PILOT SAFETY TRIAL OF PREOPERATIVE CHEMOTHERAPY COMBINED WITH DENDRITIC CELL VACCINE IN PATIENTS WITH LOCALLY ADVANCED, TRIPLE-NEGATIVE BREAST CANCER OR ER-POSITIVE, HER2-NEGATIVE BREAST CANCER

Protocol Number:	013-154
Original Date:	July 18, 2013
Amendment 1	March 06, 2014
Amendment 2	June 30, 2014
Amendment 3	November 20, 2014
Amendment 4	May 01, 2015
Amendment 5	March 21, 2016
Amendment 6	October 13, 2016
IND Number:	#15765
Principal Investigator:	**Joyce O'Shaughnessy, MD** Celebrating Women Chair in Breast Cancer Research, Baylor Sammons Cancer Center/ Texas Oncology Co-Chair, Breast Cancer Research Program, The US Oncology Network 3410 Worth Street, Suite 400 Dallas, TX 75246 (214) 370-1795

SITE INVESTIGATOR SIGNATURE PAGE

The signature below constitutes the approval of this protocol entitled "Pilot Safety Trial of Preoperative Chemotherapy Combined with Dendritic Cell Vaccine in Patients with Locally Advanced, Triple-Negative Breast Cancer or ER-Positive, HER2-Negative Breast Cancer", and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed: _____ Date: _____

Name:_____

Table of Contents

ABBREVIATIONS **7**
SYNOPSIS **11**
1 INTRODUCTION **16**
1.1 BACKGROUND ON BREAST CANCER 16
1.2 BREAST CANCER AND IMMUNOTHERAPY 16
1.3 RATIONALE FOR TARGET ANTIGEN SELECTION 17
1.4 RATIONALE FOR INTRATUMORAL DC INJECTION 19
1.5 THERAPEUTIC DC VACCINES 21
1.6 DOXORUBICIN, CYCLOPHOSPHAMIDE, AND PACLITAXEL 21
1.7 ADDITION OF CARBOPLATIN TO NEOADJUVANT CHEMOTHERAPY FOR BREAST CANCER 22
1.8 ADDITION OF CAPECITABINE AS ADJUVANT CHEMOTHERAPY FOR TNBC 23
1.8.1 Capecitabine 23
1.8.2 Adjuvant Capecitabine Improves DFS in TNBC 23
1.9 RATIONALE 24
2 TRIAL OBJECTIVES **24**
2.1 PRIMARY OBJECTIVES 24
2.2 SECONDARY OBJECTIVES 24
3 STUDY DESIGN **24**
4 SELECTION AND WITHDRAWAL OF PATIENTS **27**
4.1 SAMPLE SIZE 27
4.2 INCLUSION CRITERIA 27
4.3 EXCLUSION CRITERIA 28
4.4 PATIENT WITHDRAWAL FROM THE STUDY 28
5 PRIOR AND CONCOMITANT THERAPY **30**
5.1 PROHIBITED TREATMENTS 30
6 THERAPEUTIC AGENTS **30**
6.1 INVESTIGATIONAL PRODUCT 30
6.1.1 Dendritic Cell (DC) vaccine 30
6.1.1.1 Formulation and Supply *31*
6.1.1.2 Packaging and labeling *31*
6.1.1.3 Storage *31*
6.1.1.4 Preparation *31*
6.1.1.5 Vaccine Administration and Vaccine Schedule *33*
6.1.1.6 Increase or Reduction in Dose of the Dendritic Cell (DC) Vaccine *36*
6.1.1.7 Vaccine Manufacture *36*
6.1.1.8 Vaccine Order Preparation and Shipment *36*
6.2 NON INVESTIGATIONAL PRODUCTS 36
6.3 ACCOUNTABILITY PROCEDURES 36
6.4. POTENTIAL RISKS DURING STUDY 36
6.4.1 Apheresis 36
6.4.2 DC Vaccine administration 37
6.4.3 Lipopolysaccharide (LPS) endotoxin 37
6.5 DRUG-DRUG INTERACTIONS 38
6.5.1 Cyclophosphamide (Cytoxan®) 38
6.5.2 Paclitaxel (Taxol®) 38
6.5.3 Carboplatin (Paraplatin®) 38
6.5.4 Capecitabine (Xeloda®) 39
6.6 PREMEDICATIONS 39
6.6.1 Doxorubicin and Cyclophosphamide 39
6.6.2 Paclitaxel and Carboplatin 39
7 TREATMENT PLAN **40**
7.1 CHEMOTHERAPY TREATMENT DELAY 42
7.2 CHEMOTHERAPY DOSE MODIFICATION FOR TOXICITY 42

7.3 HEMATOLOGIC TOXICITY 43
7.4 RADIATION THERAPY 43
8 SCHEDULE OF PROCEDURES AND ASSESSMENTS 44
8.1 SCREENING 44
8.2 REGISTRATION PROCEDURES 45
8.3 APHERESIS PROCEDURES 46
8.4 PERIPHERAL BLOOD MONONUCLEAR CELL ISOLATION 46
8.5 PROCEDURES DURING STUDY DRUG TREATMENT 46
8.6 PROCEDURES FOR VACCINE TREATMENT VISITS 47
8.7 PROCEDURES FOR END OF TREATMENT VISIT (EOT) 48
8.8 PROCEDURES FOR FOLLOW-UP VISITS 48
8.9 UNSCHEDULED VISITS 49
8.10 BLOOD SAMPLES FOR IMMUNOMONITORING 49
8.11 BIOPSIES 49
9 SAFETY EVALUATIONS 50
9.1 TOXICITY ASSESSMENT 50
9.2 LABORATORY DATA 50
9.3 PATIENT DIARIES 50
9.4 ADVERSE EVENTS 50
9.4.1 Definition of Adverse Event 50
9.4.2 Definition of Serious Adverse Event 51
9.4.3 Definition of Unexpected Adverse Event 51
9.4.4 Relationship to Study Drug 51
9.4.5 Assessment of Severity 52
9.4.6 Monitoring for Adverse Events During the Clinical Trial 52
9.4.7 Reporting of Adverse Events 52
9.4.8 Reporting of Serious Adverse Events 53
9.4.9 Removal Due to Adverse Events 53
9.4.10 Removal of Patients with Disease Progression While on Study 54
9.4.11 Pregnancy 54
10 EFFICACY ASSESSMENTS 54
10.1 EVALUATION OF PATHOLOGIC RESPONSE 54
11 STATISTICAL CONSIDERATIONS 56
11.1 POPULATIONS FOR ANALYSIS 56
11.1.1 Analysis Populations 56
11.1.2 Patient Characteristics 56
11.1.3 Patient Disposition 56
11.2 HYPOTHESIS AND ENDPOINTS 56
11.3 SAMPLE SIZE 57
11.4 STATISTICAL METHODS 57
12 PROTOCOL AND DATA DEVELOPMENT 59
12.1 ETHICS 59
12.1.1 Institutional Review Board 59
12.1.2 Modification of the Protocol 59
12.1.3 Patient Informed Consent 59
12.1.4 Confidentiality of Records 60
12.2 STUDY RECORDS 60
12.2.1 Documentation 60
12.2.2 Case Report Form 60
12.2.3 Source Documents 61
12.2.4 Electronic Databases 61
12.2.5 Databases Processing 61
13 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES 61
13.1 MULTIDISCIPLINARY TRIAL OVERSIGHT MEETINGS 61
13.2 PROTOCOL INITIATION MEETING 61
13.3 BIOSTATISTICAL SUPPORT 62

14 CLINICAL MONITORING PLAN 62
14.1 INTRODUCTION 62
14.2 PROCESSES 62
14.2.1 General Guidelines 62
14.2.2 Study Monitoring Visits 62
14.2.2.1 Investigational product accountability 63
14.2.2.2 Protocol deviations and non-compliance 63
14.2.2.3 Informed consent review 63
14.2.2.4 CRF and source document verification 63
14.2.2.5 Regulatory document/binder review 64
14.2.2.6 Adverse event/serious adverse event reporting 64
15 REFERENCES 65
APPENDIX I NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE), VERSION 4.0 70
APPENDIX II SCHEDULE OF ASSESSMENTS 71
APPENDIX III ECOG PERFORMANCE STATUS SCALE 75
APPENDIX IV PATIENT DIARY 76
APPENDIX V RESEARCH BIOPSIES – COLLECTION AND HANDLING 78
APPENDIX VI 013-154 AMENDMENT 1 LIST OF CHANGES 80
APPENDIX VII 013-154 AMENDMENT 2 LIST OF CHANGES 99
APPENDIX VIII 013-154 AMENDMENT 3 LIST OF CHANGES 110
APPENDIX IX 013-154 AMENDMENT 4 LIST OF CHANGES 128
APPENDIX X 013-154 AMENDMENT 5 LIST OF CHANGES 134
APPENDIX XI 013-154 AMENDMENT 6 LIST OF CHANGES 140

In-Text Tables

Table 1. Treatment schema 42
Table 2. Dose Reduction Levels 43

ABBREVIATIONS

Abbreviation	Term
5FU	5 fluorouracil
AC	doxorubicin/cyclophosphamide
AE	Adverse event
ALL	Acute lymphoid leukemia
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
APC	Antigen-presenting cells
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BC	Breast cancer
BIIR	Baylor Institute for Immunology Research
BUN	Blood urea nitrogen
Bx	Biopsy
C	Celsius
Cb	Carboplatin
CBC	Complete blood count
CD	Cluster of differentiation
CEF	Cytomegalovirus I, Epstein-Barr (E) Virus and Influenza (F) Virus
CFR	Code of federal regulations
CHF	Congestive heart failure
CMF	Cyclophosphamide, methotrexate, and fluorouracil
CMP	Complete metabolic profile
CO_2	Carbon dioxide
CPT	Cell preparation tube
CR	Complete response
CRF	Case report form
CT	Computed tomography
CTCAE	Common terminology criteria for adverse events
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trials Management System
CYP	Cytochrome P450
DCs	Dendritic cells
DFS	Disease-free survival
DLCO	Diffusing capacity
DMARDs	Disease modifying antirheumatic drugs

DNA	Deoxyribonucleic acid
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EOT	End of treatment
ER	Estrogen receptor
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
FDG-PET	Fluorodeoxyglucose positron emission tomography
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence in situ hybridization
GGT	Gamma-glutamyl transpeptidase
Gm	Gram
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good manufacturing practices
HgB	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human immunodeficiency virus
HMGB1	High mobility group B1
HTLV	Human T-cell lymphotropic virus type 1
HSC	Hematopoietic stem cells
HSR	Hypersensitivity reactions
IFN	Interferon
IND	Investigational new drug
IGFBP	Insulin-like growth factor binding protein
IHC	Immunohistochemistry
IL-x	Interleukin-x
IL-1R	Interleukin-1 Receptor
INR	International normalized ratio
IRB	Institutional Review Board
ISR	Injection site reaction
IT	Intratumoral
ITT	Intent to treat
IV	Intravenous
L	Liter
LA	Locally advanced
LD	Longest diameter
LFU	Lost to follow-up
LVEF	Left ventricular ejection fraction

Mg	Milligram
Mm	Millimeter
M2	Type 2 polarized macrophages
MBC	Metastatic breast cancer
mDC	Myeloid dendritic cells
MDS	Myelodysplastic syndromes
Mg	Milligram
mL	Milliliter
MNC	Mononuclear cells
MRI	Magnetic resonance imaging
MUC	Mucin
NCI	National Cancer Institute
NE	Not evaluable
NYHA	New York Heart Association
OTC	Over the counter
PBMC	Peripheral blood mononuclear cells
pCR	Pathologic complete response
PI	Principal Investigator
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression-free survival
PO	Per os (oral administration)
PR	Partial response
PS	Performance scale
RCB	Residual cancer burden
RECIST	Response Evaluation Criteria in Solid Tumors
RT	Radiation therapy
SAE	Serious adverse event
SC/SQ	Subcutaneous
SD	Stable disease
SOJIA	Systemic Onset Juvenile Idiopathic Arthritis
SOP	Standard operating procedure
T	Paclitaxel
TAC	Docetaxel, doxorubicin, and cyclophosphamide
TLR	Toll-like receptor
TNBC	Triple-negative breast cancer
TNF	Tumor necrosis factor
TSH	Thyroid stimulating hormone
TSLP	thymic stromal lymphopoietin

ULN	Upper limits of normal
USP	United States Pharmacopeial Convention
WBC	White blood cells
WLN	Within normal limits
WOCBP	Women of child bearing potential
WT	Wilms tumor (antigen)

SYNOPSIS

Summary: Women with TNBC who do **not** achieve a pathologic complete response (pCR; residual disease after neoadjuvant chemotherapy) have an increased risk of recurrence, decreased overall survival, and post-recurrence survival as compared to women with non-TNBC who do **not** achieve a pCR. Women with ER+/HER2- breast cancer typically have a low likelihood of developing a pCR, and if these women have residual disease with high levels of Ki67 after preoperative therapy, this predicts for poor overall and progression-free survival with subsequent endocrine therapy. Immunotherapy could be an attractive strategy for these patients and some preliminary studies have been carried out. Recent studies have shown that human breast cancers can be immunogenic, and that enhancing the immune effector function already present may augment the cytotoxic effects of standard therapies.

There are numerous strategies under investigation aimed at overcoming a patient's immunologic resistance to cancer. Yet, vaccination remains the most attractive strategy because of its expected inducement of both therapeutic T cell immunity (effector T cells) and protective T cell immunity (tumor-specific memory T cells that can control tumor relapse). Several clinical studies have now demonstrated that immunity against tumor antigens can be enhanced in cancer patients by vaccination with ex vivo-generated tumor antigen-loaded dendritic cells (DCs). This strategy capitalizes on the unique capacity of DCs to prime lymphocytes and to regulate and maintain immune responses.

Our goals are to boost T cell immunity targeted against breast cancer utilizing a tumor antigen-loaded DC vaccine, to enhance chemotherapy effectiveness and decrease tumor metastagenicity, and to decrease the recurrence rates of LA TNBC and ER+/HER2– BC. Patients will be treated with a combination of antigen-loaded DC vaccinations along with standard preoperative chemotherapy, to improve immunogenicity and to increase the pCR rate achieved with standard therapy. The trial will consist of 2 patient cohorts: TNBC and ER+/HER2– BC.

Objectives: The primary objective of this study is to determine the safety and feasibility of combining cyclin B1/WT1/CEF (antigen)-loaded DC vaccination with preoperative chemotherapy.

The secondary objectives of this trial are to determine pathologic complete response rates; disease-free survival; to assess immune biomarkers of immunity (antigen-specific CD8+ T cell immunity and T_H2 T cells) in breast cancer biopsy specimens and blood samples in patients receiving DC vaccinations; and to assess the feasibility of immunizing LA TNBC and ER+/HER2– BC patients with patient-specific tumor antigens.

Number of patients: 20

Inclusion Criteria:

A patient will be considered for enrollment in this study if all of the following criteria are met:

1. Female patients ≥18 years of age.
2. Have either:
    a. **locally advanced TNBC** defined as invasive ductal cancer; ER- tumors with <10% of tumor nuclei immunoreactive; PR- tumors with <10% of tumor nuclei immunoreactive; T3 or T4 disease, regardless of nodal status (T2 disease is eligible if there are positive lymph nodes present by physical exam or imaging evaluation or histological evaluation, OR
    b. **High-risk ER+ breast cancer** defined as grade 3 invasive ductal or mixed ductal/lobular cancers, or grade 2 with Ki67 ≥20%; node positive as evidenced by physical exam or imaging evaluation or histological evaluation.
3. HER2-negative breast cancer. If HER2-, it is defined as follows:
    a. FISH-negative (FISH ratio <2.0), or
    b. IHC 0-1+, or
    c. IHC 2+ AND FISH-negative (FISH ratio<2.0)
4. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-1
5. Adequate hematologic function, defined by:
    a. Absolute neutrophil count (ANC) >1500/mm^3
    b. Platelet count ≥100,000/mm^3
    c. Hemoglobin >9 g/dL (in the absence of red blood cell transfusion)
6. Adequate liver function, defined by:
    a. AST and ALT ≤2.5 x the upper limit of normal (ULN)
    b. Total bilirubin ≤1.5 x ULN
7. Adequate renal function, defined by:
    a. Serum creatinine ≤1.5 x ULN or calculated creatinine clearance of ≥60 ml/min
8. Patients with previous history of invasive cancers (including breast cancer) are eligible if definitive treatment was completed more than 5 years prior to initiating current study treatment, and there is no evidence of recurrent disease.
9. Eligible for treatment with paclitaxel, doxorubicin, cyclophosphamide, carboplatin, and capecitabine.
10. Patient must be accessible for treatment and follow-up.
11. All patients must be able to understand the investigational nature of the study and give written informed consent prior to study entry.

Exclusion Criteria:

A patient will be ineligible for inclusion in this study any of the following criteria are met:

1. Evidence of metastatic disease on bone scan and CT scan of chest/abdomen (or PET CT scan). Patients with intrathoracic metastatic adenopathy are eligible.
2. Active infection or unexplained fever >38.5°C during screening.
3. Active infections including viral hepatitis and HIV.
4. Active asthma or other condition requiring steroid therapy.
5. Autoimmune disease including lupus erythematosus or rheumatoid arthritis. Topical or inhaled corticosteroids are allowed.
6. Patients who are currently receiving or who have received previous systemic therapy for breast cancer (eg, chemotherapy, antibody therapy, targeted agents). The use of an LHRH agonist during chemotherapy in premenopausal women who wish to preserve ovarian function is allowed, but is not required.
7. Women who are pregnant or lactating. All patients with reproductive potential must agree to use effective contraception from time of study entry until at least 3 months after the last administration of study drug.
8. Have a NYHA Class III or IV CHF or LVEF <55%. Patients with significant cardiac disease history within 1 year or ventricular arrhythmias requiring medication are also excluded.
9. Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation such as:
    a. severe impaired lung functions as defined as spirometry and DLCO that is 50% of the normal predicted value and/or O_2 saturation that is 88% or less at rest on room air
    b. liver disease such as cirrhosis or severe hepatic impairment (Child-Pugh class C).
10. History of any other disease, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates use of an investigational drug, or that might affect interpretation of the results of this study, or render the patient at high risk for treatment complications.
11. Any other investigational or anti-cancer treatments while participating in this study.
12. Any other cancer

Medication and Doses:	This exploratory pilot safety, open label trial will evaluate the combination of preoperative chemotherapy and Dendritic Cell (DC) vaccinations in 2 cohorts of patients with LA TNBC or ER+/HER2– BC. • LA TNBC patients will be enrolled to receive DC vaccinations during the 24 weeks of standard preoperative dose-dense doxorubicin/cyclophosphamide (AC) followed by paclitaxel and carboplatin (TCb) chemotherapy; • ER+/HER2– BC patients will receive DC vaccinations during the 22 weeks of standard preoperative dose-dense AC followed by weekly paclitaxel (T) chemotherapy. • Study procedures will be similar in both groups. LA TNBC patients will receive standard preoperative dose-dense AC (4 cycles)

	followed by TCb (4 cycles) chemotherapy, administered for 24 weeks. ER+/HER2– BC patients will receive standard preoperative dose-dense AC (4 cycles) followed by weekly T (12 cycles), administered for a total of 22 weeks. In both cohorts, chemotherapy will be combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 cells/mL), for a total of 4 time points prior to definitive surgery. • During the AC cycles, both cohorts will receive vaccines administered on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-dense AC. • For TNBC patients, vaccines will be administered on any one individual day between Days 11-15 of Cycles 1 and 3 of TCb. • For ER+/HER2– patients, vaccines will be administered Day 1 during either Cycle 2 or Cycle 3 and on Day 1 during either Cycle 8 or Cycle 9 of T. Vaccine is to be administered after T infusion is completed in this cohort of patients. Standard pegfilgrastim support will be given for each AC treatment; however, no pegfilgrastim will be given during TCb or T cycles. Patients will undergo research biopsies of their breast cancer prior to the start of treatment and 1-2 days prior to or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Patients who will have their definitive surgery **outside** a Baylor hospital will have a third research biopsy at least 1 week following the last chemotherapy dose and prior to surgery. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment. After preoperative treatment, patients will undergo definitive surgery, generally with mastectomy, and if available, fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer. After definitive surgery and during locoregional radiation therapy to the breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL (at 15 x 10^6 cells/mL), rotating injection sites in the dorsal or ventral surface of the upper arm, with antigen-loaded DCs. The timing of the boosters is the same for TNBC and ER+/HER2– cohorts. The first vaccination booster will occur once after the surgery and up to 3 days prior to radiation; the second booster will occur 30 days ± 3 days after radiation is completed; and the third booster will occur 90 days ± 3 days after the 2nd boost. TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (cycle length per physician discretion).

Duration of Study:	Patients will be on study treatment for up to 1 year, and will be followed every 3 months thereafter for 3 years.

Efficacy Assessments:	**Safety Assessments:**
Pathologic complete response rate Disease-free survival	Toxicity

1 INTRODUCTION

1.1 BACKGROUND ON BREAST CANCER

Women with breast cancer who are treated with preoperative chemotherapy have the same survival as those who receive adjuvant therapy; however, pathologic complete response (pCR) after preoperative chemotherapy is a predictor of improved outcomes.[1,2] Those treated with preoperative therapy who achieve a pCR or near pCR have significantly better distant relapse-free survival than those with extensive residual disease independent of pathologic subtype. In addition, the degree of proliferation as indicated by expression levels of Ki67 is also a predictive factor for achieving a pCR. High Ki67 in pretreatment breast cancer tissue is associated with an increase in pCR, whereas high Ki67 in post-treatment residual disease is correlated with poorer disease-free and overall survival.[3]

Women with triple-negative breast cancer (TNBC) have an increased pCR rate as compared to women with non-TNBC, and those with pCR have a 90% disease-free survival.[4,5] However, women with TNBC who do **not** achieve a pCR (residual disease after neoadjuvant chemotherapy) have an increased risk of recurrence, decreased overall survival, and post-recurrence survival as compared to women with non-TNBC who do **not** achieve a pCR. The risk of recurrence and death is time-dependent and significantly higher for women with TNBC in the first 3 years of follow-up, versus women with non-TNBC.[4]

Women with ER+/HER2- breast cancer typically have a low likelihood of developing a pCR[6], and if these women have residual disease with high levels of Ki67 after preoperative therapy, this predicts for poor overall and progression-free survival with subsequent endocrine therapy.[3] ER+ patients whose cancers have high expression levels of Ki67 have a high rate of disease recurrence and new treatment options are necessary to improve their outcome.[7]

These patients have a great unmet medical need as there is no known effective therapy which can improve outcome. Therefore, a high priority for clinical research in patients is to increase the pathologic complete response (pCR) rate in breast and axilla following preoperative therapy. Patients with T2, T3 and T4 cancers and with clinically N1/N2 axillary disease are at highest risk of not achieving a pCR with standard therapy, and of developing metastatic disease.

1.2 BREAST CANCER AND IMMUNOTHERAPY

Immunotherapy could be an attractive strategy for overcoming chemotherapy resistance in TNBC or ER+/HER2– BC patients and some preliminary studies have been carried out.[8-11] Briefly, recent studies have shown that human breast cancers can be immunogenic, and that enhancing the immune effector function already present may augment the cytotoxic effects of standard therapies.[10,11] In one preclinical study, IGFBP-2 was found at elevated levels in breast cancer patients' sera, and an IGFBP-2–specific T-cell response inhibited tumor growth in a breast cancer mouse model.[8] In a phase I clinical trial, 19 patients with HER2-overexpressing breast cancer were vaccinated with HER2 peptide-specific T-cells, resulting in the generation of both CD4 and CD8 T-cell immunity. The resulting peptide-specific T-cells recognized

endogenous HER2 protein and the immunity was maintained for a median of 12 months after the last vaccination.[9] More recently, breast cancer tissues from a phase III clinical trial were analyzed for lymphocytic infiltrate, and the results demonstrated that increased infiltration of lymphocytes in tumor and stroma was associated with an overall good prognosis in ER-negative/HER2-negative breast cancer patients. These findings were consistent regardless of the chemotherapy type administered, demonstrating that greater lymphocytic infiltration was a prognostic factor for ER-negative/HER2-negative breast cancer.[12]

The presence of naturally occurring immunity against a broad range of tumor-associated antigens including HER-2/neu, MUC1, cyclin B1 and survivin has now been documented in patients with breast cancer.[13] However, the natural immune response to the cancer co-exists with the cancer, and is therefore not protective, either because of tumor escape, for example, through clonal evolution, or because it might have been generated in and/or elicited an inappropriate immunosuppressive microenvironment.

There are numerous strategies under investigation aimed at overcoming a patient's immunologic resistance to cancer. Among these are 1) non-specific activation of the immune system with microbial components or cytokines; 2) antigen-specific adoptive immunotherapy with antibodies and/or T cells; and 3) antigen-specific active immunotherapy (vaccination). The major limitation of antibodies is that target proteins must be expressed on the cell surface whereas targets for T cells can be intracellular proteins whose peptides are presented on the cell surface in complexes with MHC molecules.[14] The identification of defined tumor antigens in humans[15, 16] prompted the development of adoptive T cell therapy. Yet, vaccination remains the most attractive strategy because of its expected inducement of both therapeutic T cell immunity (effector T cells) and protective T cell immunity (tumor-specific memory T cells that can control tumor relapse). [13,17,18]

Several clinical studies have now demonstrated that immunity against tumor antigens can be enhanced in cancer patients by vaccination with ex vivo-generated tumor antigen-loaded dendritic cells (DCs). This strategy capitalizes on the unique capacity of DCs to prime lymphocytes and to regulate and maintain immune responses. Whereas a number of antigen-presenting cells can activate memory T cells, only DCs can prime naive T cells. This feature is essential to successful vaccination as it might allow generation of a "new" immune response, possibly not compromised by the cancer.[19]

1.3 RATIONALE FOR TARGET ANTIGEN SELECTION

Transcriptional profiling of triple negative breast cancers demonstrates a very strong proliferation signature[20,21] including enhanced transcription of *cyclin B1*. Cytoplasmic accumulation of cyclin B1 has been identified as an early event in breast cancer development.[22] Furthermore, *cyclin B1* genes are among the transcripts analyzed in the 21-gene assay Oncotype Dx, the first clinically validated multigene assay that quantifies the likelihood of breast cancer recurrence.[23]

Cyclin B1 is a regulatory protein that is an essential component of the mitotic cell cycle. The natural peak of cyclin B1 occurs between the G2-M phases of the cell cycle, and is reduced to near zero afterwards. However, in cancer cells, this protein is over-expressed during all phases of the cell cycle. Additionally, cyclin B1 is found in normal cells in the nucleus, whereas in cancer

cells it is found in the cytoplasm.[24] Several studies have shown that inactivation of the tumor suppressor gene p53, which occurs in all triple negative breast cancers, directly contributes to the aberrant regulation of cyclin B1 in tumor cells.[25] Cyclin B1 has been found to be over-expressed in multiple forms of cancer, including breast cancer, and in most cancer cell lines.[24] While studies involving the immunogenicity of cyclin B1 are limited, there are some indications that it is an important antigen to pursue.[25] Cyclin B1-specific antibodies are found in the blood of patients with many cancer types, at both the premalignant and established phases.[26] Cyclin B1-specific T cells can be also found in healthy volunteers.[27] Both antibodies and T cells against cyclin B1 protect from cancer in mouse models.[27] Because cyclin B1 is necessary for cancer cell division, loss of the antigen is an unlikely means of tumor escape. Cyclin B1 is one of the cancer-related genes that comprises the Oncotype DX Recurrence Score assay.[28] This assay, developed from the National Surgical Adjuvant Breast and Bowel Project (NSABP) trials, quantifies the likelihood of breast cancer recurrence, and also can predict for chemotherapy benefit (higher recurrence scores are indicative of greater therapy benefit).[29] High Cyclin B1 levels are associated with highly proliferative ER+ breast cancers and predict for a high risk of distant disease recurrence.

In our lab at the Baylor Institute for Immunology Research (BIIR), we have shown that DCs loaded with killed breast cancer cells which express cyclin B1 induce differentiation of cyclin B1-specific T cells, and that these T cells are able to kill breast cancer tumors in vitro.[27,30] In preliminary studies preparatory to the clinical trial proposed herein, we have also found that patients with various breast cancer subtypes can display a cyclin B1-specific memory T cell repertoire in their blood. These observations further support the targeting of this antigen for breast cancer immunotherapy. Herein, we propose to immunize breast cancer patients with cyclin B1 peptide-loaded DC vaccines, along with standard preoperative chemotherapy.

Another important antigen that we will include in the DC vaccines is Wilm's tumor antigen (WT1). The zinc finger transcription factor WT1 is expressed at 10–1000x fold higher levels in leukemic cells compared to normal $CD34^+$ cells, and the magnitude of expression correlates with clinical aggressiveness of acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and acute lymphoid leukemia (ALL).[31] Although essential during embryogenesis, WT1 expression after birth is limited to low levels predominantly in kidney podocytes and CD34+ hematopoietic stem cells (HSC). WT1-specific $CD8^+$ T lymphocytes can distinguish over-expressing targets from normal cells and have been demonstrated to inhibit the growth of and to lyse leukemic but not normal CD34+ cells.

