trial enrolled 3753 patients, analyzed blood from 2026 patients for CTCs, and banked CTCs, serum, and plasma for molecular analyses. CTCs were analyzed following complete resection of the primary tumor and prior to the initiation of systemic adjuvant treatment. All patients were randomized in the SUCCESS A Study to receive 3 cycles of epirubicin, fluorouracil and cyclophosphamide chemotherapy followed by 3 cycles of either docetaxel (FEC-D) or docetaxel and gemcitabine (FEC-DG). Patients were followed for a median of 35 months (range 0 to 54 months). Cox regression models were used to assess the prognostic significance of CTCs for disease-free and overall survival. CTCs were detected in 21.5% of patients (n=435; median 1.3, range 1-827). 114 patients developed recurrent breast cancer and 66 patients died of their disease. The presence of CTCs (≥1 CTC per 23 ml blood) was an independent predictor of poor disease-free survival ($p<0.0001$), distant disease-free survival ($p<0.001$) and overall survival ($p=0.0002$). Multivariate analysis revealed that CTC detection was an independent prognostic factor with worst prognosis in patients with ≥ 5 CTCs (HR 4.0 [95%CI 2.21-7.07] for DFS; HR 3.1 [95%CI 1.51-8.28] for OAS). This is the first study to demonstrate the independent prognostic relevance of CTCs for early relapse in a large prospective study of primary breast cancer patients.

1.2. DTCs and CTCs as Prognostic Markers in the Neoadjuvant Setting

In neoadjuvant trials, where participants typically have larger tumors with more aggressive features, reported CTC detection rates are higher, on the order of 20-30%[20,29,30]. In this patient population, two of three studies showed that the presence of CTCs before and after neoadjvuant therapy is prognostic for outcome[20,30] and this endpoint is increasingly being incorporated into neoadjuvant trials. In the neoadjuvant setting, both pCR and RCB have been validated as prognostic surrogates for relapse-free survival, but little is known about the relationship between pathologic response, presence/absence of DTCs or CTCs and clinical outcome. In the recently completed German neoadjuvant trials "GeparQuattro" and "GeparQuinto", which represent the largest published trials to date in this context, CTC determinations were performed before and after primary systemic chemotherapy. The positivity rate, defined as the detection of one or more CTCs/7.5 mL blood, was 22% before chemotherapy and 11% after chemotherapy[29,34]. Mathieson and colleagues also have examined the presence of and alterations in DTC and CTC status in locally advanced breast cancer patients undergoing neoadjuvant chemotherapy and to evaluate their prognostic impact, albeit with NACT consisting of either single-agent epirubicin or paclitaxel, regimens not currently the standard of care [35,36]. Bone marrow and peripheral blood were collected before NACT (BM1: n = 231/PB1: n = 219), at surgery (BM2: n = 69/PB2: n = 71), and after 12 months from start of NACT (BM3: n = 162/PB3: n = 141). Patients were included from 1997 to 2003 and followed until 2009 (or ten years follow-up). DTC- and CTC-status were determined by morphological evaluation of immunocytochemically detected cytokeratin-positive cells. The prognostic significance of DTCs/CTCs was assessed by univariate and multivariate Cox-regression analyses. Before NACT, DTCs and CTCs were detected in 21.2% and 4.9% of the patients, respectively. At surgery, 15.9% and 1.4% had

DTC- and CTC-presence, compared to 26.5% and 4.3% at 12 months from start of NACT. Of patients for whom DTC results both before NACT and at 12 months were available, concordant results were observed in 68%, and 14 out of 65 had positive DTC-status at both time points. Presence of ≥ 1 DTC 12 months from start of NACT, but not at other time points, predicted reduced disease-free survival (DFS; HR 2.3, p = 0.003), breast cancer-specific survival (BCSS; HR 3.0, p < 0.001) and overall survival (OS; HR 2.8, p < 0.001). Before NACT, presence of ≥ 3 DTCs was also associated with unfavorable outcome, and reduced BCSS was observed for CTC-positive patients (HR 2.2, p = 0.046). In multivariate analysis, DTC status (</≥ 1 DTC) at 12 months after start of NACT remained as a prognostic factor for both DFS (HR 2.2, p = 0.005), BCSS (HR 2.6, p = 0.002) and OS (HR 2.6, p = 0.002). The survival for patients with change in DTC-status was determined by the DTC-status at 12 months. Understanding the relationship between these surrogates, DTCs or CTCs and disease recurrence would be invaluable as a way to identify those patients likely to have early relapse, as well as providing the opportunity to intervene before overt metastatic disease occurs.