Recent whole genome and transcriptome sequencing analysis of metastatic tumor tissue obtained from 14 TNBC patients, has delineated the wide array of somatic genomic alterations in these advanced tumors. Genes mutated in multiple tumors included TP53, LRP1B, HERC1, CDH5, RB1, and NF1. WT1 was among the genes that contained focal structural mutations as were CTNNA1, PTEN, FBXW7, BRCA2, FGFR1, KRAS, HRAS, ARAF, BRAF, and PGCP. Furthermore, WT1 was found to be overexpressed on RNA sequencing in all 14 samples.[32] Furthermore, the analysis of public microarray datasets of 266 early breast cancer patients showed that WT1 mRNA expression was correlated with higher histological grades, ER-negative and basal-like and ERBB2 molecular breast cancer subtypes.[33] Disease-free survival analysis showed worse prognosis the WT1 high expression group, and WT1 was found to be an

independent prognostic indicator in multivariate analysis. Finally, WT1 promotes proliferation and oncogenicity, and loss of expression is disadvantageous for the tumor, making outgrowth of antigen-loss variants less likely.

In the ER+, HER2- breast cancer cell line, MCF-7, WT1 was correlated with high expression of ERα and HER2, which indicated that it may play a role in cancer progression and development.[34] In addition, high levels of WT1 are associated with aggressive breast cancer biology, and in vitro, WT1 promoted estrogen-independent growth and anti-estrogen resistance.[35] This finding indicates that WT1 is, in part, mechanistically responsible for the switch from estrogen-dependent to -independent breast cancer growth and survival. Thus, WT1 is a very interesting candidate for therapeutic vaccination.

1.4 RATIONALE FOR INTRATUMORAL DC INJECTION

Although it is generally believed that cytotoxic antineoplastic agents mediate their therapeutic effects in a cancer cell-autonomous fashion, recent results indicate that at least some chemotherapeutics inhibit tumor growth also indirectly, via the immune system.

Indeed, our collaborators Drs Zitvogel and Kroemer demonstrated that a variety of transplantable or chemically induced, primary mouse cancers respond more efficiently to anthracyclines when they develop in hosts carrying an intact immune system.[36] Tumors evolving in immunodeficient mice fail to show a reduction in growth after anthracycline treatment in conditions in which the same tumors growing in immunocompetent mice do exhibit a significant inflection in their progression. Accordingly, clinical studies indicate that anthracycline-killed tumor cells are particularly efficient in stimulating a therapeutic immune response in cancer patients. Anthracycline-based neoadjuvant therapy of breast cancer patients is more effective when the tumor is infiltrated by T cells before chemotherapy is initiated as well as if chemotherapy causes a significant influx of $CD8^+$ T cells into the tumor bed and/or reduces the presence of immunosuppressive T regulatory (Treg) cells.[36] The reason why anthracyclines provoke this complex anticancer immune response has only been partially elucidated. In contrast to many other cytotoxic chemotherapeutics, anthracyclines stimulate immunogenic cell death that is characterized by a compendium of subtle biochemical changes in the plasma membrane surface and in the microenvironment of dying cancer cells. These changes include the pre-apoptotic exposure of calreticulin on the plasma membrane surface (to facilitate the engulfment of portions of the dying cells by antigen-presenting cells, APC) and the post-apoptotic exodus of high mobility group B1 (HMGB1) from the nucleus (to engage with TLR4 receptors and to stimulate antigen presentation).[36] Moreover, ATP release by autophagy-competent dying tumor cells (positive for LC3-II) is essential for the induction of an anticancer immune response, both by stimulating the recruitment of inflammatory cells ($CD11b^+LyC6^{high}$ $CD11c^{low}$ CD86+) into the tumor bed and by ligating P2RX7 receptors on dendritic cells, hence facilitating the activation of the NLRP3 inflammasome and the consequent secretion of IL-1b by APC.[36]

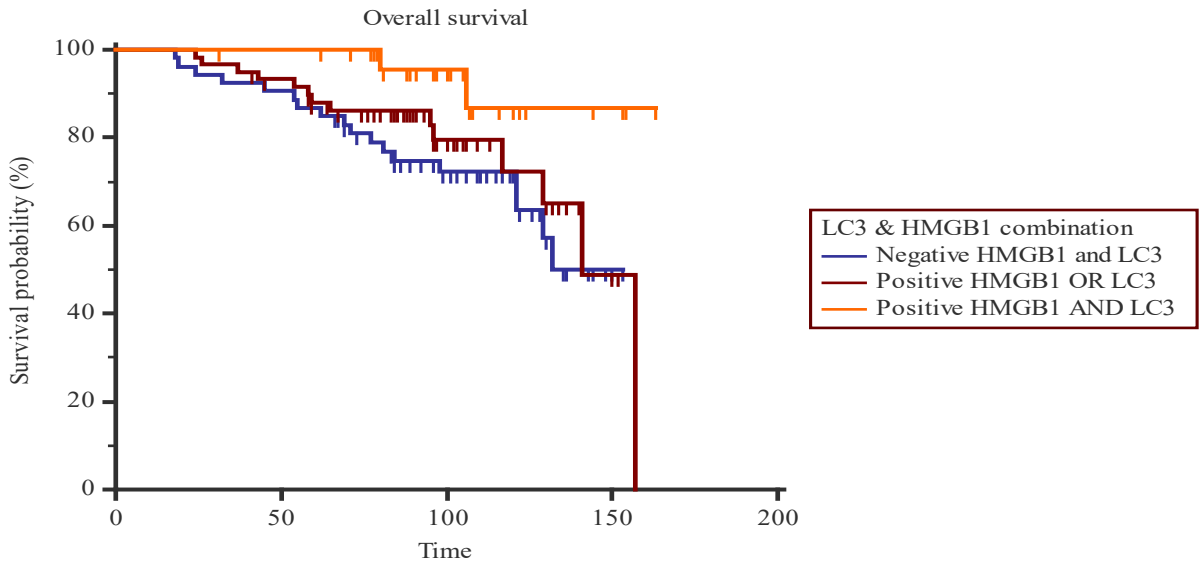
How chemotherapy-induced cell death leads to efficient antigen presentation to T cells has remained an open conundrum. Studies from Drs Zitvogel and Kroemer showed in the mice that intratumoral $CD11c^+CD11b^+Ly6C^{high}$ cells, which shared some characteristics of inflammatory dendritic cells (DC) and contained granulomonocytic precursors, were crucial for the induction of anticancer immunity post-chemotherapy.[37] First, ATP released by dying tumor cells is

essential for the recruitment of myeloid cells into tumor beds and for the local differentiation of inflammatory DC. Second, manipulations aimed at avoiding the intratumoral accumulation of these $CD11c^{+}CD11b^{+}Ly6C^{high}$ cells, such as local overexpression of the ATP-degrading enzyme CD39, pharmacological blockade of purinergic receptors, or neutralization of CD11b, abolished the immune-dependent inhibition of tumor growth by anthracyclines. Third, $CD11c^{+}CD11b^{+}Ly6C^{high}$ were efficient in capturing and presenting tumor cell antigen to T cells and protected mice upon their adoptive transfer against challenge with cancer cells. Altogether, the results identify a population of tumor-infiltrating leukocytes as therapy-relevant antigen-presenting cells.

Drs. Zitvogel and Kroemer evaluated the 2 immunogenic cell death markers HMGB1 and LC3-II, on paraffin-embedded BC specimen in a test (50 early BC treated with adjuvant anthracyclines that relapse at 3 years paired with 50 cases that were disease-free at 10 years) and a validation cohort on 150 HER2-negative early BC treated with adjuvant anthracyclines. Preliminary data suggest that LC-3-II staining was negative in the vast majority of cases of early breast cancers (>70%). These "autophagy deficient" cancers are also less infiltrated with CD8+ T cells but contained more CD68+ cells and had a greater chance of recurrence following adjuvant chemotherapy. A larger across Europe validation study is ongoing.

Blocking ectoATPases (CD39) restored the recruitment of DC in tumors and the efficacy of chemotherapy in autophagy deficient murine cancers.[38] However, anti-CD39 Ab are not available for the use in the human at this time. We propose to substitute the functional DCs via adoptive transfer of ex vivo generated autologous mature DCs injected locally into LA TNBCs and ER+/HER2– breast cancer patients post-systemic anthracycline therapy. Whereas we will not stratify in this early phase of DC vaccine assessment, all samples will be tested for LC3-II staining (and others such as CD68, CD8, Foxp3, phosphoSTAT6). Data will be used to properly power the follow up study.

Another objective for intratumoral vaccination is the possibility to enhance the access of DCs to draining lymph nodes. Indeed, recent studies suggest that the rout of DC injection might determine the homing of elicited T cells. Indeed, for mucosal cancer vaccines, the homing to and retention of CD8+ T cells in the mucosa are critical for efficacy.[39] In this context, the growth of orthotopic head and neck or lung cancers can be inhibited by a cancer vaccine provided that it is administered by the intranasal mucosal route, but not the intramuscular route. This is explained by the induction through intranasal vaccination of mucosal CD8+ T cells expressing the mucosal integrin CD49a, the expression of which is essential for the efficacy of cancer vaccines. Whereas in this pilot study we will not monitor DC migration, in the follow up study this parameter can be objectively assessed.



Number at risk

Group: 0				
53	48	28	1	0
Group: 1				
60	54	21	2	0
Group: 2				
28	27	13	3	1

1.5 THERAPEUTIC DC VACCINES

Investigators within the Ralph M. Steinman Center for Cancer Vaccines at BIIR have carried out more than 7 phase I/II clinical trials testing ex vivo-generated DC vaccines in patients with stage IV melanoma, HIV, and more recently pancreatic cancer. We have found that a fraction of patients can experience durable tumor regressions as well as prolonged survival.[40] We have also developed a DC vaccine optimized for $CD8^+$T cell responses, ie, GM-CSF/IFN-α-generated DCs activated with TLR ligands and CD40L. We have developed a closed system for vaccine generation and a frozen vaccine that has been successfully administered in multicenter clinical trials in patients with melanoma and in patients with HIV. This vaccine was used, on compassionate basis, in a patient with a partially resected stage IV pancreatic cancer who was also treated with gemcitabine and 5FU. DC vaccine was loaded with patient-specific mutant peptides whose sequences were identified by the analysis of autologous tumor. Repeated vaccinations were delivered one day after the last day of each chemotherapy cycle. The analysis of immune responses revealed the expansion of $CD8^+$ T cells specific to the pancreatic cancer antigens resulting from the vaccination.

1.6 DOXORUBICIN, CYCLOPHOSPHAMIDE, AND PACLITAXEL

Adjuvant chemotherapy can substantially reduce the risk of breast cancer recurrence and death in high-risk patients,[41] and there are many chemotherapy regimens with established efficacy and safety data. The value of chemotherapy is established from the data from individual randomized

trials and from the Early Breast Cancer Trialists' Collaborative Group's (EBCTCG) 15-year meta-analyses of randomized chemotherapy trials.[42] The meta-analyses established that anthracycline-containing therapies, such as doxorubicin and cyclophosphamide (AC) and docetaxel, doxorubicin, and cyclophosphamide (TAC), offer superior efficacy, reducing the risk of recurrence by 11% and the risk of death by 16% compared with cyclophosphamide, methotrexate, and fluorouracil (CMF) combinations.[42]

Significant improvements in disease-free survival (DFS) were reported with adjuvant dose-dense chemotherapy in women with node-positive breast cancer in the Phase III CALGB 9741 study of 2005 women. Citron et al[43] showed that when the taxane, paclitaxel (Taxol) (T), was given sequentially following standard chemotherapy, doxorubicin (A) and cyclophosphamide (C), in an every two-weekly dose-dense regimen, the rate of recurrence was significantly reduced by 26% (P=0.010) and the rate of death was reduced by 31% (P=0.014), compared to standard every 3-week administration, with an acceptable toxicity profile.

In a 2005 report of the findings of NSABP B-28, the addition of a taxane, adjuvant paclitaxel, to AC resulted in significant improvement in DFS. NSABP B-28 was conducted to determine whether 4 cycles of adjuvant T after 4 cycles of adjuvant AC (AC→T) would increase the DFS and OS compared with 4 cycles of AC alone in patients with resected operable, node-positive breast cancer.[44] Patients (N=3060) were randomly assigned to the 2 groups. The addition of AC→T significantly reduced the hazard for developing a DFS event by 17% (relative risk [RR], 0.83; 95% CI, 0.72 to 0.95; P=0.006). Five-year DFS was 76% ±2% for patients randomly assigned to AC→T compared with 72% ±2% for those randomly assigned to AC. Improvement in OS was small and not statistically significant (RR, 0.93; 95% CI, 0.78 to 1.12; P=0.46). Five-year OS was 85% ±2% for both groups. Toxicity with the AC→T regimen was found to be acceptable in the adjuvant setting.

Thus, the combination of AC, followed by a taxane such as paclitaxel (Taxol) is now widely accepted as an effective adjuvant treatment for early-stage breast cancer.

Advances in adjuvant chemotherapy have resulted in improved outcomes in patients with ER- breast cancers to a greater extent than for those with ER+ breast cancers.[45] Many of these have been implemented as neoadjuvant therapy. Standard AC→T given preoperatively to TNBC patients results in pathologic complete response rates of 30%-40%.[5,46]

1.7 ADDITION OF CARBOPLATIN TO NEOADJUVANT CHEMOTHERAPY FOR BREAST CANCER

Carboplatin is approved for the treatment of ovarian cancer and small cell lung carcinoma, and is commonly used for the treatment of non-small cell lung cancer (NSCLC), head and neck cancer, and other tumors. In breast cancer, administration of carboplatin to previously untreated patients with metastatic disease results in response rates of 20% to 50%.[47] Paclitaxel in combination with carboplatin is also highly active in breast cancer, with response rates of approximately 39% to 62% for first-line treatment of metastatic disease.[48] Notably, data suggest that the administration of carboplatin in combination with paclitaxel results in less thrombocytopenia than is expected from the use of carboplatin alone.[47]

Emerging data suggests that TNBC tumors may be more sensitive to DNA damaging agents, including carboplatin. Two small single arm trials with cisplatin combination neoadjuvant

therapy or neoadjuvant monotherapy for patients with TNBC have reported complete tumor regressions or minimal residual disease at the time of surgery in 21% and 44% of patients.[49,50] The CALGB study 40603 evaluated the efficacy of neoadjuvant paclitaxel with or without carboplatin and/or bevacizumab followed by AC in patients with TNBC. The addition of carboplatin to the standard neoadjuvant chemotherapy regimen improved pathologic complete response in the breast in patients to 60% vs. 46% of patients who did not receive carboplatin, concluding that the addition of carboplatin was a reasonable addition to the standard neoadjuvant chemotherapy for TNBC.[51]

1.8 ADDITION OF CAPECITABINE AS ADJUVANT CHEMOTHERAPY FOR TNBC

1.8.1 Capecitabine

Capecitabine is a prodrug that is enzymatically converted to 5-fluorouracil in the tumor where it inhibits DNA synthesis and slows growth of tumor tissue. Capecitabine is approved in both colorectal and breast cancer. Capecitabine demonstrated single-agent activity in subjects with MBC with an ORR of about 20% in subjects whose disease had progressed during or following anthracycline and taxane-based therapy.[52]

Capecitabine is also indicated as a combination treatment with docetaxel in early-line treatment for MBC and in combination with ixabepilone or lapatinib as second-line treatment after failure of prior anthracycline and taxane-containing chemotherapy.[52]

The recommended, approved dose of capecitabine is 1250 mg/m^2 daily BID for 14 days, followed by 7 days without treatment. This dose/schedule necessitates dose interruptions or reductions in approximately 30% of patients, and in clinical trials, approximately 17% of patients discontinued the drug due to toxicities (primarily hand-foot syndrome, diarrhea and stomatitis).[53,54]

1.8.2 Adjuvant Capecitabine Improves DFS in TNBC

Lee and colleagues recently published results from a 5 year follow up of a Phase III trial of adjuvant capecitabine in breast cancer patients with HER2-negative pathological residual invasive disease after neoadjuvant chemotherapy. Oral capecitabine was administered for 8 cycles at 1250 mg/m^2 PO BID after standard neoadjuvant chemotherapy that contained an anthracycline and/or a taxane, when patients did not have a complete pathologic response. Overall, disease-free survival (DFS) rate and overall survival increased with capecitabine as compared to standard therapy (DFS: 74.1% vs 67.6% and 89.2% vs 83.9%, respectively). Specifically in patients with triple-negative breast cancer that were non-pathologic complete responders following surgery and neoadjuvant treatment, there was a 42% reduction in the risk of recurrence with the addition of adjuvant capecitabine.Since women with TNBC who do **not** achieve a pCR have an increased risk of recurrence, decreased overall survival, and post-recurrence survival, the addition of capecitabine provides an additional benefit and option for this population.

1.9 RATIONALE

Our goals are to boost T cell immunity targeted against breast cancer utilizing a tumor antigen-loaded DC vaccine, to enhance chemotherapy effectiveness and decrease tumor metastagenicity, and to decrease the recurrence rates of LA TNBC and ER+/HER2– BCs. Patients with LA TNBC and ER+/HER2– BC will be treated with a combination of antigen-loaded DC vaccinations along with standard preoperative chemotherapy, to improve immunogenicity and to increase the pCR rate achieved with standard therapy. The trial will consist of 2 patient cohorts: TNBC and ER+/HER2– BC.

2 TRIAL OBJECTIVES

2.1 PRIMARY OBJECTIVES

The primary objective of this study is to determine the safety and feasibility of combining cyclin B1/WT1/CEF (antigen)-loaded DC vaccination with preoperative chemotherapy.

2.2 SECONDARY OBJECTIVES

The secondary objectives of this trial are to determine pathologic complete response rates; disease-free survival; to assess immune biomarkers of immunity (antigen-specific CD8+ T cell immunity and T_H2 T cells) in breast cancer biopsy specimens and blood samples in patients receiving DC vaccinations; and to assess the feasibility of immunizing LA TNBC and ER+/HER2– BC patients with patient-specific tumor antigens.

3 STUDY DESIGN

This exploratory pilot safety, open label trial will evaluate the combination of preoperative chemotherapy and Dendritic Cell (DC) vaccinations in 2 cohorts of patients with LA TNBC or ER+/HER2– BC.

- LA TNBC patients will be enrolled to receive DC vaccinations during the 24 weeks of standard preoperative dose-dense doxorubicin/cyclophosphamide (AC) followed by paclitaxel and carboplatin (TCb) chemotherapy;
- ER+/HER2– BC patients will receive DC vaccinations during the 22 weeks of standard preoperative dose-dense AC followed by weekly paclitaxel (T) chemotherapy.
- Study procedures will be similar in both groups.

The screening period is from signature of the informed consent form to final eligibility assessments. Eligible patients will undergo apheresis after registration and entry into the study. After collection of peripheral blood mononuclear cells, dendritic cell will be manufactured from the monocyte fraction, aliquoted and frozen. Patients will be given a total of 7 DC vaccinations.

Patients will undergo research biopsies of their breast cancer prior to the start of treatment, and 1-2 days prior to or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Patients who will have their definitive surgery **outside** a Baylor hospital will have a third research biopsy at least 1 week following the last chemotherapy dose and prior to

surgery. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment.

LA TNBC patients will receive standard preoperative dose-dense AC (4 cycles) followed by TCb (4 cycles) chemotherapy, administered for 24 weeks. ER+/HER2– BC patients will receive standard preoperative dose-dense AC (4 cycles) followed by weekly T (12 cycles), administered for a total of 22 weeks. In both cohorts, chemotherapy will be combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 cells/mL), for a total of 4 time points prior to definitive surgery.

- During the AC cycles, both cohorts will receive vaccines administered on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-dense AC.
- For TNBC patients, vaccines will be administered on any one individual day between Days 11-15 of Cycles 1 and 3 of TCb.
- For ER+/HER2– patients, vaccines will be administered Day 1 during either Cycle 2 or Cycle 3 and on Day 1 during either Cycle 8 or Cycle 9 of T. Vaccine is to be administered after T infusion is completed in this cohort of patients.

Timing of the vaccinations is based on data that tumor cell death associated with doxorubicin treatment increases the generation and functional activation of CD8 T cells required for the antitumor activity of doxorubicin.[56] Standard pegfilgrastim support may be given for each AC treatment; however, no pegfilgrastim will be given during TCb or T cycles.

After preoperative treatment, patients will undergo definitive surgery, generally with mastectomy, and if available, fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer. Patients will be known to have axillary node positive disease at study entry based on biopsy or clinical criteria and will generally undergo level 1/2 axillary dissection at definitive surgery. However, patients may undergo SLN biopsy before or after chemotherapy at the physician's discretion.

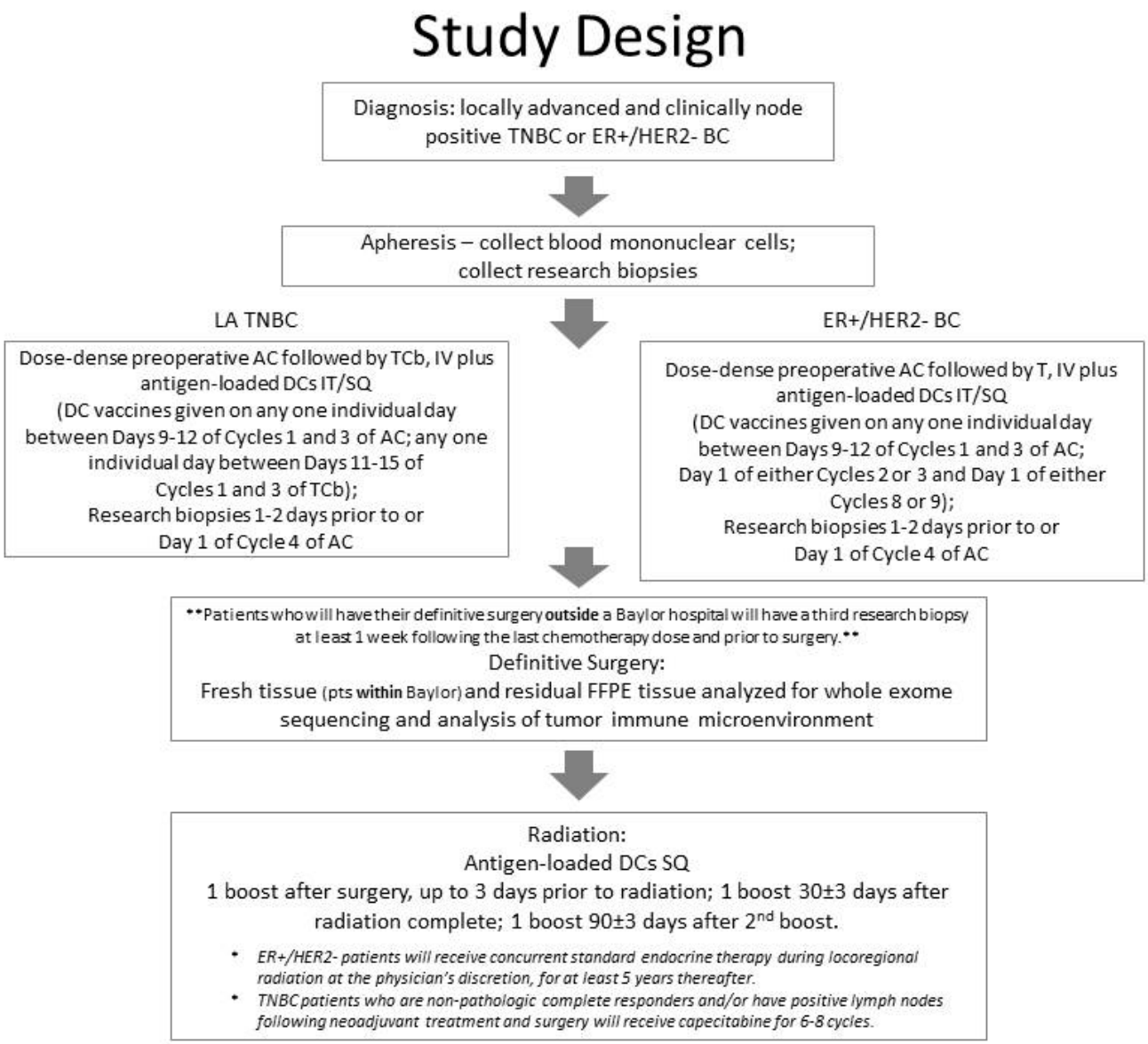
After definitive surgery and during locoregional radiation therapy to the breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL (at 15 x 10^6 cells/mL), rotating injection sites in the dorsal or ventral surface of the upper arm, with antigen-loaded DCs. The timing of the boosters is the same for TNBC and ER+/HER2– cohorts. The first vaccination booster will occur once after the surgery and up to 3 days prior to radiation; the second booster will occur 30 days ± 3 days after radiation is completed; and the third booster will occur 90 days ± 3 days after the 2nd boost.

ER+/HER2– BC patients will also receive standard endocrine therapy during locoregional radiation therapy, at the physician's discretion, and for at least 5 years thereafter.

TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (number of

cycles per physician discretion). It will be the physician's discretion to begin capecitabine treatment either during radiation or after radiation is complete.

Blood samples for immunomonitoring studies will be obtained at baseline, prior to each DC vaccination, prior to surgery, prior to radiation, 2 weeks ± 3 days after the last DC vaccination, 6 months and 1 year after the last DC vaccination.



4 SELECTION AND WITHDRAWAL OF PATIENTS

4.1 SAMPLE SIZE

Twenty patients with newly diagnosed locally advanced TNBC or ER+/HER2– BC will be enrolled over 20 months.

If a patient's apheresis product or manufactured vaccine is suboptimal after a second apheresis procedure, the patient will be withdrawn from the study and replaced. See Section 8.3.

4.2 INCLUSION CRITERIA

A patient will be considered for enrollment in this study if all of the following criteria are met:

1. Female patients ≥18 years of age.
2. Have either:
    a. **locally advanced TNBC** defined as invasive ductal cancers; ER- tumors with <10% of tumor nuclei immunoreactive[57]; PR- tumors with <10% of tumor nuclei immunoreactive; T3 or T4 disease, regardless of nodal status (T2 disease is eligible if there are positive lymph nodes present by physical exam or imaging evaluation or histological evaluation, OR
    b. **High-risk ER+ breast cancer** defined as grade 3 invasive ductal or mixed ductal/lobular cancers, or grade 2 with Ki67 ≥20%; node positive as evidenced by physical exam or imaging evaluation or histological evaluation.
3. HER2-negative breast cancer. If HER2-, it is defined as follows:
    a. FISH-negative (FISH ratio <2.0), or
    b. IHC 0-1+, or
    c. IHC 2+ AND FISH-negative (FISH ratio<2.0)
4. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-1
5. Adequate hematologic function, defined by:
    a. Absolute neutrophil count (ANC) >1500/mm^3
    b. Platelet count ≥100,000/mm^3
    c. Hemoglobin >9 g/dL (in the absence of red blood cell transfusion)
6. Adequate liver function, defined by:
    a. AST and ALT ≤2.5 x the upper limit of normal (ULN)
    b. Total bilirubin ≤1.5 x ULN
7. Adequate renal function, defined by:
    a. Serum creatinine ≤1.5 x ULN or calculated creatinine clearance of ≥60 ml/min
8. Patients with previous history of invasive cancers (including breast cancer) are eligible if definitive treatment was completed more than 5 years prior to initiating current study treatment, and there is no evidence of recurrent disease.
9. Eligible for treatment with paclitaxel, doxorubicin, cyclophosphamide, carboplatin, and capecitabine.
10. Patient must be accessible for treatment and follow-up.

11. All patients must be able to understand the investigational nature of the study and give written informed consent prior to study entry.

4.3 EXCLUSION CRITERIA

A patient will be ineligible for inclusion in this study if any of the following criteria are met:

1. Evidence of metastatic disease on bone scan and CT scan of chest/abdomen (or PET CT scan). Patients with intrathoracic metastatic adenopathy are eligible.
2. Active infection or unexplained fever >38.5°C during screening.
3. Active infections including viral hepatitis and HIV.
4. Active asthma or other condition requiring steroid therapy.
5. Autoimmune disease including lupus erythematosus or rheumatoid arthritis. Topical or inhaled corticosteroids are allowed.
6. Patients who are currently receiving or who have received previous systemic therapy for breast cancer (eg, chemotherapy, antibody therapy, targeted agents). The use of an LHRH agonist during chemotherapy in premenopausal women who wish to preserve ovarian function is allowed, but is not required.
7. Women who are pregnant or lactating. All patients with reproductive potential must agree to use effective contraception from time of study entry until at least 3 months after the last administration of study drug.
8. Have a NYHA Class III or IV CHF or LVEF <55%. Patients with significant cardiac disease history within 1 year or ventricular arrhythmias requiring medication are also excluded.
9. Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation such as:
    a. severe impaired lung functions as defined as spirometry and DLCO that is 50% of the normal predicted value and/or O_2 saturation that is 88% or less at rest on room air
    b. liver disease such as cirrhosis or severe hepatic impairment (Child-Pugh class C).
10. History of any other disease, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates use of an investigational drug, or that might affect interpretation of the results of this study, or render the patient at high risk for treatment complications.
11. Any other investigational or anti-cancer treatments while participating in this study.
12. Any other cancer

4.4 PATIENT WITHDRAWAL FROM THE STUDY

Patients will be withdrawn from the study ("off study") if any of the following occur:

1. Withdrawal of consent (patient will not be contacted and no further information will be collected). If the patient withdraws consent, then no additional data will be collected without his/her explicit consent; all data collected prior to withdrawal of consent may be used in the data analysis.
2. Termination of study by Baylor IRB or Principal Investigators

3. Disease progression
4. Intolerable toxicity
5. An intercurrent illness, which would in the judgment of the Investigator, affect assessments of clinical status to a significant degree or require discontinuation of study treatment
6. Apheresis product or manufactured vaccine is suboptimal after a second apheresis procedure.
7. Non-protocol therapy (chemotherapy, radiotherapy, hormonal therapy, immunotherapy, or surgery) that is administered during study
8. Noncompliance with protocol or treatment
9. Pregnancy
10. Lost to follow-up (3 attempts should be documented in the patient's source document before the site considers the patient as LFU.)
11. Patients who experience any of the following treatment- or vaccine-related symptoms or signs as outlined in NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (Appendix I) will be removed from study treatment, but will be followed for outcomes and long-term toxicity, per protocol:

    - Grade 2 or higher allergic reactions including bronchospasm or generalized urticaria
    - Grade 3 or greater allergic toxicity
    - Grade 2 or greater autoimmune toxicity
    - Grade 2 allergic reactions related infusion
    - Grade 3 or greater hematologic or non-hematologic toxicity including site reactions

NOTE: The treating physician may adjust chemotherapy doses based on individual patient histories to optimize dose delivery, per standard of care. However, if any patient requires more than one dose reduction of AC **OR** the need for more than one dose reduction of paclitaxel or 2 dose reductions of carboplatin due to treatment delays for hematologic toxicity or for Grade 3 or 4 non-hematologic toxicity, they will be removed from the study treatment.

The date of and reason for discontinuation must be noted on the Case Report Form (CRF). Every effort should be made to complete the appropriate assessments.

If the patient is withdrawn for any reason, the end of treatment assessments must be completed (see Section 8.7). Patients who withdraw from the study treatment due to intolerable toxicity will still be followed for outcome and toxicity, per protocol.

Patients must still be followed for adverse events (AEs) for 30 calendar days after their last dose of study drug. All new AEs occurring during this period must be reported and followed until resolution, or after 30 days (whichever comes first), unless, in the opinion of the investigator, these values are not likely to improve because of the underlying disease. In this case, the investigators must record his or her reasoning for this decision in the patients' medical records and as a comment on the CRF.

All patients who have CTCAE grade 3 or 4 laboratory abnormalities at the time of withdrawal must be followed until the laboratory values have returned to grade 1 or 2, or until 30 days after the date of withdrawal (whichever comes first), unless it is, in the opinion of the investigator, not

likely that these values are to improve because of the underlying disease. In this case, the investigator must record his or her reasoning for making this decision in the patients' medical records and as a comment on the CRF.

5 PRIOR AND CONCOMITANT THERAPY

All medications (prescribed or over the counter) and concomitant treatments including blood and blood products, taken at the time of signing the informed consent form or during the study must be reported on the source documentation and the Concomitant Medications page of the CRF.

- Blood and blood products
- Prophylactic antibiotics
- Premedications (ranitidine, diphenhydramine)
- Antifungals
- Growth factors
- LHRH agonist

5.1 PROHIBITED TREATMENTS

Patients may not use any of the following therapies during the study:

- Administration of other chemotherapy
- Administration of other immunotherapy
- Any other investigational agents
- Herbal therapies or alternative therapies
- Administration of pegfilgastrim during treatment with paclitaxel, and paclitaxel/carboplatin
- Administration of coumarin-derived anticoagulants and phenytoin during treatment with capecitabine
- Bisphosphonates or denosumab during the study, but may be used to treat osteoporosis after study therapy has been completed.

Supportive care may be administered at the discretion of the investigator.

6 THERAPEUTIC AGENTS

6.1 INVESTIGATIONAL PRODUCT

In this protocol, the investigational product is the Dendritic Cell (DC) vaccine.

6.1.1 Dendritic Cell (DC) vaccine

Immunity against tumor antigens can be boosted in cancer patients by vaccination with ex vivo-generated tumor antigen-loaded dendritic cells (DCs). This strategy capitalizes on the unique capacity of DCs to prime lymphocytes and to regulate and maintain immune responses. Whereas a number of antigen-presenting cells can activate memory T cells, only DCs can prime naive T cells. This feature is essential to successful vaccination as it might allow generation of a "new" immune response, possibly not compromised by the cancer.

6.1.1.1 Formulation and Supply

Final Formulation. The BIIR-BrcaVax-001 DC vaccine is prepared for injection into the patient by thawing the requisite number of frozen vials of DC vaccine and diluting the contents with USP injection grade sterile Lactated Ringer's to wash the cells by centrifugation. The cells are washed 3 times with Lactated Ringer's. Prior to the third wash, a sample is taken to determine the cell count and viability. After the third wash, the cells are resuspended in Lactated Ringer's at 15 x 10^6 viable cells/mL. The cell suspensions are filled into a 2 mL sterile glass vaccine vial sealed with a serum stopper and metal cap, for delivery to the clinic. Therefore, the final formulation is comprised of DCs suspended in 100% Lactated Ringer's.

6.1.1.2 Packaging and labeling

The vaccine primary container, the glass vaccine vial, will be used to contain both the frozen DC vaccine product and the washed DC vaccine prepared for injection into the patient, and is labeled with the patient's name, vaccine batch number, vaccine product name, manufacture date, name of the manufacturer, volume in the vaccine vial dose (concentration of cells in the vaccine vial or syringe), storage temperature, and the warning statements: "For Autologous Use Only" , and "Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use". On the glass vaccine vial containing the DC vaccine that has been prepared for injection, a separate product expiration date and time label is attached. The expiration time is 2 hours after the washed, resuspended DC vaccine is been filled into the glass vaccine vial.

The Vaccine Product container that is delivered to the bedside is a glass vaccine vial of 1.5 mL of prepared final Vaccine Product (15 x 10^6 viable cells/mL) to be drawn into a syringe for the intratumoral and/or subcutaneous injection. The vaccine-filled vial is placed in a clear biohazard plastic zip-lock bag and hard-foam shipping container for transport from the GMP facility to the clinic. Upon delivery to the clinic, the clinician inspects the vial to determine that it holds the correct Vaccine Product for the patient, dose and volume; and that no leak of the Vaccine Product has occurred. Before injection, the patient is asked to read aloud the name written on the glass vaccine vial label containing the vaccine preparation and to confirm that it is his/her name.

6.1.1.3 Storage

Vaccines will be kept in a locked area with limited access at BIIR. Vaccines will be kept frozen in liquid nitrogen (vapor phase) until use (removal of vials to prepare the Vaccine Product for inoculation).

6.1.1.4 Preparation

Vaccines will be prepared by the GMP Lab at BIIR. The BIIR-BrcaVax-001 DC vaccine product is prepared by culturing the breast cancer patient's monocytes with granulocyte-macrophage colony stimulating factor (GM-CSF) and Interferon alpha (IFN-α) to generate the DC that are then loaded with tumor and control antigens. Tumor antigens include long peptides covering immunogenic spots of Cyclin B1 and WT1. Control antigens include a mix of CEF peptides covering the most common CD8+ T cell epitopes of CMV (C), EBV (E) and Flu (F) viruses. The antigen loaded DC are then activated with purified lipopolysaccharide (LPS), CD40 ligand (CD40L) and the toll-like receptor (TLR) 7/8 agonist CL075 prior to fill/freezing.

The BIIR-BrcaVax-001 DC vaccine product manufacturing process is conducted in a closed system, that is, the patient's cells are processed in sterile cell collection and culture bags with media and cell transfers made using sterile tubing and connections. Addition of cytokines and antigens to the cell culture bags and collection of in-process samples for QC testing are performed with sterile needles and syringes. The cells are harvested into and washed in sterile plastic centrifuge tubes using sterile, disposable plastic pipettes to transfer cells and media. Manufacture of the BIIR-BrcaVax-001 DC vaccine product is conducted in the GMP vaccine manufacturing facility (Suite 105) located at BIIR (3434 Live Oak Street, Dallas, TX).

The DC vaccine product is manufactured according to SOP VP142.01, titled "Procedure for manufacture of BIIR-BrcaVax-001, a dendritic cell vaccine product for the treatment of breast cancer". To manufacture this frozen, autologous DC vaccine monocytes are enriched by elutriation from white blood cells that are obtained from the patient by apheresis. The patient's monocytes, at a concentration of $1x10^6$ viable cells/mL, are placed in cell culture in CellGro® DC culture media (CellGenix) supplemented with GM-CSF (LEUKINE®, Bayer) at 100 ng/mL and IFN-α (INTRON®A, Schering Corporation, now Merck) at 500 IU/mL. The cells are cultured in 100 mL cell culture bags (AFC) in a 37°C, 5% CO_2 incubator. An odd number of cell culture bags will be set up to initiate the culture of each DC vaccine batch. Depending on the total number of monocytes obtained from the elutriation step the number of cell culture bags to be set up may vary.

After 24 hours, GM-CSF and IFN-α are added to the cell culture bags to replenish the cell culture media at concentrations of 100 ng/mL and 500 IU/mL, respectively. After 48 hours of culture the antigen peptides are added to the cell culture bags in the following manner. CEF peptides are added to one of the cell culture bags (*e.g.*, Bag #1 of 15) to give a final concentration of 2 µM. To half of the remaining cell culture bags (*e.g.*, Bags #2-8 of 15) the Cyclin B1 peptides are added to give a final concentration of 2 µM. To the other half of the remaining cell culture bags (e.g., Bag #9-15 of 15) the WT1 peptides are added to give a final concentration of 3 µM. After adding the antigens the following DC activating agents are added to all of the cell culture bags; LPS at 5 EU/mL, CD40L at 100 ng/mL and CL075 at 1 µg/mL. The cells are then cultured for an additional 24 hours ± 1 hour. After completing the incubation the antigen-loaded and activated DC are harvested from the cell culture bags, combined, washed by centrifugation with Lactated Ringer's, and then suspended in cell freezing solution (consisting of 10% dimethyl sulfoxide, 80% heat-inactivated autologous serum and 10% Plasma-Lyte A with dextrose) at $30x10^6$ viable cells/mL for filling into glass vaccine vials. The vials containing 1 mL of the DC vaccine suspension are then frozen for storage in a liquid nitrogen tank (at -180°C in the liquid nitrogen vapor phase) prior to use. To prepare the inoculations for injection into the patient the required number of frozen DC vaccine vials are thawed, DMSO is washed out and the cell suspension is filled into a sterile glass vaccine vial for transport to the clinic. Each DC vaccine vial containing frozen cells is labeled with the following information: the patient's name, DC vaccine product name, DC vaccine batch number, storage conditions, manufacture date (date frozen), volume in the vaccine vial or syringe, dose (concentration of cells in the vaccine vial or syringe), vial number (frozen vaccine vials), manufacturer's name, and the warning statements: "For Autologous Use Only" , and "Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use". The same label information is attached to

the glass vaccine vial containing the Vaccine Product that is prepared for injection, along with a separate product expiration date and time label. All batches of frozen BIIR-BrcaVax-001 DC vaccine product and vaccine product prepared for injection remain in the custody of the Quality Assurance/Control Unit until they are released to the clinic or shipper.

Extensive QC release testing of the frozen vaccine will include:

a) Cell Count (Recovery) and Viability
b) Evaluation of DC morphology by Giemsa staining of cytospun cells
c) Evaluation of DC phenotype by multiparameter flow cytometry analysis
d) Sterility testing (mycoplasma, gram stain, bacteria/fungus growth and endotoxin)
e) Potency testing by phenotype and cytokine secretion.

QC release testing of the washed DC vaccine for injection will include:

a) Cell Count and Viability
b) Sterility testing: gram stain and endotoxin (results available prior to injection)
c) Sterility testing: bacterial and fungal growth (results available after injection)

6.1.1.5 Vaccine Administration and Vaccine Schedule

LA TNBC patients will receive standard preoperative dose-dense dense AC (4 cycles) followed by TCb (4 cycles) chemotherapy, administered for 24 weeks. ER+/HER2– BC patients will receive standard preoperative dose-dense AC (4 cycles) followed by weekly T (12 cycles), administered for a total of 22 weeks. In both cohorts, chemotherapy will be combined with antigen-pulsed DC vaccinations administered for a total of 4 time points prior to definitive surgery.

- During the AC cycles, both cohorts will receive vaccines administered on any one individual day between Days 9-12 of Cycle 1 and 3 of dose-dense AC.
- For TNBC patients, vaccines will be administered on any one individual day between Days 11-15 of Cycle 1 and 3 of TCb.
- For ER+/HER2– patients, vaccines will be administered Day 1 during either Cycle 2 or Cycle 3 and on Day 1 during either Cycle 8 or Cycle 9 of T. Vaccine is to be administered after T infusion is completed in patients.

At each scheduled vaccination time point during the preoperative phase, the patient will receive a total of 2 injections. Each vaccination will consist of:

- One intratumoral injection of 0.2 mL (3×10^6 cells/mL)
- One subcutaneous injection of 1 mL (15×10^6 cells/mL), rotating injection sites in the dorsal or ventral surface of the upper arm (***ipsilateral***).

NOTE: Needles used for drawing vaccine product into the syringe and for injection **must be 23-gauge or larger**. Smaller sized needles (eg, 25- or 27-gauge) will either rupture the cells or cause them to clump.

DC vaccines are timed with chemotherapy. All vaccine doses within a cycle are scheduled to be given approximately every 2 weeks apart. If necessary, a vaccine dose may be delayed for up to

2 weeks. In this case, subsequent doses should continue on a 2 week schedule, from the time of the delayed vaccine. If a vaccine dose is delayed more than 2 weeks, the PI and the BIIR Project Manager must be contacted for further instructions on continued dosing. Additional delays or modifications to the dosing schedule must be approved by the sponsor scientific chair.

Timing of the vaccinations is based on data that tumor cell death associated with doxorubicin treatment increases the generation and functional activation of CD8 T cells required for the antitumor activity of doxorubicin.[56] Standard pegfilgrastim support may be given for each AC treatment; however, no pegfilgrastim will be given during TCb or T cycles. DC vaccinations will be administered to the patient prior to administering chemotherapy on the given day, with the exception of weekly T for ER+/HER2– patients. Vaccine is to be administered after T infusion is completed in this cohort of patients.

After definitive surgery and during locoregional radiation therapy to breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL each (15×10^6 cells/mL), rotating injection sites in the dorsal or ventral surface of the upper arm (***contralateral***). The timing of the boosters is the same for TNBC and ER+/HER2– cohorts.

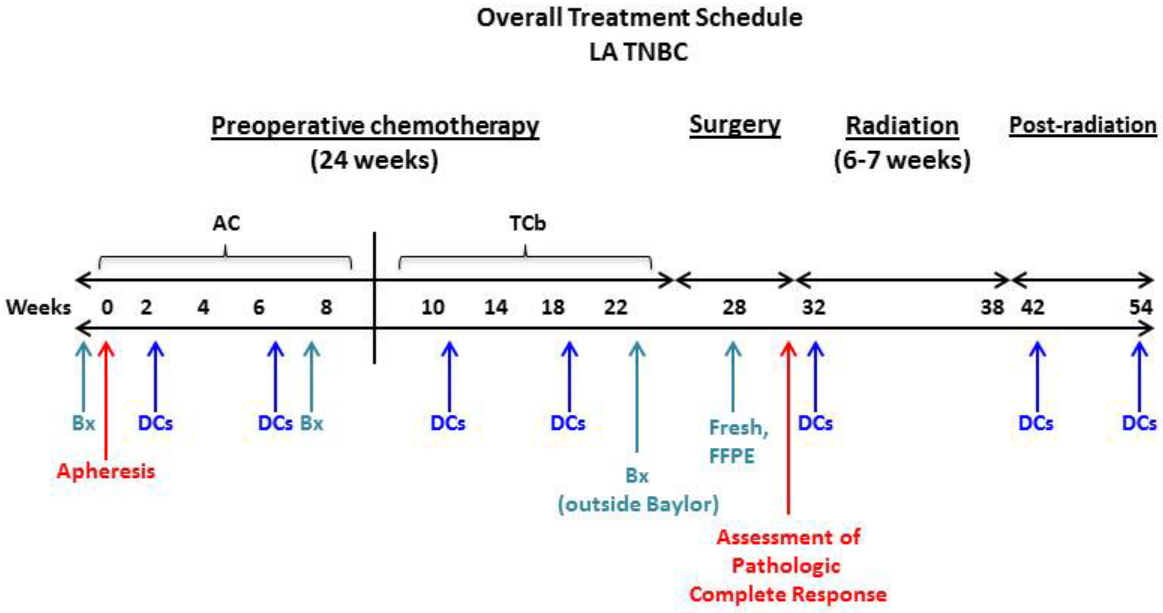
- The first vaccination booster will occur once after the surgery and up to 3 days prior to radiation.
- The second booster will occur 30 days ± 3 days after radiation is completed.
- The third booster will occur 90 days ± 3 days after the 2nd boost.

Patients will be monitored post DC infusion for any signs of infusion related reaction every 15 minutes for 1 hour.

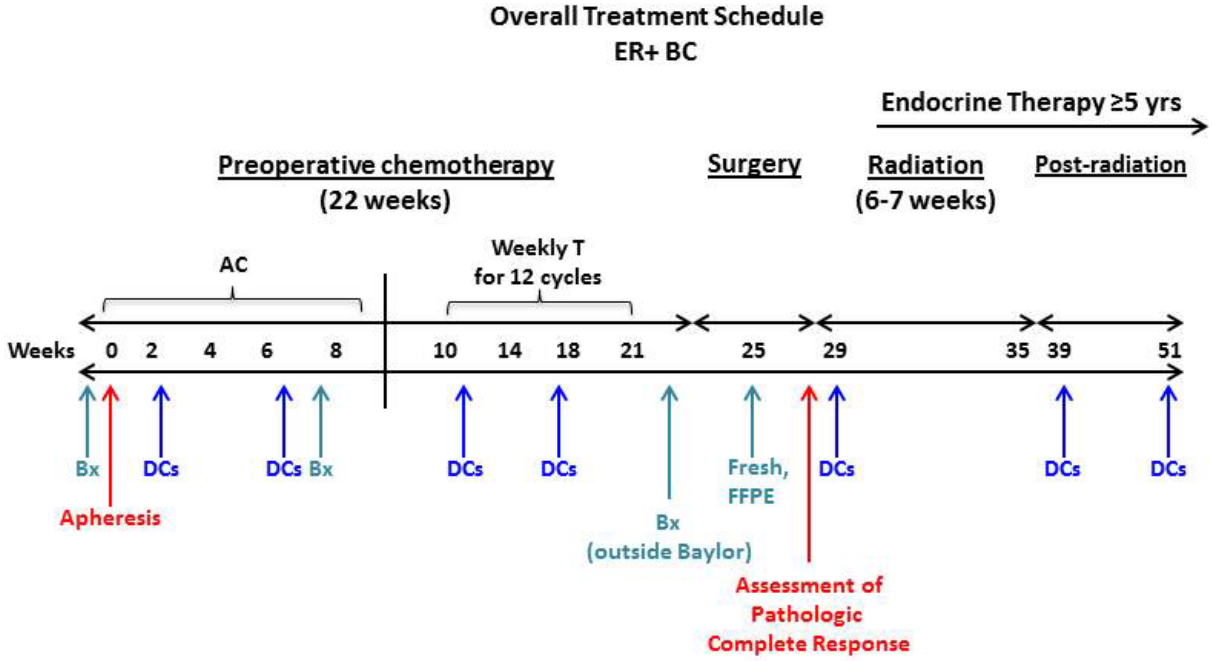
ER+/HER2– BC patients will also receive standard endocrine therapy during locoregional radiation therapy, at the physician's discretion, and for at least 5 years thereafter. TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (number of cycles per physician discretion). It will be the physician's discretion to begin capecitabine treatment either during radiation or after radiation is complete. These adjuvant treatments will not alter the vaccine schedule delineated above.

To reconcile the DC vaccine use and disposal, the procedure will be to ship the vial of 1.5 mL of prepared final Vaccine Product to the clinic and following vaccination of the patient, the unused portion of the Vaccine Product will be disposed of at the clinic by BIIR or Clinical Research Staff coordinating the study visit.

The DC vaccination schedule for **LA TNBC** is shown below.



The DC vaccination schedule for **ER+/HER2– BC** is shown below.



6.1.1.6 Increase or Reduction in Dose of the Dendritic Cell (DC) Vaccine

An increase or reduction in the target dose of the dendritic cell (DC) vaccine is not permitted during the study.

6.1.1.7 Vaccine Manufacture

The vaccine that is the subject of this IND will be manufactured in an equipped space in the BIIR at 3434 Live Oak Street, Dallas, Texas 75204, Room #105 GMP Facility, as described in the FDA IND application for this protocol.

6.1.1.8 Vaccine Order Preparation and Shipment

Vaccine shipment will follow SOP VP132 "Formulated Liquid DC Vaccine Shipment at Ambient Temperature".

6.2 NON INVESTIGATIONAL PRODUCTS

In this protocol, the non-investigational products are:

- Preoperative chemotherapy with preoperative dose-dense doxorubicin/cyclophosphamide (AC) and paclitaxel/carboplatin (TCb) or paclitaxel alone (T)
- Radiation therapy to breast or chest wall and regional lymphatics
- For ER+/HER2– BC patients only: standard endocrine therapy during and after radiation, per physician's discretion
- For TNBC non-pathologic complete responders: capecitabine treatment to begin either during radiation or after radiation is complete.
- Supportive therapy (eg, pegfilgrastim, dexamethasone, cimetidine, and dyphenhydra)

6.3 ACCOUNTABILITY PROCEDURES

The Investigator (or his/her designee) is responsible for maintaining accurate records of the receipt, dispensing, and use of all investigational materials, and may administer the study drug only to patients enrolled at the study.

The study drug accountability record includes the study identification of the patient to whom the drug is administered, the quantity and the date of administration, and any returned, disposed of, or unused drug. This record is in addition to any drug accountability information recorded on the Case Report Form.

6.4. POTENTIAL RISKS DURING STUDY

6.4.1 Apheresis

The use of apheresis for the collection of human blood mononuclear cells is commonly done in the practice of hematology and oncology. For venous access, patients will either undergo venipuncture of the antecubital veins in both arms or a central venous catheter will be inserted in those patients whose venous access is insufficient to undergo apheresis by venipuncture. Short-term side effects of apheresis include dysesthesias secondary to citrate anticoagulant,

ecchymoses at the site of venipunctures and nausea. The risk of infection related to venipuncture involving antecubital veins or insertion of central venous catheters is extremely low (approximately 1%). Other potential side effects of apheresis include hypotension, fever, catheter related infection and pneumothorax.

6.4.2 DC Vaccine administration

It is anticipated that patients who receive DC vaccinations will experience no significant adverse events related to administration of the vaccine. DC vaccinations have been administered to approximately 2,000 cancer patients worldwide in medical trials and in some healthy volunteers without signs of significant toxicity other than mild injection site reactions (redness, induration and pain) and constitutional symptoms (chills, pyrexia, fatigue and myalgias) within 24 hours post-immunization that were managed on an outpatient basis and resolved within 48 hours. Patients may experience enlarged and tender lymph nodes contiguous with the subcutaneous and/or intradermal injection of DCs. Two out of approximately 30 patients vaccinated show exacerbation of pre-existing allergic reactions. DC vaccinations were not associated with any evidence of dose limiting toxicity.

6.4.3 Lipopolysaccharide (LPS) endotoxin

No toxicity is anticipated from the use of LPS in the vaccine manufacturing process as the amount of residual endotoxin that could remain after extensive washing of the vaccine product will be quantitated prior to release. If the specifications for the vaccine are not met, the vaccine will not be administered. In process development studies using monocytes from normal donors to manufacture the DC vaccines, the total amount/concentration of endotoxin in sonicated vaccine cells has been measured and has been calculated that the amount to be injected would reach the concentration of 3.6 EU/10 mL, well below the 5 EU/10 mL as specified in FDA guidelines. The endotoxin preparation that will be used ex vivo to activate the DC vaccine has been certified by the FDA for in vivo use in healthy patients.

Reference endotoxin administered as a 4 ng/kg IV bolus is associated with an acute inflammatory response that may consist of fever, chills, rigors, arthralgias, myalgias, malaise, fatigue, somnolence, mild anxiety, headache, nausea, vomiting, hypotension, hyperdynamic cardiac response, hyperglycemia and leukopenia/leukocytosis. Symptoms peak 2 to 3 hours after injection, lessen considerably by 6 to 8 hours post injection and resolve completely by 24 hours post administration. In the current study, however, endotoxin will be used for ex vivo activation of DCs and the maximum injected dose will not exceed a limit of <0.5 EU/mL.

The endotoxin preparation used to activate the DC vaccine ex vivo is a reference endotoxin that has been certified by the FDA for in vivo use in healthy patients. The total amount/concentration of endotoxin in sonicated vaccine exposed to this concentration has been measured and it has been calculated that the total amount that would be injected subcutaneously will represent a maximum concentration of 3.6 EU/10 mL, well below the 5 EU/10 mL maximum specified in FDA guidelines. This amount of endotoxin is equivalent to a subcutaneous injection of 0.025 ng/kg, well below the dose level that elicits an acute inflammatory response.

6.5 DRUG-DRUG INTERACTIONS

6.5.1 Cyclophosphamide (Cytoxan®)

The rate of metabolism and the leukopenic activity of cyclophosphamide reportedly are increased by chronic administration of phenobarbital.

The Treating Physician should be alert for possible combined drug actions, desirable or undesirable, involving cyclophosphamide even though cyclophosphamide has been used successfully with other drugs, including other cytotoxic drugs. Cyclophosphamide treatment, which causes a marked and persistent inhibition of cholinesterase activity, potentiates the effect of succinylcholine chloride.

If a patient has been treated with cyclophosphamide within 10 calendar days prior to general anesthesia, the anesthesiologist must be alerted.[58]

6.5.2 Paclitaxel (Taxol®)

In a Phase 1 trial using escalating doses of paclitaxel (110–200 mg/m^2) and cisplatin (50 or 75 mg/m^2) given as sequential infusions, myelosuppression was more profound when paclitaxel was given after cisplatin than with the alternate sequence (ie, paclitaxel before cisplatin). Pharmacokinetic data from these patients demonstrated a decrease in paclitaxel clearance of approximately 33% when paclitaxel was administered following cisplatin.

The metabolism of paclitaxel is catalyzed by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. Caution should be exercised when paclitaxel is concomitantly administered with known substrates (eg, midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, and triazolam), inhibitors (eg, atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin), and inducers (eg, rifampin and carbamazepine) of CYP3A4.

Caution should also be exercised when paclitaxel is concomitantly administered with known substrates (eg, repaglinide and rosiglitazone), inhibitors (eg, gemfibrozil), and inducers (eg, rifampin) of CYP2C8.

Potential interactions between paclitaxel, a substrate of CYP3A4, and protease inhibitors (ritonavir, saquinavir, indinavir, and nelfinavir), which are substrates and/or inhibitors of CYP3A4, have not been evaluated in clinical trials.

A sample list of drugs that have known interaction with cytochrome P450 can be found here: http://medicine.iupui.edu/flockhart/table.htm

Reports in the literature suggest that plasma levels of doxorubicin (and its active metabolite doxorubicinol) may be increased when paclitaxel and doxorubicin are used in combination.[59]

6.5.3 Carboplatin (Paraplatin®)

The renal effects of nephrotoxic compounds may be potentiated by carboplatin.[60]

6.5.4 Capecitabine (Xeloda®)

Anticoagulants

Altered coagulation parameters and/or bleeding have been reported in patients taking capecitabine concomitantly with coumarin-derivative anticoagulants such as warfarin and phenprocoumon. These events occurred within several days and up to several months after initiating capecitabine therapy and, in a few cases, within 1 month after stopping capecitabine. These events occurred in patients with and without liver metastases. In a drug interaction study with single-dose warfarin administration, there was a significant increase in the mean AUC of S-warfarin. The maximum observed INR value increased by 91%. This interaction is probably due to an inhibition of CYP2C9 by capecitabine and/or its metabolites.

Phenytoin

The level of phenytoin should be carefully monitored in patients taking capecitabine and phenytoin dose may need to be reduced. Postmarketing reports indicate that some patients receiving capecitabine and phenytoin had toxicity associated with elevated phenytoin levels. Formal drug-drug interaction studies with phenytoin have not been conducted, but the mechanism of interaction is presumed to be inhibition of the CYP2C9 isoenzyme by capecitabine and/or its metabolites.

Leucovorin

The concentration of 5-fluorouracil is increased and its toxicity may be enhanced by leucovorin. Deaths from severe enterocolitis, diarrhea, and dehydration have been reported in elderly patients receiving weekly leucovorin and fluorouracil.