1.3. Linking Surveillance Markers to Metastatic Biology

Current theories of metastasis suggest an important role for tumor cell dissemination from the primary tumor, though precise mechanisms remain unclear [7-9]. It is increasingly recognized that tumor biology of metastatic relapse differs from the primary tumor in a subset of cases. This can range from discordance in ER/PR or Her2 expression[37,38] to extensive genomic differences [39]. However, it is currently unknown whether residual tumors after neoadjuvant chemotherapy more closely resemble cells that are circulating after therapy and/or those that subsequently manifest as distant metastases. Preclinical studies in transgenic mouse models from the laboratory of Dr. Lewis Chodosh demonstrate a distinct population of cells that arise after complete regression of primary tumors and cessation of oncogenic blockade. These cells demonstrate a variety of molecular features that differ from both the primary tumor and subsequent metastases. Preliminary human studies have recently shown that CTCs and metastatic tumors in patients after relapse can share some features, but data remain sparse in this area. We have demonstrated that potential targets can be identified within residual tumors of patients from I-SPY1 (unpublished data), and that these tumors are fundamentally different in their gene expression patterns than the primary tumors from which they arose. For example, a comparison of baseline (T1) and residual tumors (T4) revealed differential expression of 41 probes (34 unique annotated genes) (n=44, paired t-test, limited to probes with at least 3 evaluable pairs, with Benjamini Hochberg FDR-corrected p < 0.05). Genes found to be more highly expressed in T4 relative to T1 included Jun, Fos, EGFR, CYR61 and CTGF, while those found to have decreased expression at T4 included CD6, IL24, and TRAF3.

1.4. Development of novel approaches to the isolation and analysis of CTCs, DTCs and CTMs to compare to residual tumors

While CellSearch™ is the most well-validated approach to enumerating circulating tumor cells to date, other platforms are being developed for improved capture and the molecular and genetic analysis of these cells and DTCs. For example, CellSearch detects cells based upon expression of EPCAM, limiting detection of cells that may be undergoing EMT. Many alternative capture methods are focused on either increasing capture rate through expanded antibody approaches or identifying cells that have undergone EMT, and therefore no longer express epithelial markers. The former would be useful to reduce the amount of blood currently needed to perform the CellSearch assay. The latter holds appeal given experimental evidence that minimal residual disease and dormant cancer cells pass through a period of EMT that provides mechanisms of self-renewal and resistance to chemotherapy [40,41].

In addition, numerous alternative technologies that negate the need to capture whole cells are also currently under development or undergoing validation, including the isolation of micro-RNA[42-44] and cell-free DNA (cfDNA) [45]. These alternative approaches to measuring circulating tumor markers (CTMs)

have advantages and disadvantages. Early studies have shown that cfDNA is a sensitive marker of disease in the metastatic breast cancer setting, and may be better than CA-27.29 or CA15.3 in determining changes in tumor burden[45]. Pilot studies have determined that cfDNA can identify known genetic mutations seen in primary tumors. However, controversy exists as to whether this material is viable, and thus whether it constitutes a reservoir of material that ultimately gives rise to metastatic disease.

The goals of this study are to examine all of these types of circulating material to determine the extent to which each reflects the primary or residual tumor in the breast, to determine whether there are unique molecular markers in these cells that represent markers of tumor dormancy and whether the presence of these markers adds prognostic information over and above that obtained from the surgical status of the primary tumor after neoadjuvant therapy. These primary objectives are a necessary foundation to the development of therapeutic approaches in patients with incomplete response to neoadjuvant chemotherapy who are at high risk of recurrence.

2.0 Study Objectives

2.1. Primary Objective

2.1.1. To determine the incidence and frequency of bone marrow and circulating tumor biomarkers in patients who are undergoing neoadjuvant chemotherapy for primary breast cancer and are participating in the I-SPY 2 TRIAL.

2.2. Secondary Objectives

2.2.1. To compare the molecular and genetic features between primary tumor, residual tumor, and DTCs/CTCs/CTMs (Circulating Tumor Markers) in those participants in whom they are detectable

2.2.2. To determine the relationship between detection of DTCs, CTCs, or CTMs and 3-year relapse free survival (RFS), by pathologic response status and receptor status

2.2.3. To determine if the presence or absence of DTCs, CTCs or CTMs adds independent prognostic information to tumor pathologic response (RCB).