CYP2C9 substrates

Other than warfarin, no formal drug-drug interaction studies between capecitabine and other CYP2C9 substrates have been conducted. Care should be exercised when capecitabine is coadministered with CYP2C9 substrates.[52]

6.6 PREMEDICATIONS

6.6.1 Doxorubicin and Cyclophosphamide

There are no required/recommended premedications to prevent infusion reactions or life-threatening events for the administration of doxorubicin and cyclophosphamide. However, all patients must be premedicated to prevent nausea and vomiting. The regimen selected is at the discretion of the Treating Physician.

6.6.2 Paclitaxel and Carboplatin

Standard premedications for paclitaxel and carboplatin should be administered prior to each dose of paclitaxel and carboplatin:

- Dexamethasone 8-20 mg PO (physicians may administer their standard HSR steroid prophylaxis regimen)
- Diphenhydramine 25-50 mg IV (or equivalent)

- Cimetidine (300 mg) or ranitidine 50 mg IV (or equivalent)

7 TREATMENT PLAN

There are 2 patient cohorts in this study: LA TNBC patients and ER+/HER2– BC patients.

LA TNBC:

- DC vaccine plus preoperative chemotherapy for 24 weeks

ER+/HER2– BC:

- DC vaccine plus preoperative chemotherapy for 22 weeks; standard endocrine therapy during radiation, at the physician's discretion, for at least 5 years thereafter.

1. Once patients sign informed consent, they will be evaluated with a complete history, physical examination as well as initial studies as described in Section 8.1.
    a. LA TNBC patients will be enrolled to receive DC vaccinations during the 24 weeks of standard preoperative dose-dense doxorubicin/cyclophosphamide (AC) followed by paclitaxel and carboplatin (TCb) chemotherapy;
    b. ER+/HER2– BC patients will receive DC vaccinations during the 22 weeks of standard preoperative dose-dense AC followed by weekly paclitaxel (T) chemotherapy.
    c. Study procedures will be similar in both groups.
2. Eligible patients will then undergo apheresis to commence with vaccine manufacturing. After collection of peripheral blood mononuclear cells, dendritic cells will be manufactured from the monocyte fraction, aliquoted and frozen.
3. Patients will also undergo research biopsies prior to the start of treatment and 1-2 days prior to or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Patients who will have their definitive surgery **outside** a Baylor hospital will have a third research biopsy at least 1 week following the last chemotherapy dose and prior to surgery. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment.
4. LA TNBC patients will receive standard preoperative dose-dense AC (4 cycles) followed by TCb (4 cycles) chemotherapy, administered for 24 weeks. ER+/HER2– BC patients will receive standard preoperative dose-dense AC (4 cycles) followed by weekly T (12 cycles), administered for a total of 22 weeks. In both cohorts, chemotherapy will be combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 cells/mL), for a total of 4 time points prior to definitive surgery.
    a. During the AC cycles, both cohorts will receive vaccines administered on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-dense AC.

b. For TNBC patients, vaccines will be administered on any one individual day between Days 11-15 of Cycles 1 and 3 of TCb.

c. For ER+/HER2– patients, vaccines will be administered Day 1 during either Cycle 2 or Cycle 3 and on Day 1 during either Cycle 8 or Cycle 9 of T. Vaccine is to be administered after T infusion is completed in patients.

d. Standard pegfilgrastim support will be given for each AC treatment; however, no pegfilgrastim will be given during TCb or T cycles.

5. After preoperative treatment, patients will undergo definitive surgery, generally with mastectomy, and if available, fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer. Patients will be known to have axillary node positive disease at study entry based on biopsy or clinical criteria and will generally undergo level 1/2 axillary dissection at definitive surgery. However, patients may undergo SLN biopsy before or after chemotherapy at the physician's discretion.

6. After definitive surgery and during locoregional radiation therapy to the breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL (at 15 x 10^6 cells/mL), rotating injection sites in the dorsal or ventral surface of the upper arm, with antigen-loaded DCs. The timing of the boosters is the same for TNBC and ER+/HER2– cohorts. The first vaccination booster will occur once after the surgery and up to 3 days prior to radiation; the second booster will occur 30 days ± 3 days after radiation is completed; and the third booster will occur 90 days ± 3 days after the 2^{nd} boost.

7. ER+/HER2– BC patients will also receive standard endocrine therapy during locoregional radiation therapy, at the physician's discretion, and for at least 5 years thereafter.

8. TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (number of cycles per physician discretion). It will be the physician's discretion to either begin capecitabine treatment during radiation or after radiation is complete.

The treatment schema is shown in Table 1.

Table 1. Treatment schema

Agent	Dose*	Frequency of administration	Route of administration
Doxorubicin	60 mg/m^2	Cycle 1-4, Day 1, q14 days	IV
Cyclophosphamide	600 mg/m^2	Cycle 1-4, Day 1, q14 days	IV
Paclitaxel for **LA TNBC**	80 mg/m^2	Cycle 5-8, Days 1, 8, 15, q28 days	IV
Paclitaxel for **ER+/HER2– BC**	80 mg/m^2	Cycles 1-12, Day 1, q7 days	IV
Carboplatin	AUC=6	Cycle 5-8, Day 1, q28 days	IV
Capecitabine	1000 mg/m^2	Cycles 6-8[#], Days 1-14, q21 days	PO

***Note:** The treating physician may adjust chemotherapy doses based on individual patient histories to optimize dose delivery, per standard of care.
[#] cycle number per physician discretion.

7.1 CHEMOTHERAPY TREATMENT DELAY

If a treatment day variation is needed for reasons other than toxicity, an attempt should be made to keep the variation within the following parameters: **±4 calendar days.** Any delay within this window is NOT a deviation. **Note**: This delay window **does not** apply to Cycle 1.

Patients will continue with study therapy, at reduced chemotherapy doses at the physician's discretion, when treatment is resumed after a delay in treatment.

7.2 CHEMOTHERAPY DOSE MODIFICATION FOR TOXICITY

Standard of care dose reductions are to be made according to the system showing the greatest degree of toxicity. Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 as linked in Appendix I. Each chemotherapy treatment cycle will begin only when:

- ANC ≥1000
- Platelets ≥100,000
- Resolution of non-hematologic toxicities to ≤Grade 1 or baseline

Dose reductions will be at the physician's discretion. One dose reduction will be permitted for doxorubicin, cyclophosphamide, and paclitaxel. Two dose reductions are permitted for carboplatin. Capecitabine dose reductions are permitted per standard of care, drug package insert, or physician's standard practice. No dose reductions for DC vaccines will be permitted. Patients will resume treatment with the next planned cycle (provided improvement/resolution in toxicities) as outlined below. Dose reductions are permanent.

If doxorubicin, cyclophosphamide, paclitaxel, carboplatin, and/or capecitabine treatment is interrupted, DC vaccinations may still be administered following consultation with the study PI, Dr. O'Shaughnessy. A patient will be deemed to be off study treatment if the patient stops DC vaccinations, but will continue to be followed for clinical outcome.

The dose reduction levels doxorubicin, cyclophosphamide, paclitaxel, and carboplatin are shown in Table 2.

Table 2. Dose Reduction Levels

Level	Doxorubicin	Cyclophosphamide	Paclitaxel	Carboplatin
Starting Dose (Level 0)	60 mg/m^2	600 mg/m^2	80 mg/m^2	AUC=6
Level -1[a]	48 mg/m^2	480 mg/m^2	70 mg/m^2	AUC=5
Level -2[a]	N/A	N/A	N/A	AUC=4

[a] If patients cannot tolerate Level -1 (or Level -2 for carboplatin), there will be no further dose reduction; patients will be taken off chemotherapy but may continue with the vaccinations after the Treating Physician and study PI confer (per Section 9.4.9).

7.3 HEMATOLOGIC TOXICITY

Treatment decisions and any potential dose reductions on standard of care drug should be made based on ANC and platelet counts on the day of treatment administration.

- Patients must have ANC of ≥1000/mm^3 on Day 1 of each cycle (if pegfilgrastim or filgrastim is being used) or of ≥1200/mm^3 on Day 1 (if no pegfilgrastim is being used) to receive scheduled treatment. Treatment may be delayed to allow sufficient time for recovery.
- Patients must have a platelet count of ≥100,000/mm^2 on Day 1 of each cycle to receive scheduled chemotherapy treatment. Doxorubicin, cyclophosphamide, paclitaxel, and carboplatin should be delayed until platelet counts recover to ≥100,000/mm^2 and then treated with either full dose or reduced dose chemotherapy at the physician's discretion. Treatment may be delayed to allow sufficient time for recovery.

Use of hematopoietic growth factors to ameliorate hematologic toxicity is at the discretion of the physician investigator and should be in accordance with the American Society of Clinical Oncologists (ASCO) guidelines.

7.4 RADIATION THERAPY

All patients who undergo lumpectomy or who undergo mastectomy and have 4 or more positive axillary nodes must be treated with radiation therapy (RT) according to the institutional or practice standards. All decisions regarding post mastectomy RT in patients with 1 to 3 positive nodes, as well as the specific radiation portals are at the discretion of the Treating Physician. The following data must be captured on the CRF: sites of radiation (breast, lymph node beds, boost site(s)); dates of radiation and doses administered; and list of regional lymphatic beds that were included in the field.

ER+/HER2– BC patients will also receive standard endocrine therapy during locoregional radiation therapy, at the physician's discretion, for at least 5 years thereafter. The type of endocrine therapy, dosing, and duration must be captured on the CRF.

TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (number of cycles per physician discretion). It will be the physician's discretion to begin capecitabine treatment either during radiation or after radiation is complete.

8 SCHEDULE OF PROCEDURES AND ASSESSMENTS

The schedule of assessments for the trial is shown in Appendix II. If a required observation or procedure is missed, documentation is required in the source records, on the Protocol Deviation Form, and on the CRF, to explain the reason for this protocol deviation.

For scheduling purposes, Day 1 is the first day of chemotherapy administration. Return appointments must always be scheduled from Day 1 of the study. It is imperative that all visits occur within the specified windows.

8.1 SCREENING

Note: Assessments that are part of the standard of care of breast cancer patients and obtained within 4 weeks of the screening visit, are acceptable as part of the screening tests. Results of such tests will be acceptable even if obtained prior to the execution of the Inform Consent.

Prior to entry into the study, the following assessments will be performed to determine if patient is eligible to continue in the study as per section 4.2 and 4.3 describing the inclusion and exclusion criteria for the study.

1. A signed Patient Informed Consent Form and HIPAA Form must be obtained.
2. It has been confirmed that the patient meets **all** inclusion criteria and **none** of the exclusion criteria.
3. Assign patient screening number.
4. Obtain demographic information (date of birth, ethnicity, race and sex).
5. Within 4 weeks prior to enrollment obtain the following assessments:
    a) Complete medical history, including family history of breast and ovarian cancer, and collection of BRCA1/2 status, if applicable or known.
    b) Complete physical examination (including vital signs, height, and body weight)
    c) ECOG Performance Status Scale (Appendix III)
    d) Review and record of prior and concomitant medications
    e) Complete blood count (CBC) with differential and platelet count
    f) Complete metabolic profile (CMP) including: serum chemistries (creatinine, glucose, total protein, blood urea nitrogen [BUN], total carbon dioxide [CO_2], albumin, total bilirubin, alkaline phosphatase, and aspartate transaminase [AST] and alanine transaminase [ALT]) and electrolytes (total calcium, chloride, potassium, sodium)

g) Clinical assessment of the patient's locoregional disease (ie, by physical examination)
h) Radiological assessment of tumor stage (eg, chest and abdomen CT, radionuclide bone scan). A PET/CT scan is acceptable for the initial staging evaluation.

6. Females of childbearing potential must have a serum or urine pregnancy test performed within 7 calendar days prior to enrollment.
7. Baseline LVEF (left ventricular ejection fraction) determination must be obtained by echocardiogram **within 6 weeks before the first dose of study drugs** and must be ≥55%
8. Apheresis Processing Profile Test (Hepatitis B Core AB, Hepatitis B surface AG, Hepatitis C Virus AB, HIV Virus 1/2 AB, HTLV and syphilis).
9. Schedule appointment for apheresis procedure.
10. A toxicity assessment/assessment of adverse events, since the day the informed consent was signed.
11. Whole blood collection (40 mL) for immunomonitoring studies.

8.2 REGISTRATION PROCEDURES

The enrollment process begins when the Coordinator has obtained a signed informed consent.

Patients who continue to meet eligibility criteria at the screening visit, the study coordinator will complete a Patient Registration Form which will be e-mailed to the Project Manager at BIIR (contact details and forms will be provided at the study initiation visit) for sponsor representative review to approve registration into the study. The Project Manager will respond back to the site by email with the form advising approval from Sponsor Representative. The Sponsor Representative may also require additional information or clarifications prior to advising of his/her opinion.

Patients who fail to meet the entrance criteria as detailed in Section 4.2 and 4.3 are defined as "screen failures". For patients defined as "screen failure", the screening log will include the following information: protocol number, investigator, patient number (screening number), date of birth, gender, screen failure date and reason for failure.

A screening log will be maintained by research staff during the entire time of the study. The screening log will be kept in a binder for review for any reason (ie, monitoring/audit).

The PI may be allowed the opportunity to review and grant exceptions for minor deviations in eligibility, in order to maximize patient accrual without jeopardizing patient safety or scientific integrity of these studies. Examples might include minor deviations of baseline labs, timing of prior treatment or tests, etc. It is recognized that these questions arise frequently. The procedure to be followed is for the Investigator or his/her representative (ie, study coordinator) to e-mail the request to the PI and Project Manager at BIIR for an exception. The PI would then make a determination, which is binding. In no instance should this exception constitute a safety issue for the patient or a significant deviation from the scientific purpose of the study.

Treatment must begin within 20±7 working days (not counting the day of dosing) after the patient's registration on study.

8.3 APHERESIS PROCEDURES

The procedure used to collect monocytes during apheresis was developed by Gambro, Inc. Refer to the BIIR SOP Number VP 113.05 for the Gambro system of collection. The use of apheresis in the collection of human blood mononuclear cells (MNC) is commonplace. The procedure involves a standard venipuncture of an antecubital vein with return venipuncture in the opposite extremity and lasts approximately 3-4 hours (corresponding to 6-10L). Patients will have apheresis performed at Baylor University Medical Center (BUMC) Apheresis Collection Center and the apheresis bag will be transported to Baylor Institute for Immunology Research (BIIR) cGMP Facility for processing. If necessary, a central venous line will be placed for venous access in patients who have inaccessible antecubital veins.

The goal of the MNC collection is to collect a product that meets the following specifications:

- White blood cell (WBC) content - $\geq 5 \times 10^9$ to 30×10^9
- Monocyte content - $\geq 1 \times 10^9$
- Granulocyte content - <3%
- Red blood cell (RBC) content - **<7.5 Ml**

One blood draw will be performed at the time of apheresis:

1. Peripheral blood will be collected in Red Top (non-anticoagulated) tubes (60 mL) for serum to be used in vaccine manufacturing.

Failure of peripheral venous access during apheresis will not be considered a collection of cells, as, with the subject's consent, a central venous line can be placed and an apheresis completed. In the event that the apheresis is suboptimal, ie, that an inadequate monocyte collection is achieved or that the vaccine cannot be manufactured from the product collected, one additional apheresis will be allowed no less than 7-10 days after the first apheresis. Apheresis product **must** meet required specifications for vaccine to be manufactured. Manufactured vaccine **must** meet required specification to be released for injection.

Refer to the "Guidelines for the Collection of Mononuclear Cell (MNC) Products for the Elutra® Cell Separation System Monocyte Enrichment Protocol", SOP Number: VP 122.04.

8.4 PERIPHERAL BLOOD MONONUCLEAR CELL ISOLATION

After the mononuclear cells are collected from the patients, and received by the cGMP Facility at BIIR they will undergo, for further processing for generation of the DC vaccine. Specifically the monocytes will be separated from other mononuclear cells using a closed elutriation system ELUTRA (Gambro).

Refer to the "Apheresis Product Processing by Elutriation Using the Gambro Elutra™", SOP Number: VP 113.05.

8.5 PROCEDURES DURING STUDY DRUG TREATMENT

The following evaluations will be performed at the beginning of each cycle, unless otherwise specified:

NOTE: For ER+/HER2– patients during the T cycles, the following evaluations will be performed on Day 1 every 3 weeks (Day 1, Cycles 1, 4, 7, and 10).

There is a window (up to 4 calendar days prior to the scheduled time point) for assessments during the study. Assessments that are to be done on days when study drug is administered must be done prior to dosing as these assessments (CBC, CMP, assessment of response, PS, etc) may determine whether or not standard of care drug is administered, or if a dose reduction is necessary.

1. An abbreviated medical history on Day 1 of each cycle (unless otherwise indicated), to capture events that have occurred since the last cycle. Events that were not captured in the baseline complete medical history should be recorded on the AE page of the CRF.
2. A physical examination, including vital signs and body weight.
3. Assessment of PS on the ECOG scale (Appendix III).
4. Assessment of concomitant medications Day 1 of each cycle.
5. CBC with differential and platelet count
6. CMP
7. Assessment of LVEF by ECHO, as clinically indicated
8. Tumor response by clinical assessment of the patient's breast and axillary disease (ie, by physical examination) must be performed every 2 weeks during therapy. The largest diameter of the breast mass and axillary LN(s) will be assessed and recorded every 2 weeks during preoperative chemotherapy.
9. Research biopsies of the patient's breast tissue must be obtained prior to the first day of treatment and also 1-2 days prior or on Day 1 of Cycle 4 of AC for assessment of modulation of the immune microenvironment. Patients who will have their definitive surgery **outside** of a Baylor hospital will have a third research biopsy at least 1 week following the last chemotherapy dose and prior to surgery.
10. Immunomonitoring studies: Obtain approximately 30 mL of whole blood prior to all vaccinations; prior to surgery; and prior to radiation (see Section 8.10).
11. Breast cancer tissue will be collected for assessment of the immune microenvironment and for tumor whole exome sequencing **at time of definitive surgery** for patients who have their definitive surgery **within** a Baylor hospital.
12. A toxicity assessment/assessment of adverse events must be performed Day 1 of each cycle of chemotherapy and Day 1 of each vaccination.
13. Patient diary assessment

8.6 PROCEDURES FOR VACCINE TREATMENT VISITS

The following evaluations will be performed at the time of vaccine administration, unless otherwise specified:

1. Assessment of PS on the ECOG scale (Appendix III), only during visits prior to definitive surgery. No assessment of ECOG is required at vaccine visits post-surgery.
2. Assessment of concomitant medications

3. Assessment of LVEF by ECHO, as clinically indicated
4. A toxicity assessment/assessment of adverse events
5. Patient diary assessment
6. Immunomonitoring studies: Obtain approximately 30 mL of whole blood prior to all vaccinations.

8.7 PROCEDURES FOR END OF TREATMENT VISIT (EOT)

A final study visit (End of Treatment or Off Treatment Visit) will be completed for all patients after their last dose of the DC vaccine. The End of Treatment Visit will occur within 14 days ± 2 days after the patient has either:

- Completed all the treatment specified under this Study
- When patient is withdrawn from the study for whatever reason excepting those withdrawing consent. Patients who withdraw consent may not want any further assessment; however, they should be encouraged to have these final assessments done.

NOTE: End of treatment, or off treatment, is NOT considered off study or withdrawn from the study.

The following evaluations will be performed at this visit:

1. A medical history
2. A physical examination, including vital signs and body weight
3. Assessment of concomitant medications
4. CBC with differential and platelet count
5. CMP
6. Immunomonitoring studies: obtain approximately 40 mL of whole blood 2 weeks ± 3 days after the last DC vaccination (see Section 8.10).
7. Tumor clinical assessment of the patient's disease (ie, by physical examination)
8. Toxicity assessment/assessment of adverse events
9. Patient diary assessment

8.8 PROCEDURES FOR FOLLOW-UP VISITS

Patients will be followed every 3 months thereafter for 3 years following the last DC vaccination. The following evaluations will be performed at these visits:

1. An abbreviated medical history
2. A physical examination, including vital signs and body weight.
3. CBC with differential and platelet count.
4. CMP
5. A toxicity assessment/assessment of adverse events
6. Obtain approximately 30 mL of whole blood for immune response to vaccine at 6 months and 1 year after the last DC vaccination.

8.9 UNSCHEDULED VISITS

In special cases (ie, follow up on adverse events) as judged by the Investigator, an additional visit to those scheduled can be performed. This visit will be recorded in the patient's records and on the "Unscheduled visit" CRF pages.

If the patient is discontinued prematurely, refer to Section 8.7 for required assessments. The resulting data should be documented on the appropriate CRF pages. If, at an unscheduled visit, the investigator determines the patient may remain on study drug, the visit assessments should be documented on the Unscheduled Visit CRF pages.

NOTE: Assessments for an Unscheduled Visit are identical to Section 8.5.

8.10 BLOOD SAMPLES FOR IMMUNOMONITORING

Whole blood collection for immunomonitoring studies will be obtained as follows:

- 40 mL (4 CPT tubes and 2 tempus tubes) at baseline
- 30 mL prior to each treatment-vaccination cycle; prior to surgery and prior to radiation (3 CPT tubes and 2 tempus tubes).
- 40 mL (4 CPT tubes and 2 tempus tubes) 2 weeks ± 3 days after the last DC vaccination
- 30 mL at 6 months and 1 year after the last DC vaccination.

Some of the investigational procedures that could be conducted in the future on the blood and blood cell samples taken from patients who participate in this study will be done in laboratories that work closely with BIIR. Patients will be asked to give their permission for these blood samples to be used for future research projects. If the patient agrees that their blood samples can be shared, no information that identifies the patient will be given to any of the laboratories. Any material shared with any investigator within BIIR or other investigators outside of BIIR will be supplied with code number identifiers only, without the patient's name or other identifying information. Access to this database is limited to study investigators and project managers only and is safeguarded by a password protection system. Passwords are not shared. If research findings are published from this study the research patient will not be identified by name.

8.11 BIOPSIES

Research biopsies will be obtained at the following time points (See Appendix V for tissue collection and handling):

- A minimum of four*14-gauge needle biopsy cores will be collected at baseline, prior to the initiation of preoperative chemotherapy (2 cores frozen, and 2 cores in RNAlater®).
- A minimum of four* 14-gauge needle biopsy cores will be collected 1-2 days prior to or on Day 1 of Cycle 4 of AC (2 cores frozen, and 2 cores in RNAlater®).
- A minimum of four*14-gauge needle biopsy cores will be collected at least 1 week following the last chemotherapy dose and prior to surgery (2 cores frozen, and 2 cores in RNAlater®), for those patients who will have their definitive surgery **outside** a Baylor hospital.

- Fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and FFPE breast tissue from definitive surgery will be collected at the time of surgery and through the pathology department, respectively.

***Note:** if 4 research biopsy cores cannot be obtained due to limited tumor tissue, only 2 cores are essential for RNAlater®.

9 SAFETY EVALUATIONS

9.1 TOXICITY ASSESSMENT

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 as linked in Appendix I. This document can also be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page <http://ctep.cancer.gov>. Events must be documented on the AE page of the CRF.

Follow up for toxicity will be recorded the first 30 days following the last DC vaccination and any long-term toxicity will be followed for 3 years after completing study therapy.

Note: Patients who die or withdraw consent are considered withdrawn from the study and no further information will be collected.

9.2 LABORATORY DATA

For the treatment of the patient, laboratory data will be obtained according to the schedule of assessments. Only the laboratory data requested on the CRF need to be recorded on the appropriate laboratory CRF page. In addition, abnormal results that are associated with an AE will be documented on the Adverse Event page of the CRF if the laboratory abnormality fits the definition of an AE or can potentially result in an AE.

9.3 PATIENT DIARIES

All enrolled patients will be requested to complete patient diaries (see Appendix IV, Patient Diary) for review and collection of any adverse events during administration of vaccine.

All patients will be instructed to begin completing the diary the evening following administration of the DC vaccine and continue to record any new symptoms, whether or not they may be related to the vaccine, every evening for 1 week.

Patients will be instructed to return the completed diary to the clinic and give to the study coordinator at the next scheduled clinical visit. The study coordinator will record any adverse events documented in the diary in the Case Report Form.

9.4 ADVERSE EVENTS

9.4.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product during the course of a study and which does not necessarily have to have a causal relationship with this treatment. Adverse events may include

changes in laboratory parameters during the course of the study. Disease progression will not be considered an adverse event.

9.4.2 Definition of Serious Adverse Event

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- Results in death,

  (NOTE: Any death from any cause while a patient is receiving treatment on this protocol, or ≤ 30 days following the last dose of protocol treatment must be reported.)

- Is life-threatening,

  (NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient 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,

  (NOTE**:** Hospitalization is considered an overnight stay, therefore visits to hospital, without admission, are not considered serious unless they meet another criterion, (eg, medically significant). Planned surgery, or planned admission for study drug, is not considered an SAE unless, the hospitalization is prolonged or if another criterion is met.)

- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Additionally, important medical events that may not be immediately life threatening or result in death or hospitalization, but that may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

  Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

9.4.3 Definition of Unexpected Adverse Event

An adverse reaction is considered unexpected if it is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

9.4.4 Relationship to Study Drug

The following classifications should be used to assess the relationship between an adverse event and the study drugs:

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

- Probably related: There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.

- Possibly related: There is reasonable causal relationship between the investigational product and the AE. Dechallenge information is lacking or unclear.

- Definitely related: There is a reasonable causal relationship between the investigational product and the AE. The event responds to withdrawal of investigational product (dechallenge), and recurs with rechallenge when clinically feasible

9.4.5 Assessment of Severity

Adverse events are to be recorded on the AE page of the CRF. The severity (intensity) of AEs will be graded according to the following definitions:

- Mild: The patient experiences awareness of symptoms but these are easily tolerated or managed without specific treatment.

- Moderate: The patient experiences discomfort enough to cause interference with usual activity, and/or the condition requires specific treatment.

- Severe: The patient is incapacitated with inability to work or do usual activity, and/or the event requires significant treatment measures.

Changes in the severity of an AE should be documented to allow an assessment to be made of the duration of the event at each level of intensity. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

9.4.6 Monitoring for Adverse Events During the Clinical Trial

Patients will have baseline vital signs including temperature, pulse, respiration rate and blood pressure prior to each DC vaccination. Patients will receive 7 DC vaccinations intratumorally and/or subcutaneously. Patients will have vital signs taken approximately every 15 minutes for 1 hour post injection prior to patient's release. These patients will be followed at frequent intervals during the vaccination period and instructed prior to discharge to contact the study coordinator if unusual symptoms should develop at any time before the next scheduled office visit.

All AEs, regardless of severity, will be followed up by the Investigator until resolution is satisfactory. All AEs will be recorded for up to 30 days following the last study treatment.

All serious adverse events that occur while a patient is still enrolled in this study will be followed by study personnel until resolution or stabilization of the event regardless of the patient's study status.

9.4.7 Reporting of Adverse Events

Adverse events will be recorded for the duration of a patient's study treatment, and for up to 30 days following the last study treatment. All AEs, regardless of causal relationship are to be

recorded in the source documentation. The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. The diagnosis should be documented as the AE/SAE and not the individual signs /symptoms. The events, and the relationship of each event to treatment, will be assessed by the Treating Physician and recorded on the CRF. Additional information about each event, such as treatment required, eventual outcome, and whether or not therapy had to be interrupted or dosages reduced, will also be recorded on the CRF.

All Grade 2, 3 and 4 adverse events (AEs), Grades 1 and 2 alopecia, and all grades of neutropenia will be recorded in the CRF throughout the trial.

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 as linked in Appendix I. The event, and the relationship of each event to treatment, will be assessed by the Investigator and recorded on the CRF. Additional information about each event, such as treatment required, whether or not therapy had to be interrupted or dosages reduced, and eventual outcome will also be recorded on the CRF.

Pre-existing conditions will be recorded at baseline on the Medical History Form. If a pre-existing condition does not change, it does not have to be reported as an AE on subsequent cycles.

9.4.8 Reporting of Serious Adverse Events

All SAEs and unexpected adverse events will be reported per Baylor standard operating procedure (SOP) within 24 hours of becoming aware of the event. All SAEs will be reported by completing a SAE Report Form and emailing the form to the PI and BIIR Project Manager. Baylor IRB will be notified via iRIS.

All SAEs and unexpected adverse events must be documented on the Serious Adverse Event Report Form and on the adverse event CRF page. The event term used on the SAE report should match the term in the CRF. Follow-up information for SAEs, unexpected adverse events and information on non-serious AEs that become serious should also be reported to the as soon as it is available; follow up of a patient should be conducted until resolution of the adverse event.

The Sponsor (or delegate) is required to report all serious and unexpected adverse events associated with the use of Study Drug to FDA, in compliance with CFR 312.32. All SAEs that are unexpected and associated with use of the study cellular therapy are to be reported to MedWatch at http://www.fda.gov/medwatch/index.html. Form FDA 3500A will be completed, printed and submitted online at this URL. (Form FDA 3500 includes biologics (including human cells, tissues, and cellular and tissue-based products).