3.0. Study Population

3.1. Inclusion Criteria:

Eligible participants will be drawn from those participating in the I-SPY 2 TRIAL who meet the following criteria:

3.1.1. Consented to the treatment phase of the I-SPY 2 TRIAL. Participants who have discontinued assigned treatment on I-SPY 2 are still considered eligible to participate and complete study procedures.

3.1.2. Willing to undergo bone marrow aspiration and blood specimen collection per protocol specifications

3.1.3. No clinical evidence of distant metastatic disease. Pre-chemotherapy staging scans are sufficient in the absence of any symptoms or subsequent clinical evidence suggesting distant metastases

3.2. Subject Recruitment and Screening
The study team will describe the SURMOUNT study to patients at any point prior to the patient's final ISPY scheduled surgical procedure. Patients will need to be consented at a visit prior to the scheduled surgery in order to collect the pre-operative research blood..

3.3. Consent Process
Patients can be approached for consent as soon as the site has obtained IRB approval is obtained. The study team will discuss the study and study procedures with the study participant and ask if the potential study participant has any questions. After answering any questions, the study team will ask the potential study participant to provide consent to participate. .

3.4. Early Withdrawal of Participants

At any time a study participant may request to discontinue SURMOUNT participation, either independently or in the context of withdrawing consent from the ISPY2 TRIAL. Participants may elect to (1) Suspend future participation only or (2) Suspend participation and have her specimens destroyed. If the participant elects option 1, the study coordinator will document the participant's decision within the study file. The participant will no longer be contacted for SURMOUNT follow-up, but use of all data collected up to that time, and ongoing medical record reviews may continue. Should the participant opt for option 2, the participant must make this request directly to the Principal Investigator of the SURMOUNT, Angela DeMichele, MD, MSCE or other designated study staff (i.e. project manager or research coordinator or other site principal investigator/co-investigator). The participant has several options for withdrawal. The participant must request in writing that their specimens be destroyed. If data has already been generated from their specimens, the data will remain in the study database. If the specimens have not been used at the time of the request, the specimens will be destroyed. The requests for withdrawal are required to be documented in the RedCap Database and the letter is required to be submitted within 5 business days to the study lead investigator and the project manager. Additional procedures for withdrawal are outlined in the study manual of operations.

Requests for withdrawal of consent from SURMOUNT may be submitted in writing to:

Angela DeMichele, M.D., M.S.C.E.
Rowan Breast Center of the University of Pennsylvania
Perelman Center, 3rd Floor (West)
3400 Civic Center Blvd
Philadelphia, PA 19104

3.5 Return of Research Results
Study participants will be offered the opportunity to receive research results at the completion of the study. If the study participant would like to receive their research results, they will provide consent and address for which to send the results at the completion of the study. All study participants will be informed that the research testing performed in this study is not performed in a CLIA approved laboratory and should not be used to make treatment decisions. They will be informed that these research findings have not been validated and will not be placed in the medical record.

4.0 Study Procedures:

4.1. General Procedures and Study Time-points

There is no therapeutic component to the SURMOUNT substudy. Study participants will provide clinical data, blood and bone marrow samples according to the schedule of study activities shown below in Table 1. Table 1 shows the samples that will be collected at each time-point.

Table 1: Schedule of Study Activities:

Study Visit/Timepoint					
Activity	Pre-op	Surgery	Post-op	Follow Up	Recurrence
Informed Consent	X				
Blood Draw	X CellSave Tube (2 x 10ml)	X EDTA Tube (3 x 10ml)	X Serum Separator Tube (1x 8.5ml) EDTA Tube (1 x 10ml)	X Cell Save Tube (1x 10ml) Serum Separator Tube (1x 8.5ml) EDTA Tube (2 x10ml)	X Cell Save Tube (1x 10ml) Serum Separator Tube (1x 8.5ml) EDTA Tube (2 x10ml)
Bone Marrow Aspirate*		X EDTA Tube 1 x 10ml			X EDTA Tube 1 x 10ml
Medical Chart Abstraction/Clinical Data Collection				X (Beginning year 6)	X

* Bone marrow aspirates may be collected in a setting outside of the surgical setting. This decision will be at the discretion of the clinical team and patient

4.2. Study Visits

Consented participants will be asked to provide a blood sample at several pre-specified time points: 1) "Pre-op" (at the completion of neoadjuvant chemotherapy before surgery), 2) "Surgery" (a blood sample in the operative setting of the participants' scheduled surgery), 3) "Post-op" (a blood sample during the post-operative visit), 4) "Follow up" (annual blood draws), and 5) "Recurrence" (the collection of blood, and bone marrow if a recurrence occurs). Shipping instructions for all samples are included in the SURMOUNT study Manual of Operations (MOP). The study participant will also be asked to provide a bone marrow aspirate sample at the time of the scheduled surgical procedure removing the residual breast tumor. The participant's clinical data will be updated through data collected on the I-SPY 2 TRIAL and by SURMOUNT study personnel. If the participant consents, they may also be contacted in the future regarding participation in other research studies for which they may qualify. The study participant will also be asked to allow residual specimens to be kept and banked.