The Sponsor (or delegate) will notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening events associated with the use of the cellular therapy as soon as possible but no later than 7 calendar days after the event.

9.4.9 Removal Due to Adverse Events

Patients who experience any of the following vaccine-related symptoms or signs as outlined in NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (Appendix I) will be

removed from study treatment, but will be followed for outcomes and long-term toxicity, per protocol:

- Grade 2 or higher allergic reactions including bronchospasm or generalized urticaria
- Grade 3 or greater allergic toxicity
- Grade 2 or greater autoimmune toxicity
- Grade 2 allergic reactions related infusion
- Grade 3 or greater non-hematologic toxicity including site reactions.

NOTE: The treating physician may adjust chemotherapy doses based on individual patient histories to optimize dose delivery, per standard of care. However, if any patient requires more than one dose reduction of doxorubicin/cyclophosphamide (AC) **OR** the need for more than one dose reduction of paclitaxel or 2 dose reductions of carboplatin due to treatment delays for hematologic toxicity or for Grade 3 or 4 non-hematologic toxicity, they will be removed from the study treatment.

9.4.10 Removal of Patients with Disease Progression While on Study

Disease progression is **not** a criterion for removal from study. A patient may be withdrawn if in the opinion of their physician, continuation of DC infusion is not in their best interest, or if they require salvage treatment that may interfere with the response to DC infusion.

9.4.11 Pregnancy

Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study in such a manner that the risk of pregnancy is minimized.

Before study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and the potential risk factors for an unintentional pregnancy. The patient must sign an informed consent form documenting this discussion.

If pregnancy is confirmed in a patient during the course of the study, the patient must be withdrawn from the study. The pregnancy must immediately be recorded on the source documents and CRF and will be reported by completing the Pregnancy Notification Form and emailing the form to the PI and BIIR Project Manager. In addition, the Investigator must report to the Baylor IRB any follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants will be followed for a minimum of 8 weeks.

10 EFFICACY ASSESSMENTS

10.1 EVALUATION OF PATHOLOGIC RESPONSE

Pathologic response to therapy is a secondary endpoint of the study protocol. Patients will undergo surgical resection of residual breast and axillary malignant tissue after protocol-directed treatment. The pathologic specimen will be graded according to the tumor regression grading schema called the Residual Cancer Burden (RCB).[61] The following parameters are required from pathologic examination in order to calculate RCB after neoadjuvant treatment:

http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

1. The largest two dimensions (mms) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)
2. Submission of the entire largest cross-sectional area of the residual tumor bed for histologic mapping, with specific identification of those slides in the pathology report (eg, "the largest cross-sectional area of primary tumor bed was submitted in cassettes A5 - A9")
    - If the residual tumor is large (i.e. largest diameter > 5 cm), then at least 5 representative cassettes from the largest cross-sectional area are sufficient, but should be identified in the original pathology report (e.g. "representative sections from the largest cross-sectional area of primary tumor bed were submitted in cassettes A5 - A9")
3. Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following:
    - 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%
    - To assess cellularity it is helpful to scan across the sections of tumor bed and then estimate the average cellularity from the different microscopic fields.
    - When estimating percentage cancer cellularity in any microscopic field, compare the involved area with obvious standards, e.g. more or less than half, one quarter, one fifth, one tenth, one twentieth, etc.
    - Expect there to be variable cellularity within the cross section of any tumor bed, but estimate the overall cellularity from the average of the estimates in different microscopic fields of the tumor bed.
    - e.g. if cellularity in different fields of the tumor bed were estimated as 20%, 10%, 20%, 0%, 20%, 30%, then an average estimate of overall cellularity would be 20%.
4. Histologic estimate of the percentage of the carcinoma in the tumor bed that is in situ, select one of the following:
    - 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%
5. The number of positive (metastatic) lymph nodes
6. The largest diameter (mm) of the largest nodal metastasis

A pathologic complete response (pCR) is defined as **NO** pathologic evidence of invasive disease in the breast or axillary lymph nodes.

The presence or absence of a pCR will be assessed separately for the tumor and the lymph nodes. For patients who do not achieve a pCR, the size of the residual cancer in the tumor, on pathologic exam, will be documented in the as well as the number of positive lymph nodes. Patients will have their pathologic response scored using the RCB scale.

11 STATISTICAL CONSIDERATIONS

11.1 POPULATIONS FOR ANALYSIS

11.1.1 Analysis Populations

Intent-To-Treat (ITT) Population: Includes all patients registered on the study (eligible and ineligible). This population will be included in overall patient listings, in summary tables of patient demographics and baseline disease characteristics, and also in the list of treatment discontinuations after enrollment.

Evaluable Population: Includes all eligible patients who meet the protocol-specified efficacy analyses requirements and who have received at least 1 inoculation of DC vaccine therapy. This population will comprise those patients who will be assessed for pathologic response. Early discontinuation of treatment (in Cycles 1-2), secondary to toxicity, will be considered a treatment failure. If death occurs before the completion of 1 cycle, the patient will be reported as evaluable, early death, but deemed not evaluable for response.

Safety Population: Includes all patients (eligible and ineligible) who receive at least 1 inoculation of DC vaccine therapy. This safety population will also be used for the summaries and analysis of all safety parameters (drug exposure, tables of adverse events information, including serious adverse events, etc.). Adverse events that are unrelated to treatment and that occur >30 days after the administration of treatment will not be reported or analyzed.

11.1.2 Patient Characteristics

Patient characteristics including demographics and pretreatment characteristics, breast mass and axillary lymph node size, staging evaluation at baseline, medical history, and family history will be summarized. Descriptive statistics including sample size, mean, standard deviation, median, and minimum and maximum values will be presented for continuous variables. Frequency distributions will be presented for categorical variables.

11.1.3 Patient Disposition

Patient disposition including the number of patients enrolled, completed, and discontinued from the study will be summarized overall and by study site. The reasons for discontinuation will also be summarized, and patients who discontinued will be listed.

11.2 HYPOTHESIS AND ENDPOINTS

This is an exploratory and descriptive clinical trial in which the primary objective is to demonstrate the safety and feasibility of combining DC vaccines with preoperative chemotherapy.

The secondary objectives of this trial are to determine pathologic complete response rates; disease-free survival; to assess immune biomarkers of immunity (antigen-specific CD8+ T cell immunity and T_H2 T cells) in breast cancer biopsy specimens and blood samples in patients receiving DC vaccinations; and to assess the feasibility of immunizing LA TNBC or

ER+/HER2– BC patients with patient-specific tumor antigens.

11.3 SAMPLE SIZE

Twenty patients will be enrolled over 20 months.

Twenty patients provides 80% power to reject the null hypothesis that more than 20% of patients will develop toxicity with the combination that leads to treatment cessation, or delay in treatment of more than 4 weeks.

If a patient's apheresis product or manufactured vaccine is suboptimal after a second attempt at apheresis, those patients will be withdrawn from the study, and replaced.

11.4 STATISTICAL METHODS

Statistical analysis will be performed by Institute for Health Care Research and Improvement (IHCRI), an affiliate of Baylor Health Care System.

The administration of DC vaccine with preoperative chemotherapy will be considered safe if fewer than 5 of the 20 patients experience a toxicity that leads to a delay in their receiving chemotherapy on schedule of more than 4 weeks, or to cessation of therapy.

Patients' toxicity will be monitored real-time, cycle by cycle, and all grade 3-4 toxicities and any delays in treatment will be reviewed by the PI. The trial accrual will be put on hold for any incident of congestive heart failure (as none is expected) and if more than 5 patients experience a delay in receiving the planned chemotherapy on schedule due to toxicity, at any time in the accrual of the 20 patients. The conduct of the trial will be monitored by the Baylor Research Institute (BRI) quality assurance personnel.

Immunologic studies will explore immune responses in the peripheral blood and the primary breast cancer tissues. Peripheral blood lymphocytes at each pre- and post-vaccination time points will be analyzed by flow cytometry for immune phenotyping and T cell subtype quantification according to standardized protocols. Breast cancer tissue analyses from the baseline biopsy and from the residual tissue obtained at definitive surgery will include: qualitative assessment of immune cell subsets such as T effectors, Tregs, NK cells, dendritic cells, macrophage subsets, B cells and expression of immune checkpoint targets such as PD-1 and PD-L1, and iT_H2 cells. Blood and breast cancer tissue samples will be analyzed by transcriptional profiling for changes over time including in the BIIR-described transcriptional IL-1 signature. Findings will be correlated with clinical endpoints.

Statistical analysis of immunologic studies*:* Continuous variables will be summarized with means or medians and standard deviations. Dichotomous and categorical variables will be summarized using counts and proportions with exact 95% confidence intervals. These summaries will be computed for each patient both pre- and post- administration of each DC vaccination. Plots will be used to show the changes in immune response over time both for each individual. For each vaccination, comparisons in the pre- and 14-day post-vaccine responses will be compared using paired t-tests (or Wilcoxon signed rank tests, if appropriate) for continuous variables. McNemar's test will be used to identify significant changes in the percentage of individuals with a dichotomous characteristic pre- and post-vaccine. Associations between

immune parameters will be explored graphically (eg, scatter plots, box plots) and numerically (eg, correlations, $\chi 2$ tests). The relationships between the immune parameters and clinical outcomes (pCR rates and DFS) will be assessed using a variety of statistical techniques. Univariate and multivariate modeling will be used to quantify the associations between immune correlates and clinical outcomes. In the case of a time-to-event clinical outcome (ie, DFS), the Cox proportional hazards model will be used. For binary and continuous outcomes, logistic and linear regression will be used. Kaplan-Meier techniques will be used to quantify time-to- event outcomes (DFS) and Cox proportional hazards models will be used to assess risk factors and compare subgroups of interest.

Interim analysis - Safety*:* An interim safety analysis will be performed after 10 patients have been enrolled, and accrual will be held at that time. If 2 of 4 or >4 of 9 patients experience the following, then accrual will be put on hold:

- Any vaccine-related toxicity as outlined in Section 9.4.9
- More than one dose reduction in AC or paclitaxel, or 2 dose reductions for carboplatin for hematologic or nonhematologic toxicity, as per Section 9.4.9.

Constitutional symptoms that are expected to occur with vaccine therapies are not included in this interim safety analysis.

The team of specialists ("DC vaccine team") involved in this study comprises physicians, scientists, medical writers, trial coordinators, data specialists, regulatory experts, and laboratory staff. All safety and efficacy data as well as the biomarker data (when available) will be reviewed monthly at multidisciplinary trial oversight meetings, where all experts will be in attendance.

In the event the safety conditions above occur and accrual is put on hold, a discussion will be held with the DC vaccine team and the FDA to discuss the safety issues, strategies to reduce toxicity, and whether to lift the accrual hold. If major delayed toxicity is observed later during the trial, the study may also be suspended or terminated for safety concerns.

12 PROTOCOL AND DATA DEVELOPMENT

12.1 ETHICS

12.1.1 Institutional Review Board

This protocol will be implemented only after review and full approval of the protocol and the Patient Informed Consent Form has been obtained from a properly constituted IRB. This written approval must be dated and it must clearly identify the protocol, any amendments, the Patient Informed Consent Form, and any applicable recruiting materials and patient compensation programs approved.

During implementation of this protocol, the PI is required to send various documents to the IRB for review:

1. Changes to the current protocol.
2. All protocol amendments and Patient Informed Consent Form revisions.
3. Required progress reports.

Particular attention is drawn to the Food and Drug Administration (FDA) regulations regarding the IRB. The PI is responsible for the initial and continuing review and approval of the proposed clinical study in accordance with these regulations. At least once a year, the IRB will be asked to review and re-approve the tissue collection protocol; the request must be documented in writing. At the end of the trial, the PI will notify the IRB that the trial has been completed.

12.1.2 Modification of the Protocol

Any changes to this protocol that affect study objectives, study design, study procedures, patient population, or significant administrative procedures will require a formal amendment to the protocol. Any proposed protocol amendments must be sent in writing to the Baylor IRB. Prior to implementation, an amendment must be agreed upon by the Sponsor and PI and approved by the IRB.

General administrative changes to the protocol are minor corrections and/or clarifications that do not affect the manner in which the study is to be conducted. Such administrative changes will be agreed upon by the Sponsor and PI and will be documented in a memorandum. The applicable IRB will be notified of administrative changes according to applicable IRB guidelines.

12.1.3 Patient Informed Consent

The informed consent should meet the requirements of the latest version of the Declaration of Helsinki and any applicable regulations and guidelines. It must be approved by an institutional ethics committee/IRB.

Prior to entry into the trial and before any protocol-required procedures are performed, the Investigator must explain the nature of the trial, its intended purpose, and the implications of participation to potential patients or to their legal representatives. They will be told about the possible risks and benefits, and the possible adverse experiences. They will be informed that patients' participation is voluntary, and that they may withdraw consent to participate at any

time. They will also be informed that if patients choose not to participate in the trial, alternative treatments are available; such refusal will not prejudice further treatment of their disease. Potential patients or their legal representatives must be given the opportunity to ask questions about the trial protocol and the procedures involved.

Finally, each patient will be told that his or her records may be accessed by authorized personnel and other authorized individuals without violating the patient's confidentiality, to the extent permitted by the applicable laws and/or regulations. By signing the written Patient Informed Consent Form, the patient or his or her legal representative is authorizing such access. Following this explanation and prior to entry into the trial, the written, dated, and signed Patient Informed Consent Form must be obtained from each patient or his or her legal representative; a copy will be given to the person signing the form.

12.1.4 Confidentiality of Records

Case report forms, study reports and all other communications relating to this study will identify patients by initials and assigned patient numbers by Baylor Institute for Immunology Research. Access to all pertinent medical records to verify data recorded on the case report forms and to audit the data collection process will be allowed only to personnel who are directly involved with this aspects of the conduct of the study. All records will be kept in a secured location in the offices of the Investigator. The US Food and Drug Administration (FDA) may also request access to study records and source documents.

12.2 STUDY RECORDS

12.2.1 Documentation

A log of all patients evaluated for this protocol must be maintained. Patients excluded from admission will be provided with a clear explanation of the specific reasons why they have been excluded from the study. Patients who are included will be assigned a patient identification number.

For each patient treated with the study drug(s), the Research Coordinator is required to prepare and maintain case histories that include all observations and other data pertinent to the investigation. This will include all source documents needed to verify the accuracy of all observations and other data contained in the CRFs on each study patient.

The Investigator or his/her designee is required to retain the records related to the trial for a period of 2 years following the date a marketing application is approved for the indication being investigated. If no application is to be filed or if the application is not approved for such indication, the records must be retained until 2 years after the investigation is discontinued and the regulatory agencies are notified.

12.2.2 Case Report Form

Data will be entered at the site using the protocol CRF. The Investigator or his/her designee is responsible for recording all data relating to the trial on the CRFs. Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained.

Paper CRFs must be completed legibly in ink. Individual case report forms will be reviewed, signed and dated by the Investigator when patients complete the study, withdraw from treatment or are withdrawn from the study by the Investigator. Corrections to entries will be crossed out with a single line and the correct value written above. The corrected entry will then be initialed and dated by the person authorized to make corrections in the case report forms.

The Investigator must verify that all data entries on the CRFs are accurate and correct. CRFs must be completed **within 15 calendar days** of the end of each cycle and **within 15 calendar days** following completion of study therapy.

12.2.3 Source Documents

Source documents consist of, but not limited to, in-patient hospital charts, clinic notes, original test results, laboratory data, worksheets, drug accountability records, consent forms, etc. Source documents must be available for review and inspection during on-site monitoring of the study by the Sponsor agent and their designee(s), IRB, and/or appropriate regulatory authorities.

12.2.4 Electronic Databases

Databases containing patient demographics and other data pertinent to the main objectives of the study will be maintained by the study Sponsor and the data management team at Baylor University Medical Center. The databases will be password protected and will be available for authorized personnel only to view the data.

12.2.5 Databases Processing

Case report form data will be entered into a quality controlled database. Data will be reviewed and validated. Data clarifications will be requested of investigators or their designees, and the database will be edited appropriately. The database will be authorized for release when all data management quality control procedures are completed. Data entered in these databases will be the main source of clinical data to be reviewed by the Data Safety Monitoring Board.

13 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

13.1 MULTIDISCIPLINARY TRIAL OVERSIGHT MEETINGS

The team of specialists ("DC vaccine team") involved in this study is comprised of physicians, scientists, medical writers, trial coordinators, data specialists, regulatory experts, and laboratory staff. All safety and efficacy data as well as the biomarker data (when available) will be reviewed monthly at multidisciplinary trial oversight meetings, where all experts will be in attendance.

13.2 PROTOCOL INITIATION MEETING

All personnel involved in the implementation of this study protocol will attend a protocol initiation meeting prior to opening the study to enrollment. During this protocol initiation meeting, each person will be informed of his/her responsibilities for the duration of the study. The procedures for assuring that adequate and correct documentation of all study activity is maintained throughout the study will be reviewed. Case report forms and other pertinent study

materials will be examined as well as procedures for reporting serious adverse events and variations from protocol.

13.3 BIOSTATISTICAL SUPPORT

Consulting and monitoring of the study design and data analysis will be provided by statistical staff at the Institute for Health Care Research and Improvement (IHCRI), an affiliate of Baylor Health Care System. IHCRI has extensive experience in clinical trial design and conduction.

14 CLINICAL MONITORING PLAN

14.1 INTRODUCTION

Clinical monitoring for the site will be conducted an interdepartmental group entitled Baylor Research Institute (BRI) Research Quality Improvement hereinafter referred to as the sponsor's agent. A monitoring plan has been designed to ensure consistent and constant oversight of the clinical trial in accordance with the principles of the International Conference on Harmonization (ICH) Guidelines (E6), FDA "Guidelines for the Monitoring of Clinical Investigations", applicable SOPs at Baylor University Medical Center, applicable SOPs at each individual clinical site and applicable 21CFR regulatory requirements.

14.2 PROCESSES

14.2.1 General Guidelines

- Prior to implementation, the study must have the approval of the FDA and of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC).
- 100% of all patients entered in the study will be monitored.
- Each patient enrolled will be monitored for 100% of the elements.
- The first monitoring visit will be scheduled to comply with the monitoring plan upon the registration of the first patient and will continue as described in the monitoring plan until all data has been monitored for every patient.

14.2.2 Study Monitoring Visits

A representative from the sponsor's agent will contact the study site a minimum of 2 weeks in advance to schedule a monitoring visit. After a monitoring visit has been scheduled the date will be confirmed in writing by the sponsor's agent. It is anticipated that each monitoring visit will require approximately 2 days. A Monitoring Visit Report will be submitted to the Sponsor of the IND and a follow-up letter outlining observations and outstanding issues noted during the visit will be submitted to the investigator at each clinical site following the monitoring visit.

Components reviewed during study monitoring consist of, but are not limited to:

- Investigational product accountability
- Protocol deviations and non-compliance
- Informed Consent review

- Case Report Form (CRF) and source document verification
- Regulatory document/binder review
- Adverse Event/Serious Adverse Event reporting

14.2.2.1 Investigational product accountability

The principal investigator, co-investigator or designated staff member will maintain accurate records of the receipt, dispensing and use of all investigational products for this study using forms provided by BIIR. The log will be monitored against source documentation for the accuracy of investigational products receipt, use and dispensation.

14.2.2.2 Protocol deviations and non-compliance

The representative from the sponsor's agent will use Data Clarification forms to notify the site of all protocol deviations. All concerns observed during routine monitoring visits that relate to significant occurrences of non-compliance to protocol or other regulatory requirements will be addressed through a Clinical Trial Escalation procedure initiated by the sponsor's agent. All such concerns will be reported to the BIIR Project Manager overseeing monitoring of the project who will in turn notify the Sponsor Representative or a delegated representative at BIIR.

14.2.2.3 Informed consent review

Proper informed consent will be obtained from all participating patients in the clinical trial. During monitoring visits, each informed consent will be reviewed by the sponsor's agent to ensure that all consent forms have been properly signed and dated by each participating patient or their authorized representative, the person obtaining the informed consent and the principal investigator. A copy of the signed document will be given to the patient, an additional copy will be filed with the regulatory documents at each site and another copy will be maintained in the patient's medical record if this procedure is in conformance with the institution's policy.

14.2.2.4 CRF and source document verification

Records related to the clinical trial will be maintained in accordance with FDA CFR 312.62. Corrections will be made on the original document so as not to obscure an audit trail and will include the date of correction, initials of the individual making the correction, and the reason for the change.

Source documents to be reviewed at each clinical site include, but are not limited to:

- medical charts
- nursing notes
- medical consultation notes
- discharge summaries
- laboratory reports
- diagnostic reports
- clinical notes

14.2.2.5 Regulatory document/binder review

100% review of all essential regulatory documents will be performed at each site visit. Such documents include, but are not limited to:

- Study protocol
- Protocol clarifications/amendments
- Investigator annual reports
- Site signature/delegation of authority logs
- Informed consent forms
- IRB review and approval documentation
- IRB correspondence
- IRB membership list
- Laboratory certificate(s) of accreditation
- Laboratory Director(s) curriculum vitae
- Laboratory(s) reference ranges
- Safety reports
- Investigator(s) curriculum vitae and medical license
- Form FDA 1572
- Financial disclosure forms
- Patient screening/enrollment log(s)

Copies of all essential documents will be maintained in the offices of the regulatory specialist and updated on an ongoing basis.

14.2.2.6 Adverse event/serious adverse event reporting

The sponsor's agent will ensure that all requirements of the overseeing IRB and of the protocol have been met and documented appropriately by the clinical site. All serious adverse events will be monitored for appropriate reporting to the Sponsor Representative so that all serious adverse events may be reported to the FDA by the Sponsor Representative according to timelines outlined in 21CFR 312 subpart D.

15 REFERENCES

1. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women and operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol. 15:2483-2493, 1997.
2. Rastogi P, Anderson SJ, Bear HD, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol. 26:778-785, 2008.
3. Yoshioka T, Hosoda M, Yamamoto M, et al. Prognostic significance of pathologic complete response and Ki67 expression after neoadjuvant chemotherapy in breast cancer. Breast Cancer Epub ahead of print, 2013.
4. Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 26:1275-1281, 2008.
5. Von Minckwitz G, Blohmer JU, Costa S, et al. Neoadjuvant chemotherapy adapted by interim response improves overall survival of primary breast cancer patients – results of the GeparTrio trial. SABCS. S3-2 abstract, 2011.
6. Houssami N, Macaskill P, von Minckwitz G, et al. Meta-analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant chemotherapy. Eur J Cancer 48(18):3342-3354, 2012.
7. von Minckwitz G, Untch M, Blohmer J-U, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 30(15):1796-804, 2012.
8. Park KH, Gad E, Goodell V, et al. Insulin-like growth factor-binding protein-2 is a target for the immunomodulation of breast cancer. Cancer Res 68, 8400-8409, 2008.
9. Knutson KL, Schiffman K, Disis ML. Immunization with a HER-2/neu helper peptide vaccine generates HER-2/neu CD8 T-cell immunity in cancer patients. J Clin Invest 107, 477-484 2001.
10. Anderson KS. Tumor vaccines for breast cancer. Cancer Invest 27, 361-368, 2009.
11. Disis ML, Schiffman K. Cancer vaccines targeting the HER2/neu oncogenic protein. Semin Oncol 28, 12-20, 2001.
12. Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 31:860-867, 2013.
13. Finn OJ. Cancer vaccines: between the idea and the reality. Nat Rev Immunol 3, 630-641, 2003.
14. Townsend AR, Gotch FM, Davey J. Cytotoxic T cells recognize fragments of the influenza nucleoprotein. Cell 42, 457-467, 1985.

15. Boon T, Cerottini JC, Van den Eynde B, et al. Tumor antigens recognized by T lymphocytes. Annu Rev Immunol 12, 337-365, 1994.

16. Rosenberg SA. Cancer vaccines based on the identification of genes encoding cancer regression antigens. Immunol Today 18, 175-182, 1997.

17. Pardoll DM. Cancer vaccines. Nat Med 4, 525-531, 1998.

18. Gilboa E. The makings of a tumor rejection antigen. Immunity 11, 263-270, 1999.

19. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer 12, 265-277, 2012.

20. Schneider BP, Winer EP, Foulkes WD, et al. Triple-negative breast cancer: risk factors to potential targets. Clin Cancer Res 14, 8010-8018, 2008.

21. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 98, 10869-10874, 2001.

22. Kao H, Marto JA, Hoffmann TK, et al. Identification of cyclin B1 as a shared human epithelial tumor-associated antigen recognized by T cells. J Exp Med 194, 1313-1323, 2001.

23. Straver ME, Glas AM, Hannemann J, et al. The 70-gene signature as a response predictor for neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat 119:551-558, 2010.

24. Egloff AM, Vella LA, Finn OJ. Cyclin B1 and other cyclins as tumor antigens in immunosurveillance and immunotherapy of cancer. Cancer Res 66, 6-9, 2006.

25. Yu M, Zhan Q, Finn OJ. Immune recognition of cyclin B1 as a tumor antigen is a result of its overexpression in human tumors that is caused by non-functional p53. Mol Immunol 38, 981-987, 2002.

26. Suzuki H, Graziano DF, McKolanis J. et al. T cell-dependent antibody responses against aberrantly expressed cyclin B1 protein in patients with cancer and premalignant disease. Clin Cancer Res 11, 1521-1526, 2005.

27. Neidhardt-Berard EM, Berard F, Banchereau J, et al. Dendritic cells loaded with killed breast cancer cells induce differentiation of tumor-specific cytotoxic T lymphocytes. Breast Cancer Res 6, R322-328, 2004.

28. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. New Engl J Med 351:2817-2826, 2004.

29. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol 24(23):3726-3734, 2006.

30. Saito H, Dubsky P, Dantin C, et al. Cross-priming of cyclin B1, MUC-1 and survivin specific CD8+T cells by dendritic cells loaded with killed allogeneic breast cancer cells. Breast Cancer Res 8, R65, 2006.

31. Chapuis AG, Ragnarsson GB, Nguyen HN, et al. Transferred WT1-reactive CD8+ T cells can mediate antileukemic activity and persist in post-transplant patients. Sci Transl Med 5(174):174ra27, 2013.

32. Craig DW, O'Shaughnessy JA, Kiefer JA, et al. Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities. Mol Cancer Ther 12(1):104-16, 2013.

33. Qi XW, Zhang F, Yang XH, et al. High Wilms' tumor 1 mRNA expression correlates with basal-like and ERBB2 molecular subtypes and poor prognosis of breast cancer. Oncol Rep 28(4):1231-6, 2012.

34. Nasomyon T, Samphao S, Sangkhathat S, et al. Correlation of Wilms' tumor 1 isoforms with HER2 and ER-α and its oncogenic role in breast cancer. Anticancer Res 34(3):1333-1342, 2014.

35. Wang L and Wang Z-Y. The Wilms' tumor suppressor WT1 induces estrogen-independent growth and anti-estrogen insensitivity in ER-positive breast cancer MCF7 cells. Oncol Rep 23:1109-1117, 2010.

36. Kroemer G, Galluzzi L, Kepp O, et al. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 31:51-72, 2013.

37. Ma Y, Adjemian S, Mattarollo SR, et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. Immunity 38(4):729-41, 2013.

38. Michaud M, Martins I, Sukkurwala AQ, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 334(6062):1573-7, 2011.

39. Sandoval F, Terme M, Nizard M, et al. Mucosal imprinting of vaccine-induced CD8+ T cells is crucial to inhibit the growth of mucosal tumors. Sci Transl Med 5:72ra20, 2013.

40. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol 5, 296-306, 2005.

41. Early Breast Cancer Trialists' Collaborative Group. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. N Engl J Med. 319:1681-1692, 1988.

42. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365:1687-1717, 2005.

43. Citron ML, Berry DA, Cirrincione C, et al. Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: first report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741. J Clin Oncol 21:1431-1439, 2003.

44. Mamounas EP, Bryant J, Lembersky B, et al. Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: results from NSABP B-28. J Clin Oncol 23:3686-3696, 2005.

45. Berry DA, Cirrincione C, Henderson IC, et al. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA 295:1658-1667, 2006.

46. Carey LA, Dees EC, Sawyer L, et al. The triple-negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 13:2329, 2007.

47. Decatris MP, Sundar S, O'Byrne KJ. Platinum-based chemotherapy in metastatic breast cancer: current status. Cancer Treat Rev 30(1):53-81, 2004.

48. Belani CP, Kearns CM, Zuhowski EG, et al. Phase 1 trial, including pharmacokinetic and pharmacodynamic correlations, of combination paclitaxel and carboplatin in patients with metastatic non-small-cell lung cancer. J Clin Oncol 17(2):676-84, 1999.

49. Telli ML, Jensen KC, Kurian AW, et al. PrECOG 0105: Final efficacy results from a phase II study of gemcitabine (G) and carboplatin (C) plus iniparib (BSI-201) as neoadjuvant therapy for triple-negative (TN) and BRCA1/2 mutation-associated breast cancer. J Clin Oncol 31 Suppl. Abstract 1003, 2013.

50. Silver DP, Richardson AL, Eklund AC, et al. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. J Clin Oncol 28(7):1145-53, 2010.