4.2.1. Pre-Operative Visit

This study visit will occur at any time after the last dose of chemotherapy and before the surgical date. Pertinent tracking information, as outlined in the study Manual of Operations, will be subsequently entered into the SURMOUNT Tracking Databases. The study participant will have the pre-operative study blood drawn during the pre-operative visit. Of note, this blood draw can occur at the same time as the required I-SPY 2 pre-surgical blood draw. With the clinical guidance of the treating physician, study participant will decide whether or not to have their bone marrow aspirate as an in-clinic procedure or during their scheduled surgical procedure.

4.2.2. Surgery Visit

This study visit will occur on the day of surgery. The blood specimen and the bone marrow aspirate may be collected before or after surgical removal of the breast tissue. These will be handed off from the OR team to the study team for processing and shipping. Residual tumor tissue will be handled as outlined in the I-SPY 2 protocol; separate tissue will NOT be collected for SURMOUNT.

4.2.3. Post Operative Visit

This visit will take place after surgery but prior to the commencement of radiation therapy. It is recommended that specimen collection occur at the time of the 30-day I-SPY 2 TRIAL follow-up appointment. However, if the site is unable to accommodate the draw at this time, this research blood draw should take place no earlier than 2 weeks post-surgery and no later than 8 weeks post-surgery. At the study participant's post-operative appointment, research blood will be drawn according to Table 1.

4.2.4. Follow-Up Visits

This study visit will occur during the participants annual surveillance follow up visit. The annual visit can take place +/- 60 days from the study participant's yearly surgical anniversary. Participants will be followed annually in order to be evaluated for cancer recurrence events. At annual follow-up, study participants will be asked to provide blood specimens as outlined in the specimen collection table (Table 1). This will continue for a period of 10 years or until recurrence of breast cancer, whichever occurs first.

4.2.5. Recurrence Visit

This visit will occur in the event that the participant develops a local or distant recurrence of breast cancer. Participants will be asked to provide the study team with blood specimens and a bone marrow aspirate specimen. Tissue collection will take place as outlined in the I-SPY 2 TRIAL protocol. These collections are outlined in the schedule of specimen collection table (Table 1).

5.0 Specimen Processing and Laboratory Procedures

The manual of operations will detail the exact methods by which the specimens will be collected, processed, shipped and delivered to the appropriate labs of the study collaborators. Specimen and participant tracking procedures are outlined in the SURMOUNT MOP.

5.1. Whole Blood – Plasma, Buffy Coat, and Serum

Participants will have blood collected at specified time points. The details of blood collection are shown in Table 2. Notably, no study participant will have more than 50 ml of blood drawn at any given specimen collection time point. Blood samples will be collected from every consenting participant enrolled in this study. The blood specimens will be collected for research purposes. In addition to nucleic acid collection, serum, buffy coat, and plasma will be extracted from blood specimens. Study participants have the option to consent to allow the researchers to save leftover blood, serum, buffy coat and plasma samples for future research. Leftover specimens will remain at study laboratories until central study storage has been implemented. Central study specimen storage will be housed at the University of Pennsylvania.

5.2. Bone Marrow Aspiration

The bone marrow sample will be obtained either in a non-operative setting (i.e. clinic) by a qualified/trained healthcare provider or at the time of the study subject's scheduled surgery. Additionally, if the participant recurs, a bone marrow aspirate will be collected using the same procedures. If the participant and physician choose to collect the bone marrow sample at the time of breast surgery, it will happen while the participant is already under anesthesia. The participant will be turned to a decubitus position and bone marrow aspirates will be obtained from the posterior iliac crest. Approximately 10mLof aspirate fluid will be obtained in an EDTA-containing tube. Specimens will be shipped overnight as outlined in the SURMOUNT MOP

5.3. Banked Specimens

While the proposed assays will be sufficient to address the objectives of the current study, our understanding of circulating and disseminated tumor biomarkers is constantly evolving, and new technologies are in development and validation. miRNA and cell-free DNA will be assessed at a later point when new technologies have been optimized. Additional banking procedures and changes in preparation and storage procedures may occur as new technologies and techniques evolve. For this reason, data and specimens obtained from study participants will be kept and stored indefinitely.

5.4. Laboratory Assays

Table 2 lists the planned study assays and the laboratories that will be performing them.. Additional details regarding specimen tracking and shipping can be found in the SURMOUNT MOP.

5.4.1. Cell Search (Janssen, Inc)

The CellSearch Epithelial Cell Test will be applied to all samples for the enrichment and enumeration of CTCs. CTCs will be captured from the peripheral blood by anti-EpCAM antibody-bearing ferrofluid and subsequently identified by cytokeratin-positivity plus negativity for the leukocyte common antigen CD45 and 4'6'-diamidino-2-phenylindole (DAPI) staining to ensure the integrity of the nucleus. We will further characterize these cells for ER, Her2 and c-MET expression within the CellSearch system by the addition of FITC-labeled antibodies and use of the additional free channels in the system. Janssen Diagnostics, LLC is providing support for some of the analysis that will be conducted on the study participant's blood samples. This has been disclosed in the informed consent document. Additionally the company may receive de-identified data from this study in accordance with the I-SPY 2 TRIAL clinical data sharing policies and guidelines. We will not be sharing with the company any of the study participant's personal and private information.

5.4.2. IE/FACS and Molecular Profiling

CTCs identified in peripheral blood by the CellSearch™ screen and all bone marrow aspirates will undergo further evaluation for genetic and expression profiling.Tumor cells will be isolated from peripheral blood using IE/FACS as previously reported[49]. We have elected to perform this procedure only on those specimens positive by CellSearch™ screen for efficiency and cost containment, given the high correlation between the CellSearch™ system and that of the Park laboratory. IE/FACs is performed using magnetic beads coated with EpCAM mAb for initial enrichment; the enriched sample is then subjected to FACS using differentially labeled mAbs to distinguish tumor cells (EpCAM+) from leukocytes (CD45+) during sorting.

5.4.3. Molecular profiling of isolated cells:

DNA from isolated tumor cells will be subjected to whole genome amplification (WGA) followed by comparative genomic hybridization (CGH). In addition, we will isolate mRNA for expression profiling across a panel of candidate genes associated with breast cancer progression. We have also used large-scale cDNA arrays to evaluate the transcriptome of CTCs and DTCs. Briefly, total RNA from isolated CTCs or DTCs will be reverse-transcribed into cDNA. 64 genes will be chosen a priori for expression analysis, based on preliminary studies from mouse models. cDNAs of these genes will be pre-amplified

and analyzed in triplicate via Taqman®™-based RT-PCR in a low-density array format. Statistical analysis of gene expression data will be performed using Realtime Statminer®. In addition, we will utilize ABI systems customized arrays to expand our assessment to genes of interest from both the gene expression arrays performed on residual tumors. We will only be conducting genetic testing on the tumor and will not be performing germline testing.

Table 2: Study Assays

Sample Type	Assay(s)	Time point(s)	Possible Biomarkers Obtained
Whole Blood	Cell Search	PreOp, Annual F/U, Recurrence	Enumeration (EPCAM markers), ER, Her2, c-MET
Bone Marrow Aspirate	IE/FACS	Surgery, Recurrence	Enumeration, genetic evaluation
Whole Blood	IE/FACS	Surgery, Annual F/U, Recurrence	Enumeration, genetic evaluation
Whole Blood Serum aliquots	Banked for miRNA and others	Post Op Visit, Annual F/U, Recurrence	miRNA profile DNA profile/mutations
Whole Blood Plasma and Buffy coat aliquots	Banked for cell-free DNA, and others	Post Op Visit Annual F/U, Recurrence	DNA profile/mutations

6.0 Clinical Data Management

6.1. Data Obtained from the Electronic Medical Record

Clinical data for SURMOUNT will be collected in the I-SPY 2 TRIAL database system using the online platform SalesForce as outlined in the study protocol. The participant's clinical data will be updated by the I-SPY 2 TRIAL in years 1 to 5 of follow up. At the completion of year 5 of follow-up, SURMOUNT study personnel will update the participant's clinical date during years 6 to 10 of the follow-up period, or at recurrence, if it occurs.