51. Sikov WM, Berry DA, Perou CM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC on pathologic complete response rates in triple-negative breast cancer. San Antonio Breast Cancer Symposium. Abstract S5-01, 2013.

52. Xeloda package insert, 2015

53. Mackean M, Planting A, Twelves C, et al. Phase I and pharmacologic study of intermittent twice-daily oral therapy with capecitabine in patients with advanced and/or metastatic cancer. J Clin Oncol 16(9):2977-85, 1998.

54. O'Shaughnessy JA, Blum J, Moiseyenko V, et al. Rnadomized, open-label, phase II trial of oral capecitabine (Xeloda) vs. a reference arm of intravenous CMF (cyclophosphamide, methotrexate and 5-fluorouracil) as first-line therapy for advanced/metastatic breast cancer. Ann Oncol 12(9):1247-54, 2001.

55. Lee S-J, Toi M, Ohtani S, et al. A phase III trial of adjuvant capecitabine in breast cancer patients with HER2-negative pathological residual invasive disease after neoadjuvant chemotherapy (CREATE-X/JBCRG-04). SABCS 2015, Abstract S1-07.

56. Mattarollo SR, Loi S, Duret H, et al. Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. Cancer Res 71:4809-4820, 2011.

57. Yi M, Huo L, Koenig KB, et al. Estrogen receptor positivity: 10% or 1%? Cancer Research 72(24): Suppl 3, 2012.

58. Cytoxan package insert, 2005

59. Taxol package insert, 2011

60. Paraplatin package insert, 2010

61. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 25:4414-4422, 2007.

Appendix I NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0

Publish Date: May 28, 2009

COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE)
Version 4.0

As of May 28, 2009 (v4.03: June 14, 2010), NCI has edited version 4.0 of the Common Terminology Criteria for Adverse Events. These may be obtained at the following web link http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.

DO NOT USE CTC VERSION 3.0 TO GRADE TOXICITIES IN THIS STUDY!

Appendix II Schedule of Assessments

LA TNBC Patients: Category	Screening	Apheresis	Pre-Operative Chemotherapy Week 1 to Week 24[a]					Surgery Week 28 ± 1 Week	RT Week 32 ± 2 Weeks			EOT 14 days ± 2 days after last DC vaccine	2 weeks ± 3 days after last DC Vaccine	Follow Up (every 3 months for 3 years)
									Vaccine Booster					
			AC Cycle 1	AC Cycle 3	TCb Cycle 1	TCb Cycle 3	Each Cycle (AC 1-4 and TCb 1-4)		Up to 3 days prior to 1st RT	30 days ± 3 days after RT is completed	90 days ± 3 days after 2nd boost			
Day of Cycle	-28 to -1		9-12[b]	9-12[b]	11-15[b]	11-15[b]	1	PER PROTOCOL						
Informed Consent and HIPAA	X													
Inclusion/ Exclusion criteria	X													
Demographics	X													
Medical History[c, d]	X		X	X	X	X	X					X		X
Vital Signs, Weight and Height[e]	X		X	X	X	X	X					X		X
Physical Exam[f]	X		X	X	X	X	X[g]					X		X
ECOG	X		X	X	X	X	X							
Pregnancy Test for WCBP[h]	X													
Concomitant Medications	X		X	X	X	X	X		X	X	X	X		
CBC	X						X					X		X
CMP	X						X					X		X
Clinical Assessment	X						X					X		
Radiological Assessment	X													
Apheresis Profile Test	X													
Blood for Vaccine Manufacturing		60 mL												
LVEF[i, j]	X		X	X	X	X	X		X	X	X			
Blood Draw for Immunomonitoring[k]	40 mL		30 mL	30 mL	30 mL	30 mL			30 mL	30 mL	30 mL		40 mL	30 mL[k]
Research Biopsies	X[l]			X[l]				X[m]						
DC Vaccine			X	X	X	X			X	X	X			
Capecitabine for TNBC no pCR pts									X[n]	X[n]	X[n]			

Instruct Diary Completion/Assessment			X	X	X	X	X		X	X	X	X		
Adverse Events	X		X	X	X	X	X	X	X	X	X	X	X	X
Toxicity Assessment			X	X	X	X	X		X	X	X	X		X[o]

[a] TNBC patients will receive preoperative dose-dense doxorubicin/cyclophosphamide followed by paclitaxel and carboplatin (AC/TCb) chemotherapy for 24 weeks combined.
[b] During the AC cycles, both cohorts will receive vaccinations on **any one individual day** between Days 9-12 of Cycles 1 and 3 of dose-dense AC. For TNBC patients, vaccinations will be administered **on any one individual day** between Days 11-15 of Cycles 1 and 3 of TCb.
[c] Medical history should include family history of breast and ovarian cancer, and collection of BRCA1/2 status, if applicable or known.
[d] Only an abbreviated medical history will be obtained to capture events that have occurred since the last cycle. Events that were not captured in the baseline complete medical history should be recorded on the AE page of the CRF.
[e] Height will only be obtained at the screening visit.
[f] Only a limited physical assessment focusing on the affected breast, including vital signs and body weight, will be performed at vaccine treatment visits.
[g] Only an abbreviated physical exam will be performed
[h] Serum or urine pregnancy test will be performed for all women of childbearing potential within 7 days prior to enrollment. Negative result must be available prior to first dose of the study drugs.
[i] Left ventricular ejection fraction determination must be obtained by echocardiogram within 6 weeks before the first dose of study drugs and must be $\geq$55%.
[j] Assessment of LVEF as clinically indicated.
[k] For 40 mL blood draws, 4 CPT tubes and 2 tempus tubes will be used for collection. For 30 mL blood draws, 3 CPT tubes and 2 tempus tubes will be used for collection. For blood draws **occurring during follow-up**, 30 mL will only be collected at 6 months and 1 year after the last DC vaccination.
[l] Patients will undergo research biopsies of their breast cancer prior to the start of treatment and 1-2 days prior or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Core biopsies will be obtained to collect tissue for future whole exome sequencing and expression analysis.
[m] Patients who will have their definitive surgery **outside** a Baylor hospital will have a third research biopsy at least 1 week following the last chemotherapy dose and prior to surgery. If available, fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer.
[n] TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (number of cycles per physician discretion). It will be the physician's discretion to begin capecitabine treatment either during radiation or after radiation is complete.
[o] Follow up for toxicity will be recorded the first 30 days after last DC vaccine and any long-term toxicity will be followed for 3 years after completing study therapy.

Schedule of Assessments

ER+/HER2- Patients: **Category**	**Screening**	**Apheresis**	**Pre-Operative Chemotherapy Week 1 to Week 22[p]**					**Surgery Week 25 ± 1 Week**	**RT Week 29 ± 2 Weeks**			**EOT 14 days ± 2 days after last DC vaccine**	**2 weeks ± 3 days after last DC Vaccine**	**Follow Up (every 3 months for 3 years)**
			AC Cycle 1	**AC Cycle 3**	**T Cycle 2 or 3**	**T Cycle 8 or 9**	**Each Cycle (AC 1-4 and T 1, 4, 7, 10)**		**Vaccine Booster**					
									Up to 3 days prior to 1st RT	**30 days ± 3 days after RT is completed**	**90 days ± 3 days after 2nd boost**			
Day of Cycle	-28 to -1		9-12[q]	9-12[q]	1[q]	1[q]	1	PER PROTOCOL						
Informed Consent and HIPAA	X													
Inclusion/ Exclusion criteria	X													
Demographics	X													
Medical History[r, s]	X		X	X	X	X	X					X		X
Vital Signs, Weight and Height[t]	X		X	X	X	X	X					X		X
Physical Exam[u]	X		X	X	X	X	X[v]					X		X
ECOG	X		X	X	X	X	X							
Pregnancy Test for WCBP[w]	X													
Concomitant Medications	X		X	X	X	X	X		X	X	X	X		
CBC	X						X					X		X
CMP	X						X					X		X
Clinical Assessment	X						X					X		
Radiological Assessment	X													
Apheresis Profile Test	X													
Blood for Vaccine Manufacturing		60 mL												
LVEF[x, y]	X		X	X	X	X	X		X	X	X			
Blood Draw for Immunomonitoring[z]	40 mL		30 mL	30 mL	30 mL	30 mL			30 mL	30 mL	30 mL		40 mL	30 mL[z]
Research Biopsies	X[aa]			X[aa]				X[bb]						
DC Vaccine			X	X	X	X			X	X	X			
Endocrine Therapy[cc]									X	X	X	X	X	X
Instruct Diary			X	X	X	X	X		X	X	X	X		

Completion/Assessment														
Adverse Events	X		X	X	X	X	X	X	X	X	X	X	X	X
Toxicity Assessment[dd]			X	X	X	X	X		X	X	X	X		X

[p] Patients will receive preoperative dose-dense doxorubicin/cyclophosphamide followed by paclitaxel (AC/T) chemotherapy for 22 weeks combined.
[q] During the AC cycles, both cohorts will receive vaccinations on **any one individual day** between Days 9-12 of Cycles 1 and 3 of dose-dense AC. For ER+/HER2– patients, vaccines will be administered Day 1 during either Cycle 2 or Cycle 3 and on Day 1 during either Cycle 8 or Cycle 9 of T. Vaccine is to be administered after T infusion is completed in this cohort of patients.
[r] Medical history should include family history of breast and ovarian cancer, and collection of BRCA1/2 status, if applicable or known.
[s] Only an abbreviated medical history will be obtained to capture events that have occurred since the last cycle. Events that were not captured in the baseline complete medical history should be recorded on the AE page of the CRF.
[t] Height will only be obtained at the screening visit.
[u] Only a limited physical assessment focusing on the affected breast, including vital signs and body weight, will be performed at vaccine treatment visits.
[v] Only an abbreviated physical exam will be performed
[w] Serum or urine pregnancy test will be performed for all women of childbearing potential within 7 days prior to enrollment. Negative result must be available prior to first dose of the study drugs.
[x] Left Ventricular Ejection fraction determination must be obtained by echocardiogram within 6 weeks before the first dose of study drugs and must be $\geq$55%.
[y] Assessment of LVEF as clinically indicated.
[z] For 40 mL blood draws, 4 CPT tubes and 2 tempus tubes will be used for collection. For 30 mL blood draws, 3 CPT tubes and 2 tempus tubes will be used for collection. For blood draws **occurring during follow-up**, 30 mL will only be collected at 6 months and 1 year after the last DC vaccination.
[aa] Patients will undergo research biopsies of their breast cancer prior to the start of treatment and 1-2 days prior or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Core biopsies will be obtained to collect tissue for future whole exome sequencing and expression analysis.
[bb] Patients who will have their definitive surgery **outside** a Baylor hospital will have a third research biopsy at least 1 week following the last chemotherapy dose and prior to surgery. If available, fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer.
[cc] ER+/HER2– BC patients will also receive standard endocrine therapy during locoregional radiation therapy, at the physician's discretion, and for at least 5 years thereafter.
[dd] Follow up for toxicity will be recorded the first 30 days after last DC vaccine and any long-term toxicity will be followed for 3 years after completing study therapy.

Appendix III ECOG Performance Status Scale

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of light or sedentary nature—(eg, light housework or office work)
2	Ambulatory and capable of all self care, but unable to carry out any work activities; up and about > 50% of waking hours
3	Capable only of limited self-care, confined to bed or chair >50% of waking hours
4	Completely disabled; cannot carry out any self care; totally confined to bed chair
5	Dead

Source: Oken MM, Creech RH, Tormey DC, et al. Toxicity and Response Criteria of The Eastern Cooperative Oncology Group. *Am J Clin Oncol*. 1982; 5:649-655.

Appendix IV Patient Diary

BAYLOR INSTITUTE FOR IMMUNOLOGY RESEARCH

SUBJECT DIARY

Baylor IRB Project #013-154 "Pilot Safety Trial of Preoperative Chemotherapy Combined with Dendritic Cell Vaccine in Patients With Locally Advanced, Triple-Negative Breast Cancer or ER-Positive, HER2-Negative Breast Cancer"

Subject ID #XXX-XXX-XX-___-___

From the Day of Injection to 7 Days After Injection

Vaccination #_____

ID #: XXXXXX -01 - |__|__|__| _ |__|__|__|

Today's Date (MM/DD/YYYY): |__|__| / |__|__| / |__|__|__|__|

Symptom	Absent	Mild	Moderate	Severe
Pain at injection site	☐	☐	☐	☐
Itching at injecting site	☐	☐	☐	☐
Redness at injection site	☐	☐	☐	☐
Discoloration at injection site	☐	☐	☐	☐
Fluid filled blisters at injection site	☐	☐	☐	☐
Blood filled blisters at injection site	☐	☐	☐	☐
Hard swelling in skin surface at or close to injection site	☐	☐	☐	☐
Fever (If yes, record highest temperature today: _____ºF)	☐	☐	☐	☐
Chills	☐	☐	☐	☐
Malaise (feeling of general discomfort or uneasiness)	☐	☐	☐	☐
Fatigue (feeling of tiredness, exhaustion, or lack of energy)	☐	☐	☐	☐
General muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐
Diarrhea	☐	☐	☐	☐
Tenderness of any lymph nodes in armpits/groin	☐	☐	☐	☐
Enlargement of any lymph nodes in armpits/groin	☐	☐	☐	☐
If you have experienced any medical problems today other than those listed above, please describe below.				
		☐	☐	☐
		☐	☐	☐
		☐	☐	☐

List any medications you have taken today.		
1.	4.	7.
2.	5.	8.
3.	6.	9.

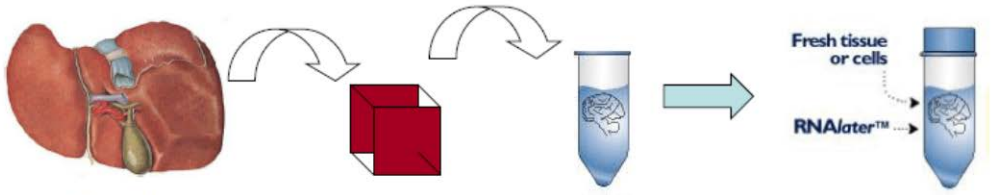
***The information you provide is very important and will remain confidential** If you feel any symptom you are experiencing is "severe", please contact the study nurse immediately at 214-820-XXXX*

Created: April 5, 2013

Appendix V Research Biopsies – Collection and Handling

RNA*later* ® example:

Tissue Collection for Gene Expression Analysis



Sample source

Sample size **<0.5cm** in every dimension

Place 2-3 pieces in vial containing 1-2ml RNA*later*

Cap vial and ensure tissue is fully submerged in the solution. Store at room temp. for 24hr.

Actual sample size: ■

After 24 hrs. use pipette tip to draw all the RNAlater solution out and leave the tissue pieces in the tube. Store frozen at -80°C.

MATERIALS REQUIRED (SPECIMEN KIT PROVIDED BY BIIR BPM CORE):

1. Specimen containers
2. Requisition Form
3. Labels
4. Specimen poly bag

LABELING AND REQUISITION:

All specimens should be clearly labeled BEFORE being sent to the BIIR BPM Core, to ensure correct identification of the patient and sample. Each specimen/container must be labeled with the labels from the KIT.

Specimen container(s) must be tightly sealed to ensure safe handling and quality patient results. Leaking specimen container(s) may result in poor fixation, illegible labeling and lost tissue samples.

All specimens must be submitted with a completed Biopsy Tissue Collection Form. Indicate the date, the time the specimen was removed from the patient and the time the specimen was placed in containers on the BPM Biopsy Tissue Collection Form where indicated.

SPECIMEN COLLECTION:

Obtain 4 to 5 core biopsies. Place 2-3 core biopsies into a non-breakable, leak proof, sterile container and the remaining 2 core biopsies into the containers containing RNA*later*®.

FRESH BIOPSY PREPARATION INSTRUCTIONS:

1. Place the biopsies on a piece of slightly moistened saline gauze. Care should be taken to keep the tissue from drying out. The tissue should NOT be immersed in saline.
2. Place the tissue on the gauze into the container tightening the screw cap (please use one container per specimen).
3. Label containers appropriately.
4. Specimens must be placed into a specimen polybag.
5. The completed Biopsy Tissue Collection Form must be placed in the separate pocket of the poly bag for this purpose (separated from the specimen container).

RNA*later*® BIOPSY PREPARATION INSTRUCTIONS:

1. Place the 2 -3 dissected tissue specimens (less than 0.5 cm in any one dimension) in a vial containing RNAlater®.
2. Cap the vial and ensure tissue is fully submerged in the solution.
3. Label containers appropriately.
4. Specimens must be placed into a specimen polybag.
5. The completed Biopsy Tissue Collection Form must be placed in the separate pocket of the poly bag for this purpose (separated from the specimen container).

DELIVERY INSTRUCTIONS:

1. Transport specimens at room temperature in a small Styrofoam container to BIIR BPM Core located at the Sammons Cancer Center, suite 600, within one hour of the procedure.

2. For inquiries contact:
    - BIIR BPM Core at 214-820-7834 or bpmcore@bswhealth.org
    - Luz Stella Muniz (Project Manager) at 214-820-7310 or luz.muniz@bswhealth.org

Appendix VI 013-154 Amendment 1 List of Changes

Protocol Section	Original Text	Revised Text	Rationale
Title Page	----------	Amendment 1, Date: January 31, 2014; IND 15765; Protocol 013-154; 3410 Worth Street, Suite 6224 Dallas, TX 75246	Updated protocol
Table of Contents, In-Text Tables, and Abbreviations	----------	Corresponding headings and pages have been changed to reflect modifications as a result of Amendment # 1.	Updated protocol
Study Synopsis – Summary	In the second group, IL-1 blockade with anakinra will be added to the treatment regimen.	In the second group, IL-1 blockade with anakinra will be added to the preoperative chemotherapy and DC vaccine.	Updated to match text in protocol.
Study Synopsis – Inclusion Criteria Section 4.2 – Inclusion Criteria	5. Adequate hematologic function, defined by: a. Absolute neutrophil count (ANC) >1500/mm^3 b. Platelet count ≥100,000/mm3 c.Hemoglobin >9 g/dL	5. Adequate hematologic function, defined by: a. Absolute neutrophil count (ANC) >1500/mm^3 b. Platelet count ≥100,000/mm3 c.Hemoglobin >9 g/dL (in the absence of red blood cell transfusion)	Clarification of inclusion criterion #5".
Study Synopsis – Exclusion Criteria; Section 4.3 – Exclusion Criteria	1. Evidence of metastatic disease on bone scan and CT scan of chest/abdomen (or PET CT scan).	1. Evidence of metastatic disease on bone scan and CT scan of chest/abdomen (or PET CT scan). Patients with intrathoracic metastatic adenopathy are	Clarification of exclusion criterion #1.

Protocol Section	Original Text	Revised Text	Rationale
		eligible.	
Study Synopsis – Medication and Doses Section 3 – Study Design (4th, 5th, and 6th paragraphs) Section 7 – Treatment Plan, #4	; the following 10 patients will receive DC vaccinations and anakinra 100 mg SC for 7 days, followed by 7 days off, then repeating during the 16 weeks of preoperative chemotherapy. … Patients will receive standard preoperative dose-dense doxorubicin/cyclophosphamide (4 cycles) followed by paclitaxel (4 cycles; AC/T) chemotherapy, administered every 2 weeks for 16 weeks combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.5 mL at 2 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 14 x 10^6 DCs) on Day 2 of Cycles 1 and 3 of dose-dense AC and on Day 3 of Cycles 1 and 3 of T (4 timepoints). Standard pegfilgrastim support will be given on Day 2 of each AC treatment. After preoperative treatment, patients will undergo definitive surgery, generally with mastectomy, and if available, the residual FFPE breast cancer tissue will be collected for	; the following 10 patients will be a staggered enrollment, and will receive DC vaccinations and anakinra 100 mg SC for 7 days, followed by 7 days off, then repeating during the 16 weeks of preoperative chemotherapy. … Patients will receive standard preoperative dose-dense doxorubicin/cyclophosphamide (4 cycles) followed by paclitaxel (4 cycles; AC/T) chemotherapy, administered every 2 weeks for 16 weeks combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 DCs) on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-dense AC and on any one individual day between Days 9-12 of Cycles 1 and 3 of T (4 timepoints). Standard pegfilgrastim support will be given for each AC treatment. After preoperative treatment, patients will undergo definitive surgery,	Modification of enrollment of patients, introducing a staggered enrollment in order to monitor the safety of the novel combination; brief description of enrollment. Modification to the vaccine specifications and timing of vaccine administration. Removal of pegfilgrastim administration specificity. Addition of fresh tissue collection at the time of definitive surgery, in addition to FFPE.

Protocol Section	Original Text	Revised Text	Rationale
	assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer. After definitive surgery and during locoregional radiation therapy to the breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL (at 14 x 10^6 cells/mL) in the ventral surface of the upper arm, with antigen-loaded DCs. The first vaccination booster will occur once after the surgery and prior to radiation; the second booster will occur one month after radiation is completed; and the third booster will occur 3 months after the 2nd boost.	generally with mastectomy, and if available, fresh tissue and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer. After definitive surgery and during locoregional radiation therapy to the breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL (at 15 x 10^6 cells/mL) in the ventral surface of the upper arm, with antigen-loaded DCs. The first vaccination booster will occur once after the surgery and up to 3 days prior to radiation; the second booster will occur 30 days ± 3 days after radiation is completed; and the third booster will occur 90 days ± 3 days after the 2nd boost.	
Section 3 – Study Design (1st paragraph) Section 6.1.2.3 – Dosing and Administration Section 7 – Treatment Plan, #1	Enrollment in Group 1 will complete before enrollment can begin in Group 2. Study procedures will be similar in both groups.	Enrollment in Group 1 will complete before enrollment can begin in Group 2. For Group 2, there will be a staggered enrollment, in order to observe the safety of AC/T	Modification of enrollment of patients, introducing a staggered enrollment in order to monitor the safety of the novel combination; extensive description of enrollment.

Protocol Section	Original Text	Revised Text	Rationale
		chemotherapy, DC vaccinations, and anakinra. After the first 3 patients have been enrolled in Group 2, enrollment will be held for observation of these patients for the 4 months of AC/T chemotherapy plus anakinra plus DC vaccine for adverse events, prior to enrolling a second set of 3 patients. Observation of these next 3 patients will occur over the 4 months of AC/T, anakinra, and DC vaccine for toxicity prior to completing enrollment of the last 4 patients in Group 2. Study procedures will be similar in both groups.	
Section 3 – Study Design Schema	(Figure)	(Figure)	Figure of study design schema was updated to reflect the changes in the protocol.
Section 4.1 – Sample Size Section 11.3 – Sample Size	Twenty patients with newly diagnosed locally advanced TNBC will be enrolled over 14 months (10 patients per group).	Twenty patients with newly diagnosed locally advanced TNBC will be enrolled over 20 months (10 patients per group).	Updated the time to accrual
Section 4.4 – Patient Withdrawal from the Study	12. Patients who experience any of the following vaccine-related symptoms or signs as outlined in NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (Appendix I) will be removed from study: • Grade II or higher allergic	12. Patients who experience any of the following treatment- or vaccine-related symptoms or signs as outlined in NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (Appendix I) will be removed from study treatment, but will be followed	Updated and clarified the criteria for withdrawing patients from study, to include study treatment in addition to vaccine therapy. In particular, decreased the autoimmune toxicity from ≥Grade 3 to ≥Grade 2, to increase safety.

Protocol Section	Original Text	Revised Text	Rationale
	reactions including bronchospasm or generalized urticaria • Grade III or greater allergic toxicity • Grade III or greater autoimmune toxicity • Grade II allergic reactions related infusion • Grade III or greater hematologic or non-hematologic toxicity including site reactions The date of and reason for discontinuation must be noted on the Case Report Form (CRF). Every effort should be made to complete the appropriate assessments. If the patient is withdrawn for any reason, the end of study assessments must be completed. After withdrawal from protocol, patients must be followed for adverse events (AEs) for 30 calendar days after their last dose of study drug.	for outcomes and long-term toxicity, per protocol: • Grade 2 or higher allergic reactions including bronchospasm or generalized urticaria • Grade 3 or greater allergic toxicity • Grade 2 or greater autoimmune toxicity • Grade 2 allergic reactions related infusion • Grade 3 or greater hematologic or non-hematologic toxicity including site reactions **NOTE:** If any patient requires more than one dose reduction of doxorubicin/cyclophosphamide (AC) **OR** the need for more than one dose reduction of paclitaxel due to treatment delays for hematologic toxicity or for Grade 3 or 4 non-hematologic toxicity, they will be removed from the study treatment. The date of and reason for discontinuation must be noted on the Case Report Form (CRF). Every effort should be made to complete the	

Protocol Section	Original Text	Revised Text	Rationale
		appropriate assessments. If the patient is withdrawn for any reason, the end of study assessments must be completed. Patients who withdraw from the study treatment due to intolerable toxicity will still be followed for outcome and toxicity, per protocol. Patients must still be followed for adverse events (AEs) for 30 calendar days after their last dose of study drug.	
Section 6.1.1.1 – Formulation and Supply	**Final Formulation.** The BIIR-BrcaVax-001 DC vaccine is prepared for injection into the patient by thawing the requisite number of frozen vials of DC vaccine and diluting the contents with USP injection grade sterile normal saline to wash the cells by centrifugation. The cells are washed 3 times with normal saline. Prior to the third wash a sample is taken to determine the cell count and viability. After the third wash the cells are divided and resuspended in normal saline at either $14x10^6$ viable cells/mL or $2x10^6$ viable cells/mL. The cell suspensions are drawn into separate syringes, that is, 1 mL at $14x10^6$ cells/mL for subcutaneous injection	**Final Formulation.** The BIIR-BrcaVax-001 DC vaccine is prepared for injection into the patient by thawing the requisite number of frozen vials of DC vaccine and diluting the contents with USP injection grade sterile Lactated Ringer's to wash the cells by centrifugation. The cells are washed 3 times with Lactated Ringer's. Prior to the third wash, a sample is taken to determine the cell count and viability. After the third wash, the cells are resuspended in Lactated Ringer's at 15×10^6 viable cells/mL. The cell suspensions are filled into a 2 mL sterile glass vaccine vial sealed with a serum stopper and metal cap, for delivery to the clinic. Therefore, the	Updated vaccine manufacturing procedure, for increased cell viability.

Protocol Section	Original Text	Revised Text	Rationale
	and 0.5 mL at $2x10^6$ cells/mL for intratumoral injection, for delivery to the clinic. Therefore, the final formulation is comprised of 100% normal saline. DC vaccines are supplied in single-use, preservative-free 1 mL syringes. Each subcutaneous injection will consist of 1 mL at $14x10^6$ cells/mL and each intratumoral injection will consist of 0.5 mL at $2x10^6$ cells/mL.	final formulation is comprised of DCs suspended in 100% Lactated Ringer's.	
Section 6.1.1.2 – Packaging and Labeling	The vaccine primary container, ie, the frozen glass vaccine vial and syringe containing the DC vaccine product for infusion into the patient, is labeled with the patient's name, vaccine batch number, vaccine product name, manufacture date, information on cell fill concentration, and the "For Investigational Use Only" warning statement. Before infusion the patient is asked to read aloud the name written on the syringe label containing the vaccine preparation and to confirm that it is his/her name. The Vaccine Product container that is delivered to the bedside is a BD 1 mL syringe for the intratumoral injection and/or a 3 mL syringe for the	The vaccine primary container, the glass vaccine vial, will be used to contain both the frozen DC vaccine product and the washed DC vaccine prepared for injection into the patient, and is labeled with the patient's name, vaccine batch number, vaccine product name, manufacture date, name of the manufacturer, volume in the vaccine vial dose (concentration of cells in the vaccine vial or syringe), storage temperature, and the warning statements: "For Autologous Use Only" , and "Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use". On the glass vaccine vial containing the DC vaccine that has been prepared for	Updated vaccine manufacturing procedure, for increased cell viability.

Protocol Section	Original Text	Revised Text	Rationale
	subcutaneous injection affixed with a syringe cap. The vaccine filled syringe(s) is/are placed in a clear biohazard plastic zip-lock bag. Upon delivery to the clinic, the clinician inspects the syringe(s) to determine that it holds the correct volume and that no leak of the Vaccine Product has occurred.	injection, a separate product expiration date and time label is attached. The expiration time is 2 hours after the washed, resuspended DC vaccine is been filled into the glass vaccine vial. The Vaccine Product container that is delivered to the bedside is a glass vaccine vial of 1.5 mL of prepared final Vaccine Product (15×10^6 viable cells/mL) to be drawn into a syringe for the intratumoral and/or subcutaneous injection. The vaccine-filled vial is placed in a clear biohazard plastic zip-lock bag and hard-foam shipping container for transport from the GMP facility to the clinic. Upon delivery to the clinic, the clinician inspects the vial to determine that it holds the correct Vaccine Product for the patient, dose and volume; and that no leak of the Vaccine Product has occurred. Before injection, the patient is asked to read aloud the name written on the glass vaccine vial label containing the vaccine preparation and to confirm that it is his/her name.	

Protocol Section	Original Text	Revised Text	Rationale
Section 6.1.1.3 – Storage	Vaccines will be kept in a locked area with limited access at BIIR. Vaccines will be kept frozen in liquid nitrogen (vapor phase) until use.	Vaccines will be kept in a locked area with limited access at BIIR. Vaccines will be kept frozen in liquid nitrogen (vapor phase) until use (removal of vials to prepare the Vaccine Product for inoculation).	Updated vaccine manufacturing procedure, for increased cell viability.
Section 6.1.1.4 – Preparation	The following table gives an example of the number of bags to be initiated for DC culture based on a different number of monocytes available for culture. ... After completing the incubation the antigen-loaded and activated DC are harvested from the cell culture bags, combined, washed by centrifugation with normal saline, and then suspended in cell freezing solution (consisting of 10% dimethyl sulfoxide, 80% heat-inactivated autologous serum and 10% Plasma-Lyte A with dextrose) at $30x10^6$ viable cells/mL for filling into glass vaccine vials. The vials containing 1 mL of the DC vaccine suspension are then frozen for storage in a liquid nitrogen tank (at -180°C in the liquid nitrogen vapor phase) prior to use. To prepare the inoculations for injection into the patient the required frozen DC vaccine vials are thawed,	An odd number of cell culture bags will be set up to initiate the culture of each DC vaccine batch. ... After completing the incubation the antigen-loaded and activated DC are harvested from the cell culture bags, combined, washed by centrifugation with Lactated Ringer's, and then suspended in cell freezing solution (consisting of 10% dimethyl sulfoxide, 80% heat-inactivated autologous serum and 10% Plasma-Lyte A with dextrose) at $30x10^6$ viable cells/mL for filling into glass vaccine vials. The vials containing 1 mL of the DC vaccine suspension are then frozen for storage in a liquid nitrogen tank (at -180°C in the liquid nitrogen vapor phase) prior to use. To prepare the inoculations for injection into the patient the required number of frozen DC vaccine vials are thawed, DMSO is washed out and the cell suspension is filled into a sterile	Corrected a misstatement of inclusion of a table. Updated vaccine manufacturing procedure, for increased cell viability.

Protocol Section	Original Text	Revised Text	Rationale
	DMSO is washed out and the cell suspension is drawn into syringes for transport to the clinic. Each DC vaccine vial and filled syringe is labeled with the following information: the patient's name, DC vaccine product name, DC vaccine batch number, storage conditions, manufacture date (date frozen), target fill volume and concentration, vial number, manufacturer's name, and the statement "Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use". All batches of frozen BIIR-BrcaVax-001 DC vaccine product remain in the custody of the Quality Assurance/Control Unit until they are released to the clinic or shipper. Extensive release testing of the frozen vaccine will include: a) Cell Count (Recovery) and Viability b) Evaluation of DC morphology by Giemsa staining of cytospun cells c) Evaluation of DC phenotype by multiparameter flow cytometry analysis d) Sterility testing	glass vaccine vial for transport to the clinic. Each DC vaccine vial containing frozen cells is labeled with the following information: the patient's name, DC vaccine product name, DC vaccine batch number, storage conditions, manufacture date (date frozen), volume in the vaccine vial or syringe, dose (concentration of cells in the vaccine vial or syringe), vial number (frozen vaccine vials), manufacturer's name, and the warning statements: "For Autologous Use Only" , and "Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use". The same label information is attached to the glass vaccine vial containing the Vaccine Product that is prepared for injection, along with a separate product expiration date and time label. All batches of frozen BIIR-BrcaVax-001 DC vaccine product and vaccine product prepared for injection remain in the custody of the Quality Assurance/Control Unit until they are released to the clinic or shipper. Extensive QC release testing of the frozen vaccine will include: a) Cell Count (Recovery) and	

Protocol Section	Original Text	Revised Text	Rationale
	e) Potency testing by phenotype.	Viability b) Evaluation of DC morphology by Giemsa staining of cytospun cells c) Evaluation of DC phenotype by multiparameter flow cytometry analysis d) Sterility testing (mycoplasma, gram stain, bacteria/fungus growth and endotoxin) e) Potency testing by phenotype and cytokine secretion. QC release testing of the washed DC vaccine for injection will include: a) Cell Count and Viability b) Sterility testing: gram stain and endotoxin (results available prior to injection) c) Sterility testing: bacterial and fungal growth (results available after injection)	
Section 6.1.1.5 – Vaccine Administration and Vaccine Schedule	At each scheduled vaccination during the preoperative phase, the patient will receive a total of 2 injections. Each vaccination will consist of: • One intratumoral injection of 0.5 mL ($2x10^6$ cells/mL) • One subcutaneous injection of 1 mL (14×10^6 cells/mL) in the ventral surface of the	At each scheduled vaccination during the preoperative phase, the patient will receive a total of 2 injections. Each vaccination will consist of: • One intratumoral injection of 0.2 mL (3×10^6 cells/mL) • One subcutaneous injection of 1 mL (15×10^6 cells/mL) in the ventral surface of the	Updated vaccine manufacturing procedure, for increased cell viability. Updated DC vaccination schedule figure to reflect changes in the protocol.

Protocol Section	Original Text	Revised Text	Rationale
	upper arm (ipsilateral). The cells are delivered along the length of the inoculation path as the cells are continuously injected as the needle is slowly withdrawn. ... After definitive surgery and during locoregional radiation therapy to breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL each (14 x 10^6 cells/mL) in the ventral surface of the upper arm (contralateral). ... (Figure)	upper arm (ipsilateral). ... After definitive surgery and during locoregional radiation therapy to breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL each (15 x 10^6 cells/mL) in the ventral surface of the upper arm (contralateral). ... To reconcile the DC vaccine use and disposal, the procedure will be to ship the vial of 1.5 mL of prepared final Vaccine Product to the clinic and following vaccination of the patient, the unused portion of the Vaccine Product will be returned to the GMP QC laboratory for reconciliation and testing. (Figure)	
Section 6.1.1.6 – Increase or Reduction in Dose of the Dendritic Cell (DC) Vaccine	An increase or reduction in the dose of the dendritic cell (DC) vaccine is not permitted during the study.	An increase or reduction in the target dose of the dendritic cell (DC) vaccine is not permitted during the study.	Clarification of the statement regarding the dose of vaccine.
Section 6.1.1.8 – Vaccine Order Preparation and Shipment	Vaccine order preparation and shipment will follow SOP VP115.04.	Vaccine shipment will follow SOP VP132 “Formulated Liquid DC Vaccine Shipment at Ambient Temperature”.	Updated vaccine manufacturing procedure, for increased cell viability.

Protocol Section	Original Text	Revised Text	Rationale
Section 6.3 – Accountability procedures	----------	For the purpose of accountability, all unused Anakinra and package inserts from used Anakinra, which was dispensed to the patient, should be returned to the clinic at each visit.	Added an accountability procedure for anakinra.
Section 7.2 – Chemotherapy Dose Modification for Toxicity	If doxorubicin, cyclophosphamide, and paclitaxel are withheld for any reason, anakinra and DC vaccinations may still be administered at the discretion of the Treating Physician and in consultation with the study PI, Dr. O'Shaughnessy. Patients may remain on-treatment regardless of which study drug is discontinued. A patient will be deemed to be off study treatment if the patient stops DC vaccinations, but will continue to be followed for clinical outcome.	If doxorubicin, cyclophosphamide, and paclitaxel treatment is interrupted, anakinra and DC vaccinations may still be administered following consultation with the study PI, Dr. O'Shaughnessy. A patient will be deemed to be off study treatment if the patient stops DC vaccinations, but will continue to be followed for clinical outcome.	Clarification on treatment interruption with regard to vaccine administration.
Section 8.3 – Apheresis Procedures Section 11.3 – Sample Size	The procedure involves a standard venipuncture of an antecubital vein with return venipuncture in the opposite extremity and lasts approximately 2-3 hours. ... In the event that the apheresis is sub-optimal, ie, that an inadequate monocyte collection is achieved or that the vaccine cannot be manufactured from the product collected, one additional apheresis	The procedure involves a standard venipuncture of an antecubital vein with return venipuncture in the opposite extremity and lasts approximately 3-4 hours (corresponding to 6-10L). ... One blood draw will be performed at the time of apheresis: 2. Peripheral blood will be collected in Red Top (non-anticoagulated) tubes (60 mL) for serum to be	Updated heading to include "Procedures". Addition of an assessment to be performed at the time of apheresis, and clarified the procedure for a suboptimal apheresis, and addressed whether a patient will be replaced or not.

Protocol Section	Original Text	Revised Text	Rationale
	will be allowed.	used in vaccine manufacturing. In the event that the apheresis is suboptimal, ie, that an inadequate monocyte collection is achieved or that the vaccine cannot be manufactured from the product collected, one additional apheresis will be allowed at least 1 week later. Apheresis product **must** meet required specifications for vaccine to be manufactured. Manufactured vaccine **must** meet required specification to be released for injection. **NOTE:** If a patient's apheresis product or manufactured vaccine is suboptimal after a second attempt at apheresis, those patients will be withdrawn from the study, and replaced.	
Section 8.5 – Procedures During Study Drug Treatment	Any delay within this window is NOT a deviation. 10. Breast cancer tissue for formalin embedding will be collected for assessment of the immune microenvironment and for tumor whole exome sequencing **at time of definitive surgery**.	10. Breast cancer tissue will be collected for assessment of the immune microenvironment and for tumor whole exome sequencing **at time of definitive surgery**.	Deleted sentence for clarity. Deleted formalin embedding from tissue collection to allow for flexible preservation methods.

Protocol Section	Original Text	Revised Text	Rationale
Section 8.6 – Procedures for Vaccine Treatment Visits	7. CBC with differential and platelet count 8. CMP	----------	Deleted unnecessary assessments.
Section 8.9 – Unscheduled Visits	In special cases (ie, suspicion of disease progression or follow up on adverse events) as judged by the Investigator, an additional visit to those scheduled can be performed. If a patient experiences a worsening in disease activity and it is the investigator's assessment that the patient's condition has worsened from baseline, then the patient may be terminated from the study. If the patient is discontinued prematurely, refer to Section 8.4 for required assessments.	In special cases (ie, follow up on adverse events) as judged by the Investigator, an additional visit to those scheduled can be performed. If the patient is discontinued prematurely, refer to Section 8.7 for required assessments. **NOTE:** Assessments for an Unscheduled Visit are identical to Section 8.5.	Clarification of assessments for an unscheduled visit.
Section 8.10 – Blood Samples for Immunomonitoring	Whole blood collection for immunomonitoring studies will be obtained as follows: • 100 mL at baseline • Approximately 30 mL prior each treatment-vaccination cycle; prior to surgery and prior to radiation. • 100 mL 2 weeks after the last DC vaccination	Whole blood collection for immunomonitoring studies will be obtained as follows: • 100 mL (11 ACD tubes and 2 tempus tubes) at baseline • Approximately 30 mL prior each treatment-vaccination cycle; prior to surgery and prior to radiation (3 ACD tubes and 2 tempus tubes). • 100 mL (11 ACD tubes and 2 tempus tubes) 2 weeks after the last DC vaccination	Added a description of the blood collection tubes to be used.

Protocol Section	Original Text	Revised Text	Rationale
Section 8.11 – Biopsies	• FFPE breast tissue from definitive surgery will be collected through the pathology department.	• Fresh tissue and FFPE breast tissue from definitive surgery will be collected at the time of surgery and through the pathology department.	Addition of fresh tissue collection at the time of definitive surgery, in addition to FFPE.
Section 9.4.9 – Removal Due to Adverse Events	Patients who experience any of the following vaccine-related symptoms or signs as outlined in NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (Appendix I) will be removed from study: • Grade II or higher allergic reactions including bronchospasm or generalized urticaria • Grade III or greater allergic toxicity • Grade III or greater autoimmune toxicity • Grade II allergic reactions related infusion • Grade III or greater hematologic or non-hematologic toxicity including site reactions.	Patients who experience any of the following vaccine-related symptoms or signs as outlined in NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (Appendix I) will be removed from study treatment, but will be followed for outcomes and long-term toxicity, per protocol: • Grade 2 or higher allergic reactions including bronchospasm or generalized urticaria • Grade 3 or greater allergic toxicity • Grade 2 or greater autoimmune toxicity • Grade 2 allergic reactions related infusion • Grade 3 or greater non-hematologic toxicity including site reactions. **NOTE:** If any patient requires more than one dose reduction of doxorubicin/cyclophosphamide (AC) **OR** the need for more than one dose	Updated and clarified the criteria for withdrawing patients from study, to include study treatment in addition to vaccine therapy. In particular, decreased the autoimmune toxicity grade from ≥Grade 3 to ≥Grade 2, to increase safety.

Protocol Section	Original Text	Revised Text	Rationale
		reduction of paclitaxel due to treatment delays for hematologic toxicity or for Grade 3 or 4 non-hematologic toxicity, they will be removed from the study treatment.	
Section 11.4 – Statistical Methods (Interim Safety Analysis)	An interim safety analysis will be performed after 4 and 9 patients have completed 4 vaccines. Accrual will be put on hold if 2 of 4 or >4 of 9 patients experience the following: • any vaccine related Grade 4 organ or injection site toxicity • a Grade 3 toxicity (other than autoimmune toxicity) that does not resolve to Grade 1 or less within 2 weeks Constitutional symptoms that are expected to occur with vaccine therapies are not included in this interim safety analysis. In the event safety conditions above occur, a discussion will be held with the DSMB and the FDA to map out further vaccine development strategies. Otherwise, enrollment will continue. If major delayed toxicity are observed later during the trial, the study may also be suspended or terminated for safety concerns.	An interim safety analysis will be performed after 4 and 9 patients have completed 4 vaccines in both Groups 1 and 2. Accrual will be put on hold if 2 of 4 or >4 of 9 patients experience the following: • Any vaccine-related toxicity as outlined in Section 9.4.9 • More than one dose reduction in AC or paclitaxel for hematologic or nonhematologic toxicity, as per Section 9.4.9. Constitutional symptoms that are expected to occur with vaccine therapies are not included in this interim safety analysis. A separate interim safety analysis will be performed for Group 1 and Group 2 separately, and the incidence of AEs in both groups will be compared. In the event the safety conditions above occur and accrual is put on hold, a discussion will be held with the DSMB and the FDA to discuss the	Modifications to perform an interim safety analysis per group rather than overall. Included additional safety measures to include treatment toxicity, and procedures for accrual hold.

Protocol Section	Original Text	Revised Text	Rationale
		safety issues, strategies to reduce toxicity, and whether to lift the accrual hold. If major delayed toxicity is observed later during the trial, the study may also be suspended or terminated for safety concerns.	
References	----------	Yi M, et al.	Addition of reference to support inclusion criteria #2 in the protocol.
Appendix II – Schedule of Assessments	----------	Addition of Apheresis blood collection assessment, 60 mL Addition to footnote C and O: Events that were not captured in the baseline complete medical history should be recorded on the AE page of the CRF. New footnote I and U, for Blood Draw for Immunomonitoring: For 100 mL blood draws, 11 ACD tubes and 2 tempus tubes will be used for collection. For 30 mL blood draws, 3 ACD tubes and 2 tempus tubes will be used for collection. Addition to footnote K and W: Fresh tissue and....	Language updated to match protocol.
Appendices IV – Mononuclear Cell Collection, and V – Gambro Elutra Apheresis	All of appendices IV and V and references therein	----------	Deleted 2 SOPs that were originally included in the protocol, as policies and procedures are not usually included in protocols, since manufacturing policies and procedures are held confidential and

Protocol Section	Original Text	Revised Text	Rationale
			proprietary by the research organization.
Appendix VI	----------	All of Appendix VI	Added the Amendment 1 list of changes as an appendix to the protocol, in order to identify changes from version to version of the protocol quickly.

Minor grammatical errors or misspellings were corrected as needed.

Appendix VII 013-154 Amendment 2 List of Changes

Protocol Section	Original Text	Revised Text	Rationale
Title Page	----------	Amendment 2, Date: June 30, 2014	Updated protocol
Table of Contents, In-Text Tables, and Abbreviations	----------	Corresponding headings and pages have been changed to reflect modifications as a result of Amendment # 2.	Updated protocol
Study Synopsis – Inclusion Criteria Section 4.2 – Inclusion Criteria	2. Have locally advanced TNBC with T3-T4 disease, and positive lymph nodes (radiologically or histologically positive), defined as invasive ductal cancers, ER- tumors with <10% of tumor nuclei immunoreactive 9. Eligible for treatment with paclitaxel, doxorubicin, and cyclophosphamide.	2. Have: a. locally advanced TNBC defined as invasive ductal cancer b. ER- tumors with <10% of tumor nuclei immunoreactive c. PR- tumors with <10% of tumor nuclei immunoreactive d. T3 or T4 disease, regardless of nodal status; T2 disease is eligible if there are positive lymph nodes present by physical exam or imagine evaluation or histological evaluation. 9. Eligible for treatment with paclitaxel, doxorubicin, cyclophosphamide, and carboplatin.	Expanded inclusion criteria to include patients with T2 disease, in order to increase accrual to the study. Also amended inclusion criteria to include the addition of carboplatin to the treatment regimen.

Protocol Section	Original Text	Revised Text	Rationale
Study Synopsis – Medication and Doses Section 3 – Study Design Section 6.1.1.5 – Vaccine Administration and Vaccine Schedule Section 7 – Treatment Plan Appendix II – Schedule of Assessments Throughout protocol	... 16 weeks of standard preoperative dose-dense doxorubicin/cyclophosphamide followed by paclitaxel chemotherapy; the following 10 patients will be a staggered enrollment, and will receive DC vaccinations and anakinra 100 mg SC for 7 days, followed by 7 days off, then repeating during the 16 weeks of preoperative chemotherapy. Four to 5 core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment. Patients will receive standard preoperative dose-dense doxorubicin/cyclophosphamide (4 cycles) followed by paclitaxel (4 cycles; AC/T) chemotherapy, administered every 2 weeks for 16 weeks combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 DCs) on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-dense AC and on any one individual	...24 weeks of standard preoperative dose-dense doxorubicin/cyclophosphamide (AC) followed by paclitaxel and carboplatin (TCb) chemotherapy; the following 10 patients will be a staggered enrollment, and will receive DC vaccinations and anakinra 100 mg SC for 7 days, followed by 7 days off, then repeating during the 24 weeks of preoperative chemotherapy. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment. Patients will receive standard preoperative dose-dense doxorubicin/cyclophosphamide (4 cycles; AC) followed by paclitaxel and carboplatin (4 cycles; TCb) chemotherapy, administered for 24 weeks combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 DCs) on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-	Addition of carboplatin to the treatment regimen. Changes are throughout the protocol. Modifications to the treatment plan are summarized: • Carboplatin added during Taxol ("TCb") • 24 weeks of preoperative chemotherapy • Vaccine during one individual day, Days 11-15 of TCb • No pegfilgrastim will be given during TCb cycles. • Research biopsy prior to or on Day 1 of Cycle 4 of AC.

Protocol Section	Original Text	Revised Text	Rationale
	day between Days 9-12 of Cycles 1 and 3 of T (4 timepoints). Standard pegfilgrastim support will be given for each AC treatment.	dense AC and on any one individual day between Days 11-15 of Cycles 1 and 3 of TCb (4 timepoints). Standard pegfilgrastim support will be given for each AC treatment; however, no pegfilgrastim will be given during TCb cycles. Patients will undergo research biopsies of their breast cancer prior to the start of treatment and 1-2 days prior to or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment.	
Section 1.8 – Addition of Carboplatin to Neoadjuvant Chemotherapy for Breast Cancer	----------	Carboplatin is approved for the treatment of ovarian cancer and small cell lung carcinoma, and is commonly used for the treatment of non-small cell lung cancer (NSCLC), head and neck cancer, and other tumors. In breast cancer, administration of carboplatin to previously untreated patients with metastatic disease results in response rates of 20% to 50%.[48] Paclitaxel in combination with	Addition of background on carboplatin, due to the addition of carboplatin to the treatment regimen.

Protocol Section	Original Text	Revised Text	Rationale
		carboplatin is also highly active in breast cancer, with response rates of approximately 39% to 62% for first-line treatment of metastatic disease.[49] Notably, data suggest that the administration of carboplatin in combination with paclitaxel results in less thrombocytopenia than is expected from the use of carboplatin alone.[48] Emerging data suggests that TNBC tumors may be more sensitive to DNA damaging agents, including carboplatin. Two small single arm trials with cisplatin combination neoadjuvant therapy or neoadjuvant monotherapy for patients with TNBC have reported complete tumor regressions or minimal residual disease at the time of surgery in 21% and 44% of patients.[50,51] The CALGB study 40603 evaluated the efficacy of neoadjuvant paclitaxel with or without carboplatin and/or bevacizumab followed by AC in patients with TNBC. The addition of carboplatin to the standard neoadjuvant chemotherapy regimen improved pathologic complete	

Protocol Section	Original Text	Revised Text	Rationale
		response in the breast in patients to 60% vs. 46% of patients who did not receive carboplatin, concluding that the addition of carboplatin was a reasonable addition to the standard neoadjuvant chemotherapy for TNBC.[52]	
Section 3 – Study Design	Blood samples for immunomonitoring studies will be obtained at baseline, prior to each DC vaccination, prior to surgery, prior to radiation, and 2 weeks after the last DC vaccination.	Blood samples for immunomonitoring studies will be obtained at baseline, prior to each DC vaccination, prior to surgery, prior to radiation, 2 weeks after the last DC vaccination, and at each follow up appointment.	Added research blood draws during the follow up portion of the study (was previously written into the protocol, but missing in this paragraph).
Section 3 – Study Design Schema	(Figure)	(Figure)	Figure of study design schema was updated to reflect the changes in the protocol.
Section 4.4 – Patient Withdrawal from Study Section 9.4.9 – Removal Due to Adverse Events	**NOTE:** If any patient requires more than one dose reduction of doxorubicin/cyclophosphamide (AC) **OR** the need for more than one dose reduction of paclitaxel due to treatment delays for hematologic toxicity or for Grade 3 or 4 non-hematologic toxicity, they will be removed from the study treatment.	**NOTE:** If any patient requires more than one dose reduction of doxorubicin/cyclophosphamide (AC) **OR** the need for more than one dose reduction of paclitaxel or 2 dose reductions of carboplatin due to treatment delays for hematologic toxicity or for Grade 3 or 4 non-hematologic toxicity, they will be removed from the study treatment.	Modification due to the addition of carboplatin to the treatment regimen.
Section 5.1 – Prohibited Treatments	Administration of pegfilgastrim during treatment with paclitaxel	Administration of pegfilgastrim during treatment with paclitaxel and carboplatin	Modification due to the addition of carboplatin to the treatment regimen.

Protocol Section	Original Text	Revised Text	Rationale
Sectoin 6.1.1.5 – Vaccine Administration and Vaccine Schedule	---------- (Figure)	DC vaccines are timed with chemotherapy. All vaccine doses within a cycle are scheduled to be given approximately every 2 weeks apart. If necessary, a vaccine dose may be delayed for up to 2 weeks. In this case, subsequent doses should continue on a 2 week schedule, from the time of the delayed vaccine. If a vaccine dose is delayed more than 2 weeks, the PI and the BIIR Project Manager must be contacted for further instructions on continued dosing. Additional delays or modifications to the dosing schedule must be approved by the sponsor scientific chair. (Figure)	Clarification of vaccine delay. Modification of DC vaccine schedule figure due to the changes in timing caused by the addition of carboplatin to the treatment regimen.
Section 6.5.4 – Carboplatin (Paraplatin®)	----------	The renal effects of nephrotoxic compounds may be potentiated by carboplatin.[58]	Addition of section due to the addition of carboplatin to the treatment regimen.
Section 6.6.3 – Paclitaxel and Carboplatin	Section 6.6.3 – Paclitaxel Standard premedications for paclitaxel and carboplatin should be administered prior to each dose of paclitaxel and carboplatin:	Section 6.6.3 – Paclitaxel Standard premedications for paclitaxel and carboplatin should be administered prior to each dose of paclitaxel and carboplatin:	Modification due to the addition of carboplatin to the treatment regimen.

Protocol Section	Original Text	Revised Text	Rationale
Section 7 – Treatment Plan, Table 1	Paclitaxel 175 mg/m^2 Cycle 5-8, Day 1, q14 days	Paclitaxel 80 mg/m^2 Cycle 5-8, Days 1, 8, 15; q28 days Carboplatin AUC=6 Cycle 5-8, Day 1; q28 days	Modification of the treatment schema due to the addition of carboplatin to the treatment regimen.
Section 7.2 – Chemotherapy Dose Modification for Toxicity	Dose reductions will be at the physician's discretion. One dose reductions will be permitted for doxorubicin, cyclophosphamide, and paclitaxel. No dose reductions for anakinra and DC vaccines will be permitted. Patients will resume treatment with the next planned cycle (provided improvement/resolution in toxicities) as outlined below. Dose reductions are permanent. If doxorubicin, cyclophosphamide, and paclitaxel treatment is interrupted, anakinra and DC vaccinations may still be administered following consultation with the study PI, Dr. O'Shaughnessy. A patient will be deemed to be off study treatment if the patient stops DC vaccinations, but will continue to be followed for clinical outcome. The dose levels doxorubicin, cyclophosphamide, and paclitaxel are shown in Table 2.	Dose reductions will be at the physician's discretion. One dose reduction will be permitted for doxorubicin, cyclophosphamide, and paclitaxel. Two dose reductions are permitted for carboplatin. No dose reductions for anakinra and DC vaccines will be permitted. Patients will resume treatment with the next planned cycle (provided improvement/resolution in toxicities) as outlined below. Dose reductions are permanent. If doxorubicin, cyclophosphamide, paclitaxel, and carboplatin treatment is interrupted, anakinra and DC vaccinations may still be administered following consultation with the study PI, Dr. O'Shaughnessy. A patient will be deemed to be off study treatment if the patient stops DC vaccinations, but will continue to be followed for clinical outcome. The dose levels doxorubicin,	Modification of the dose reductions and dose levels (including table) due to the addition of carboplatin to the treatment regimen.

Protocol Section	Original Text	Revised Text	Rationale
	(Table 2)	cyclophosphamide, paclitaxel, and carboplatin are shown in Table 2. (Table 2)	
Section 8.5 – Procedures during Study Drug Treatment	9. Research biopsies of the patient's breast tissue must be obtained prior to the first day of treatment and also 1-2 days prior or on Day 1 of Cycle 3 of AC for assessment of modulation of the immune microenvironment.	9. Research biopsies of the patient's breast tissue must be obtained prior to the first day of treatment and also 1-2 days prior or on Day 1 of Cycle 4 of AC for assessment of modulation of the immune microenvironment.	Change in research biopsy timing.
Section 8.10 – Blood Samples for Immunomonitoring	ACD tubes	CPT tubes 30 mL at the time of each follow-up appointment	Change from ACD tubes to CPT tubes. Added research blood draws during the follow up portion of the study (was previously written into the protocol, but missing in this paragraph).
Section 8.11 – Research biopsies	Four...	A minimum of four... 1-2 days prior to or on Day 1 of Cycle 4 of AC....	Allowed for a minimum amount of biopsies, in the occasion the tumor is large enough to provide additional cores. Changed the timing of the research biopsy.
Section 9.4.6 – Monitoring for Adverse Events during the Clinical Trial	Patients will receive seven DC vaccinations intravenously.	Patients will receive 7 DC vaccinations intratumorally and/or subcutaneously.	Corrected the typo/method of vaccinating.

Protocol Section	Original Text	Revised Text	Rationale
Section 11.4 – Statistical Methods	***Interim analysis - Safety****:* An interim safety analysis will be performed after 4 and 9 patients have completed 4 vaccines in both Groups 1 and 2. Accrual will be put on hold if 2 of 4 or >4 of 9 patients experience the following: • Any vaccine-related toxicity as outlined in Section 9.4.9 • More than one dose reduction in AC or paclitaxel for hematologic or nonhematologic toxicity, as per Section 9.4.9. Constitutional symptoms that are expected to occur with vaccine therapies are not included in this interim safety analysis. A separate interim safety analysis will be performed for Group 1 and Group 2 separately, and the incidence of AEs in both groups will be compared. In the event the safety conditions above occur and accrual is put on hold, a discussion will be held with the DSMB and the FDA to discuss the safety issues, strategies to reduce toxicity, and whether to lift the	***Interim analysis - Safety****:* An interim safety analysis will be performed after 4 and 9 patients have completed 4 vaccines in both Groups 1 and 2. Accrual will be put on hold if 2 of 4 or >4 of 9 patients experience the following: • Any vaccine-related toxicity as outlined in Section 9.4.9 • More than one dose reduction in AC or paclitaxel, or 2 dose reductions for carboplatin for hematologic or nonhematologic toxicity, as per Section 9.4.9. Constitutional symptoms that are expected to occur with vaccine therapies are not included in this interim safety analysis. A separate interim safety analysis will be performed for Group 1 and Group 2 separately, and the incidence of AEs in both groups will be compared. The team of specialists ("DC vaccine team") involved in this study comprises physicians, scientists, medical writers, trial coordinators, data specialists, regulatory experts,	Modified dose reduction statement due to the addition of carboplatin to the treatment regimen. Addressed the team of specialist named "DC vaccine team", which replaces the need for a DSMB.