6.2. Case Report Forms

The study case report forms (CRF) are the primary data collection instrument for the study. The CRFs for this study will be completed electronically using a RedCap database system accessible to all study sites. Training will be provided to all sites for proper use of the REDCap Database. The SURMOUNT MOP contains additional details for completing the forms in REDCap.

6.3. Records Retention

Records and specimens will be retained for the entire length of this study. As the relapse rate for many breast cancers remains elevated for over 10 years, participants in this study will be followed annually for 10 years after enrollment. Study documents will be retained by the investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPPA), and Office of Human

Research Protections (OHRP), unless the standard at the site is more stringent. Additionally, data and specimens obtained from study participants will be kept and stored indefinitely

7.0 Statistical Analysis Plan

7.1. Sample Size Determination

We anticipate enrolling and following 350 women from across the I-SPY 2 study sites. We expect 50% of these participants to have a complete pathological response (pCR or RCB=0) and approximately 50%, or 175, to have residual disease (i.e., RCB > 0). Our primary goal is to estimate the proportion of women in each RCB class with detectable CTCs and DTCs. We are primarily interested in the women with residual disease; if the rate is higher than 50% and we have accrued 175 such women before reaching the target of 350 total, we will suspend accrual. With 175 women with residual disease, the width of the 95% confidence interval around the detection rate will be no more than 0.15 units wide. We expect that among these 175 women, the proportions in the three RCB classes will be 0.35, 0.4, and 0.25, respectively. The maximum width of the 95% CIs in each group will therefore be no more than 0.25, 0.23, and 0.30 units wide, respectively. We also wish to compare the detection rate in these groups. Assuming that women in the RCB 2-3 group will have a detection rate of approximately 40%, we have more than 80% power to detect a decrease in this rate to about 18% in the RCB 1 group. Additional aims include correlation of features of DTCs/CTCs and primary or residual tumor cells. We anticipate that approximately 60% of the women with residual disease, or 105 women, will have detectable DTCs, and that approximately 20%, or 35 women, will have detectable CTCs. With 35 women, we have more than 80% power to detect a correlation coefficient of 0.5 or greater, using a two-sided, 0.05-level test. Calculations assume two-sided alpha level of 0.05 and 80% power.

7.2. Interim Analysis

We will conduct an interim analysis when we have enrolled 30 patients in order to determine if the detection rate for CTCs and DTCs is sufficiently high to continue to enroll participants. If the upper bound of the 95% confidence interval for the detection rate falls below the minimum threshold of 10%, we will attempt to restrict future enrollees using radiographic evidence of residual tumor at the completion of chemotherapy.

7.3. Final Analyses:

7.3.1 Statistical Analysis for Aim 1

In Aim 1, we will estimate and compare the CTC and DTC detection rates in several populations. We will compare detection rates in women with different RCB levels; the maximum CI widths in these groups are given above. We will also describe the detection rates in subgroups defined by receptor status, estimating the detection rates separately for hormone receptor positive patients, Her2+ patients, and triple negative patients. We will conduct exploratory logistic regression modeling to determine what additional factors may predict DTC/CTC detection. We will also consider changes in DTC/CTC detection over time using repeated measures logistic regression models.

7.3.2. Statistical Analysis for Aim 2.

In Aim 2, we will characterize the molecular and genetic features of the DTCs, CTCs, cells from the residual tumor, and cells from the primary tumor. We will estimate correlation coefficients for various features between cells of different types (e.g., DTCs and primary tumor) within the same patient.

7.3.3. Statistical Analysis for Aim 3

In Aim 3, we will estimate 3-year relapse-free survival in participants with and without detection of DTCs/CTCs at surgery. We will also explore Cox regression models using DTC/CTC detection as a predictor, first in a univariate model and then in multivariate models that include other known prognostic factors. We recognize that power is limited in this cohort to detect anything but quite large effects; these analyses are thus considered exploratory and will provide important preliminary estimates for future study

design and hypothesis generation. We will further explore characterizing DTC/CTC detection as a time-varying covariate using values at follow-up assessments in addition to the status at the time of surgery.

7.3.4. Statistical Analysis for Aim 4

In Aim 4, we will explore the value of DTC/CTC levels in predicting RCB class at the time of surgery. Initially we will use binary logistic regression to predict RCB 0/1 vs. RCB 2/3; if we find that CTC and DTC levels are useful, we will expand this model to an ordered logistic regression model and predicted membership in each class separately. Models will be assessed using the area under the ROC curve (AUC) and other measures of correct classification. These will be exploratory models that, if promising, will be validated in future independent data sets.

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