Protocol Section	Original Text	Revised Text	Rationale
	accrual hold.	and laboratory staff. All safety and efficacy data as well as the biomarker data (when available) will be reviewed monthly at multidisciplinary trial oversight meetings, where all experts will be in attendance. In the event the safety conditions above occur and accrual is put on hold, a discussion will be held with the DC vaccine team and the FDA to discuss the safety issues, strategies to reduce toxicity, and whether to lift the accrual hold.	
Section 13.1 – Multidisciplinary Trial Oversight Meetings	Section 13.1 – Data Safety Monitoring Board – entire section	The team of specialists ("DC vaccine team") involved in this study is comprised of physicians, scientists, medical writers, trial coordinators, data specialists, regulatory experts, and laboratory staff. All safety and efficacy data as well as the biomarker data (when available) will be reviewed monthly at multidisciplinary trial oversight meetings, where all experts will be in attendance.	Removal of the DSMB and replaced with an internal multidisciplinary team.
References	----------	Decatris MP et al Belani CP, et al Telli ML, et al Silver DP, et al	Additional references to support the addition of carboplatin to the treatment regimen in the protocol.

Protocol Section	Original Text	Revised Text	Rationale
		Sikov WM, et al Paraplatin package insert	
Appendix V – Research Biopsies – Collection and Handling	----------	Instructions for collection fresh tissue samples.	Added instructions for the collection of fresh tissue that were previously missing from the appendix.

Minor grammatical errors or misspellings were corrected as needed.

Appendix VIII 013-154 Amendment 3 List of Changes

Protocol Section	Original Text	Revised Text	Rationale
Title Page; Appendix IV - Patient Diaries	Title: Pilot safety trial of anakinra combined with chemotherapy and dendritic cell vaccine in patients with and locally advanced, triple-negative breast cancer.	New title: Pilot safety trial of preoperative chemotherapy combined with dendritic cell vaccine in patients with locally advanced, triple-negative breast cancer or ER-positive, HER2-negative breast cancer. Amendment 3, Date: November 17, 2014	Updated title of the protocol, amendment version and date to reflect the changes in the protocol. Updated title on the title page of the patient diary.
Table of Contents, In-Text Tables, and Abbreviations	----------	Corresponding headings and pages have been changed to reflect modifications as a result of Amendment # 3.	Updated protocol
Thoughout the protocol	---------- With and without anakinra	…and ER+/HER2- breast cancer (BC). ----------	Changes reflect the addition of patients with ER+/HER2- breast cancer and the removal of anakinra treatment from the trial. Changes are throughout the protocol.
Study Synopsis – Summary	---------- Immunotherapy could be an attractive strategy for overcoming chemotherapy resistance in TNBC patients and some preliminary studies have been carried out. In addition, blockade of IL-1β represents a novel approach to breast	Women with ER+/HER2- breast cancer typically have a low likelihood of developing a pCR, and if these women have residual disease with high levels of Ki67 after preoperative therapy, this predicts for poor overall and progression-free survival with subsequent endocrine therapy. Immunotherapy could be an attractive	Updated to reflect changes in the protocol.

Protocol Section	Original Text	Revised Text	Rationale
	cancer immunotherapy. TSLP secretion from breast cancer cells is regulated by IL-1β. Results showed high levels of IL-1β in the breast cancer microenvironment. IL-1β induces TSLP production from breast cancer cells lines in a dose and contact dependent manner. Cancer cells induce IL-1β secretion from DCs and monocytes in a contact-dependent fashion. This is mediated by cancer cell-derived TGF- β. Administration of the IL-1R antagonist, anakinra, prevents tumor growth in vivo, blocks $OX40L^{+}$ expression on DCs, and blocks iT_H2 generation in vivo. Our goals are to boost T cell immunity targeted against breast cancer utilizing a tumor antigen-loaded DC vaccine, to reverse the immune suppressive tumor microenvironment by IL-1 blockade, to enhance chemotherapy effectiveness and decrease tumor metastagenicity, and to decrease the recurrence rates of LA TNBC. Patients with LA TNBC will be treated with a combination of antigen-loaded DC vaccinations along with standard preoperative	strategy for these patients and some preliminary studies have been carried out. ---------- Our goals are to boost T cell immunity targeted against breast cancer utilizing a tumor antigen-loaded DC vaccine, to enhance chemotherapy effectiveness and decrease tumor metastagenicity, and to decrease the recurrence rates of LA TNBC and ER+/HER2– BC. Patients will be treated with a combination of antigen-loaded DC vaccinations along with standard preoperative chemotherapy, to improve immunogenicity and to increase the pCR rate achieved with standard therapy. The trial will consist of 2 patient cohorts: TNBC and ER+/HER2– BC.	

Protocol Section	Original Text	Revised Text	Rationale
	chemotherapy, to improve TNBC immunogenicity and to increase the pCR rate achieved with standard therapy. The trial will consist of 2 patient cohorts. In the first group, patients will receive DC vaccinations in combination with preoperative chemotherapy. In the second group, IL-1 blockade with anakinra will be added to the preoperative chemotherapy and DC vaccine.		
Study Synopsis – Objectives; Section 2.1 – Primary Objective; Section 2.2 – Secondary Objectives	The primary objective of this study is to determine the safety and feasibility of combining cyclin B1/WT-1/CEF (antigen)-loaded DC vaccination with preoperative chemotherapy, and to combine DC vaccination with preoperative chemotherapy in addition to IL-1 blockade with anakinra in patients with LA TNBC. The secondary objectives of this trial are to determine pathologic complete response rates, with and without anakinra; disease-free survival; to assess immune biomarkers of immunity (antigen-specific CD8+ T cell immunity and T_H2 T cells) in breast cancer biopsy specimens and blood samples in patients receiving DC	The primary objective of this study is to determine the safety and feasibility of combining cyclin B1/WT1/CEF (antigen)-loaded DC vaccination with preoperative chemotherapy. The secondary objectives of this trial are to determine pathologic complete response rates; disease-free survival; to assess immune biomarkers of immunity (antigen-specific CD8+ T cell immunity and T_H2 T cells) in breast cancer biopsy specimens and blood samples in patients receiving DC vaccinations; and to assess the feasibility of immunizing LA TNBC and ER+/HER2– BC patients with patient-specific tumor antigens.	Changes in the objectives reflect the addition of patients with ER+/HER2- breast cancer and the removal of anakinra treatment from the trial.

Protocol Section	Original Text	Revised Text	Rationale
	vaccinations, with and without IL-1 blockade with anakinra; and to assess the feasibility of immunizing LA TNBC patients with patient-specific tumor antigens.		
Study Synopsis – Inclusion Criteria; Section 4.2 – Inclusion Criteria	2. Have: a. locally advanced TNBC defined as invasive ductal cancer b. ER- tumors with <10% of tumor nuclei immunoreactive c. PR- tumors with <10% of tumor nuclei immunoreactive d. T3 or T4 disease, regardless of nodal status; T2 disease is eligible if there are positive lymph nodes present by physical exam or imaging evaluation or histological evaluation.	2. Have either: a. **locally advanced TNBC** defined as invasive ductal cancer; ER- tumors with <10% of tumor nuclei immunoreactive; PR- tumors with <10% of tumor nuclei immunoreactive; T3 or T4 disease, regardless of nodal status (T2 disease is eligible if there are positive lymph nodes present by physical exam or imaging evaluation or histological evaluation, OR b. **High-risk ER+ breast cancer** defined as grade 3 invasive ductal or mixed ductal/lobular cancers, or grade 2 with Ki67 ≥20%; node positive as evidenced by physical exam or imaging evaluation or histological evaluation.	Inclusion criteria have been modified to include patients with ER+/HER2- breast cancer.

Protocol Section	Original Text	Revised Text	Rationale
Study Synopsis – Exclusion Criteria; Section 4.3 – Exclusion Criteria	----------	6. ….The use of an LHRH agonist during chemotherapy in premenopausal women who wish to preserve ovarian function is allowed, but is not required.	Clarification of exclusion criterion #6, which would allow for the use of an LHRH agonist.
Study Synopsis – Medication and Doses; Section 3 – Study Design; Section 6.1.1.5 – Vaccine administration and vaccine schedule; Section 7 – Treatment Plan	This exploratory pilot safety, open label trial will evaluate the combination of preoperative chemotherapy and Dendritic Cell (DC) vaccinations in 2 groups of patients with LA TNBC. The first 10 patients will be enrolled to receive DC vaccinations during the 24 weeks of standard preoperative dose-dense doxorubicin/cyclophosphamide (AC) followed by paclitaxel and carboplatin (TCb) chemotherapy; the following 10 patients will be a staggered enrollment, and will receive DC vaccinations and anakinra 100 mg SC for 7 days, followed by 7 days off, then repeating during the 24 weeks of preoperative chemotherapy. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment. Patients will receive standard preoperative dose-dense	This exploratory pilot safety, open label trial will evaluate the combination of preoperative chemotherapy and Dendritic Cell (DC) vaccinations in 2 cohorts of patients with LA TNBC or ER+/HER2– BC. • LA TNBC patients will be enrolled to receive DC vaccinations during the 24 weeks of standard preoperative dose-dense doxorubicin/cyclophosphamide (AC) followed by paclitaxel and carboplatin (TCb) chemotherapy; • ER+/HER2– BC patients will receive DC vaccinations during the 22 weeks of standard preoperative dose-dense AC followed by weekly paclitaxel (T) chemotherapy. • Study procedures will be similar in both groups. LA TNBC patients will receive standard preoperative dose-dense AC (4 cycles)	Modification of the study design to reflect the addition of patients with ER+/HER2- breast cancer and the removal of anakinra treatment from the trial.

Protocol Section	Original Text	Revised Text	Rationale
	doxorubicin/cyclophosphamide (4 cycles; AC) followed by paclitaxel and carboplatin (4 cycles; TCb) chemotherapy, administered for 24 weeks combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 DCs) on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-dense AC and on any one individual day between Days 11-15 of Cycles 1 and 3 of TCb (4 timepoints)…. After definitive surgery and during locoregional radiation therapy to the breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL (at 15 x 10^6 cells/mL) in the ventral surface of the upper arm, with antigen-loaded DCs. The first vaccination booster will occur once after the surgery and up to 3 days prior to radiation; the second booster will occur 30 days ± 3 days after radiation is completed; and the third booster will occur 90 days ± 3 days	followed by TCb (4 cycles) chemotherapy, administered for 24 weeks. ER+/HER2– BC patients will receive standard preoperative dose-dense AC (4 cycles) followed by weekly T (12 cycles), administered for a total of 22 weeks. In both cohorts, chemotherapy will be combined with antigen-loaded DC vaccinations administered intratumoral (one injection of 0.2 mL at 3 x 10^6 cells/mL) and subcutaneous (one injection of 1 mL at 15 x 10^6 cells/mL), for a total of 4 time points prior to definitive surgery. • During the AC cycles, both cohorts will receive vaccines administered on any one individual day between Days 9-12 of Cycles 1 and 3 of dose-dense AC. • For TNBC patients, vaccines will be administered on any one individual day between Days 11-15 of Cycles 1 and 3 of TCb. For ER+/HER2– patients, vaccines will be administered Day 1 during either Cycle 2 or Cycle 3 and on Day 1 during either Cycle 8 or Cycle 9 of T. Vaccine	

Protocol Section	Original Text	Revised Text	Rationale
	after the 2nd boost.	is to be administered after T infusion is completed in this cohort of patients…. After definitive surgery and during locoregional radiation therapy to the breast or chest wall and regional lymphatics per standard of care, patients will receive 3 boost DC vaccinations subcutaneously of 1 mL (at 15 x 10^6 cells/mL), rotating injection sites in the dorsal or ventral surface of the upper arm, with antigen-loaded DCs. The timing of the boosters is the same for TNBC and ER+/HER2– cohorts. The first vaccination booster will occur once after the surgery and up to 3 days prior to radiation; the second booster will occur 30 days ± 3 days after radiation is completed; and the third booster will occur 90 days ± 3 days after the 2nd boost.	
Section 1.1 – Background on breast cancer	---------- Therefore, a high priority for clinical research in patients with locally advanced TNBC is to increase the pathologic complete response (pCR) rate in breast and axilla following	…In addition, the degree of proliferation as indicated by expression levels of Ki67 is also a predictive factor for achieving a pCR. High Ki67 in pretreatment breast cancer tissue is associated with an increase in pCR, whereas high Ki67 in	Addition of scientific literature in support of the addition of patients with ER+/HER2- breast cancer.

Protocol Section	Original Text	Revised Text	Rationale
	preoperative therapy. Patients with T3 and T4 cancers and with clinically N1/N2 axillary disease are at highest risk of not achieving a pCR with standard therapy, and of developing metastatic disease.	post-treatment residual disease is correlated with poorer disease-free and overall survival.[3] … Women with ER+/HER2- breast cancer typically have a low likelihood of developing a pCR[6], and if these women have residual disease with high levels of Ki67 after preoperative therapy, this predicts for poor overall and progression-free survival with subsequent endocrine therapy.[3] ER+ patients whose cancers have high expression levels of Ki67 have a high rate of disease recurrence and new treatment options are necessary to improve their outcome.[7] Therefore, a high priority for clinical research in patients is to increase the pathologic complete response (pCR) rate in breast and axilla following preoperative therapy. Patients with T2, T3 and T4 cancers and with clinically N1/N2 axillary disease are at highest risk of not achieving a pCR with standard therapy, and of developing metastatic disease.	
Section 1.3 – Rationale for target antigen selection	----------	… Cyclin B1 is one of the cancer-related genes that comprises the Oncotype DX Recurrence Score	Addition of scientific literature in support of the target antigens for addition of patients with ER+/HER2-

Protocol Section	Original Text	Revised Text	Rationale
		assay.[28] This assay, developed from the National Surgical Adjuvant Breast and Bowel Project (NSABP) trials, quantifies the likelihood of breast cancer recurrence, and also can predict for chemotherapy benefit (higher recurrence scores are indicative of greater therapy benefit).[29] High Cyclin B1 levels are associated with highly proliferative ER+ breast cancers and predict for a high risk of distant disease recurrence…. In the ER+, HER2- breast cancer cell line, MCF-7, WT1 was correlated with high expression of ERα and HER2, which indicated that it may play a role in cancer progression and development.[34] In addition, high levels of WT1 are associated with aggressive breast cancer biology, and in vitro, WT1 promoted estrogen-independent growth and anti-estrogen resistance.[35] This finding indicates that WT1 is, in part, mechanistically responsible for the switch from estrogen-dependent to -independent breast cancer growth and survival.	breast cancer.

Protocol Section	Original Text	Revised Text	Rationale
Section 1.4 – Rationale for intratumoral DC injection	We propose to substitute the functional DCs via adoptive transfer of ex vivo generated autologous mature DCs injected locally into LA TNBCs at 48 hours post-systemic anthracyclines.	We propose to substitute the functional DCs via adoptive transfer of ex vivo generated autologous mature DCs injected locally into LA TNBCs and ER+/HER2– breast cancer patients post-systemic anthracycline therapy.	Updated the timing on DC vaccine due to changes to the treatments and patient cohorts.
(Previous) Section 1.5 – Rationale for targeting IL-1	All of Section 1.5	----------	Removed background on anakinra due to the removal of anakinra as a treatment in this trial.
Section 1.8 – Rationale	Our goals are to boost T cell immunity targeted against breast cancer utilizing a tumor antigen-loaded DC vaccine, to reverse the immune suppressive tumor microenvironment by IL-1 blockade, to enhance chemotherapy effectiveness and decrease tumor metastagenicity, and to decrease the recurrence rates of LA TNBC. Patients with LA TNBC will be treated with a combination of antigen-loaded DC vaccinations along with standard preoperative chemotherapy, to improve TNBC immunogenicity and to increase the pCR rate achieved with standard therapy. The trial will consist of 2 patient cohorts. In the first group, patients will receive DC vaccinations in combination with preoperative chemotherapy. In the second group,	Our goals are to boost T cell immunity targeted against breast cancer utilizing a tumor antigen-loaded DC vaccine, to enhance chemotherapy effectiveness and decrease tumor metastagenicity, and to decrease the recurrence rates of LA TNBC and ER+/HER2– BCs. Patients with LA TNBC and ER+/HER2– BC will be treated with a combination of antigen-loaded DC vaccinations along with standard preoperative chemotherapy, to improve immunogenicity and to increase the pCR rate achieved with standard therapy. The trial will consist of 2 patient cohorts: TNBC and ER+/HER2– BC.	Modification of the overall rationale of the study, to reflect the addition of patients with ER+/HER2- breast cancer and the removal of anakinra treatment from the trial.

Protocol Section	Original Text	Revised Text	Rationale
	IL-1 blockade with anakinra will be added to the preoperative chemotherapy and DC vaccine.		
Section 3 – Study Design Schema	(Figure)	(Figure)	Figure of study design schema was updated to reflect the changes in the protocol.
Section 4.1 – Sample Size	Twenty patients with newly diagnosed locally advanced TNBC will be enrolled over 20 months (10 patients per group).	Twenty patients with newly diagnosed locally advanced TNBC or ER+/HER2– BC will be enrolled over 20 months. If a patient's apheresis product or manufactured vaccine is suboptimal after a second apheresis procedure, the patient will be withdrawn from the study and replaced. See Section 8.3.	Removed the wording for groups, to reflect the addition of patients with ER+/HER2- breast cancer and the removal of anakinra treatment from the trial. Clarified replacement patient population.
Section 4.4 – Patient Withdrawal from the Study	----------	7. Apheresis product or manufactured vaccine is suboptimal after a second apheresis procedure.	Addition of a reason to withdraw a patient due to apheresis procedure failures.
Section 5 – Prior and concomitant therapy	----------	• LHRH agonist	Modified the list of reported conmeds to allow for the use of an LHRH agonist for women who wish to preserve ovarian function.
Section 5.1 – Prohibited treatments	• Administration of hormonal therapy (with the exception of replacement steroids) • Administration of TNF blocking agents	----------	Deletion of prohibited treatments to allow for the use of an LHRH agonist and the removal of anakinra from the trial.

Protocol Section	Original Text	Revised Text	Rationale
Section 6.1 – Investigational product	In this protocol, the investigational products are the Dendritic Cell (DC) vaccines and anakinra (Kineret®).	In this protocol, the investigational product is the Dendritic Cell (DC) vaccine.	Modification of the investigational product to address the removal of anakinra from the trial.
Section 6.1.1.5 – Vaccine administration and vaccine schedule	---------- (Figure)	**NOTE:** Needles used for drawing vaccine product into the syringe and for injection **must be 23-gauge or larger**. Smaller sized needles (eg, 25- or 27-gauge) will either rupture the cells or cause them to clump. (2 Figures)	Clarified the needle size required for vaccine administration. Modification and addition of DC vaccination schedules to reflect the addition of patients with ER+/HER2- breast cancer.
Previous Section 6.1.2 – Anakinra	All of Section 6.1.2	----------	Removed anakinra as an investigational product due to its removal as a treatment in this trial.
Section 6.2 – Non investigational products	----------	In this protocol, the non-investigational products are: • Preoperative chemotherapy with preoperative dose-dense doxorubicin/cyclophosphamide (AC) and paclitaxel/carboplatin (TCb) or paclitaxel alone (T) • Radiation therapy to breast or chest wall and regional lymphatics	Modification of the non-investigational products to address the new treatment regimens that will be used for the addition of patients with ER+/HER2- breast cancer.

Protocol Section	Original Text	Revised Text	Rationale
		• For ER+/HER2– BC patients only: standard endocrine therapy during and after radiation	
Section 6.3 – Accountability procedures	For the purpose of accountability, all unused Anakinra and package inserts from used Anakinra, which was dispensed to the patient, should be returned to the clinic at each visit.	----------	Removed anakinra information in accountability procedures due to its removal as a treatment in this trial.
Previous Section 6.4.1 - Anakinra	All of Section 6.4.1	----------	Removed anakinra as a potential risk due to its removal as a treatment in this trial.
Previous Section 6.5.1 - Anakinra	All of Section 6.5.1	----------	Removed drug-drug interaction information for anakinra due to its removal as a treatment in this trial.
Previous Section 6.6.1 - Anakinra	All of Section 6.6.1	----------	Removed premedication information for anakinra due to its removal as a treatment in this trial.
Section 7 – Treatment schema, Table 1	Anakinra Paclitaxel	---------- Paclitaxel for LA TNBC Paclitaxel for ER+/HER2– BC	Modification of the treatment schema due to the addition of patients with ER+/HER2– breast cancer, and the removal of anakinra from treatment in this trial.
Section 7.1 – Chemotherapy Treatment Delay	Patients who miss a dose of anakinra by more than a few hours should be instructed to take the missed dose as soon as possible and contact the Investigator. The next day dose should **not** be doubled if a daily dose is	----------	Deleted, due to the removal of anakinra in this trial.

Protocol Section	Original Text	Revised Text	Rationale
	missed.		
Section 7.4 – Radiation therapy	----------	ER+/HER2– BC patients will also receive standard endocrine therapy during locoregional radiation therapy, at the physician's discretion, for at least 5 years thereafter. The type of endocrine therapy, dosing, and duration must be captured on the CRF.	Addition of endocrine therapy to begin during radiation therapy, to reflect the addition of patients with ER+/HER2- breast cancer.
Section 8.1 – Screening	11. Whole blood collection (100 mL) for immunomonitoring studies.	11. Whole blood collection (40 mL) for immunomonitoring studies.	Reduced the amount of blood required for whole blood collection.
Section 8.3 – Apheresis Procedures	In the event that the apheresis is suboptimal, ie, that an inadequate monocyte collection is achieved or that the vaccine cannot be manufactured from the product collected, one additional apheresis will be allowed at least 1 week later. Apheresis product **must** meet required specifications for vaccine to be manufactured. Manufactured vaccine **must** meet required specification to be released for injection. **NOTE:** If a patient's apheresis product or manufactured vaccine is suboptimal after a second attempt at apheresis, those patients	Failure of peripheral venous access during apheresis will not be considered a collection of cells, as, with the subject's consent, a central venous line can be placed and an apheresis completed. In the event that the apheresis is suboptimal, ie, that an inadequate monocyte collection is achieved or that the vaccine cannot be manufactured from the product collected, one additional apheresis will be allowed no less than 7-10 days after the first apheresis. Apheresis product **must** meet required specifications for vaccine to be manufactured. Manufactured	Modified the time that a second apheresis can occur, and clarified that the apheresis second attempt is for an apheresis procedure, and not an attempt at only accessing a patient's veins.

Protocol Section	Original Text	Revised Text	Rationale
	will be withdrawn from the study, and replaced.	vaccine **must** meet required specification to be released for injection.	
Section 8.5 – Procedures during study drug treatment	---------- 1. A medical history on Day 1 of each cycle….	**NOTE:** For ER+/HER2– patients during the T cycles, the following evaluations will be performed on Day 1 every 3 weeks (Day 1, Cycles 1, 4, 7, and 10). 1. An abbreviated medical history….	Clarification of the assessments to be performed during study, to reflect the addition of patients with ER+/HER2- breast cancer. Clarified that an abbreviated medical history is sufficient during study drug treatment.
Section 8.6 – Procedures for Vaccine Treatment Visits	1. A medical history (note changes from baseline) 2. A physical examination, including vital signs and body weight.	1. An abbreviated medical history (note changes from baseline) 2. A limited physical assessment focusing on the affected breast, including vital signs and body weight.	Clarified that an abbreviated medical history and a limited physical assessment is sufficient during vaccine treatment visits.
Section 8.8 – Procedures for Follow-Up Visits	1. A medical history	1. An abbreviated medical history	Clarified that an abbreviated medical history is sufficient during follow-up visits.
Section 8.10 – Blood Samples for Immunomonitoring	Whole blood collection for immunomonitoring studies will be obtained as follows: • 100 mL (11 CPT tubes and 2 tempus tubes) at baseline • 30 mL prior to each treatment-vaccination cycle; prior to surgery and prior to radiation (3 CPT tubes and 2	Whole blood collection for immunomonitoring studies will be obtained as follows: • 40 mL (4 CPT tubes and 2 tempus tubes) at baseline • 30 mL prior to each treatment-vaccination cycle; prior to surgery and prior to radiation (3 CPT tubes and 2	Reduced the amount of blood required for whole blood collection.

Protocol Section	Original Text	Revised Text	Rationale
	tempus tubes). • 100 mL (11 CPT tubes and 2 tempus tubes) 2 weeks after the last DC vaccination • 30 mL at the time of each follow-up appointment	tempus tubes). • 40 mL (4 CPT tubes and 2 tempus tubes) 2 weeks after the last DC vaccination • 30 mL at the time of each follow-up appointment	
Section 9.3 – Patient diaries	Patients enrolled in Group 2, in addition to collection of new symptoms after vaccine administration, will be instructed to record any new symptoms that may have developed during administration of anakinra.	----------	Removal of patient diaries for anakinra, due to its removal as a treatment in this trial.
Section 9.4.6 – Monitoring for Adverse Events During the Clinical Trial	Patients will have vital signs taken approximately 15 minutes and 30 minutes following the DC vaccinations and again at approximately 1 hour post injection prior to patient's release.	Patients will have vital signs taken approximately every 15 minutes for 1 hour post injection prior to patient's release.	Modified to include a vital sign time point at 45 minutes.
Section 11.4 – Statistical Methods	…in Group 1 patients… The administration of DC vaccine plus anakinra plus preoperative chemotherapy in Group 2 patients will be considered safe if no more than 3 of the 10 patients experience a toxicity that leads to chemotherapy dosing delay of more than 4 weeks. Quantitative and avidity evaluation of	----------	Removed statistical analyses that were associated with administration of anakinra, due to its removal as a treatment in this trial.

Protocol Section	Original Text	Revised Text	Rationale
	tumor-infiltrating and peripheral blood cyclin B1 and patient-specific mutation, antigen-specific T cells will be carried out for patients who received anakinra versus patients who did not receive anakinra. …and for patients who received anakinra versus patients who did not receive anakinra. …administration of anakinra versus no administration of anakinra, …. …in both Groups 1 and 2…. A separate interim safety analysis will be performed for Group 1 and Group 2 separately, and the incidence of AEs in both groups will be compared.		
References	(Deleted:) Coussens 2013 Teschendorff 2010 Kristensen 2012 Terabe 2004 Zhang 2008 DeNardo 2011 Galon 2006 Pascual 2005 Kineret package insert 2009	New references: Yoshioka 2013 Houssami 2012 Von Minckwitz 2012 Paik 2004 Paik 2006 Nasomyon 2014 Wang 2010 ----------	Removal of anakinra references, and addition of scientific literature to support the addition of patients with ER+/HER2- breast cancer.

Protocol Section	Original Text	Revised Text	Rationale
Appendix II - Schedule of Assessments	Group 1 Group 2	LA TNBC ER+/HER2-	Language updated to match protocol.
Appendix V – Research Biopsies – Collection and Handling	Specimens must not be subjected to freezing or extreme temperatures	----------	Removed this statement, due to a change in processes.

Minor grammatical errors or misspellings were corrected as needed.

Appendix IX 013-154 Amendment 4 List of Changes

Protocol Section	Original Text	Revised Text	Rationale
Title Page	----------	Amendment 4, Date: May 01, 2015	Updated amendment version and date to reflect the changes in the protocol.
Table of Contents, In-Text Tables, and Abbreviations	----------	Corresponding headings and pages have been changed to reflect modifications as a result of Amendment # 4.	Updated protocol
Study Synopsis – Inclusion Criteria Section 4.2 – Inclusion Criteria	11. Patients must be willing to undergo research biopsies to obtain breast cancer tissue for whole exome sequencing and evaluation of tumor immune microenvironment.	----------	Patients will be encouraged to donate a portion of their tissue, but they can refuse without being off study.
Study Synopsis – Exclusion Criteria Section 4.3 – Exclusion Criteria	9. Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation such as: a. severe impaired lung functions as defined as spirometry and DLCO that is 50% of the normal predicted value and/or O_2 saturation that is 88% or less at rest on room air b. uncontrolled diabetes as defined by fasting serum glucose >1.5 x ULN c. liver disease such as	9. Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation such as: a. severe impaired lung functions as defined as spirometry and DLCO that is 50% of the normal predicted value and/or O_2 saturation that is 88% or less at rest on room air b. liver disease such as cirrhosis or severe hepatic impairment (Child-Pugh class C).	Removal of fasting serum glucose >1.5 x ULN as an exclusion criterion.

Protocol Section	Original Text	Revised Text	Rationale
	cirrhosis or severe hepatic impairment (Child-Pugh class C).		
Synopsis - Medication and Doses; Section 3 - Study Design Section 7 - Treatment Plan Section 8.5 - Procedures During Study Drug Treatment	Patients will undergo research biopsies of their breast cancer prior to the start of treatment and 1-2 days prior to or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment. After preoperative treatment, patients will undergo definitive surgery, generally with mastectomy, and if available, fresh tissue and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify cancer-associated mutations in the residual, chemotherapy-refractory cancer.	Patients will undergo research biopsies of their breast cancer prior to the start of treatment and 1-2 days prior to or on Day 1 of Cycle 4 of AC to analyze the composition of the immune microenvironment. Patients who will have their definitive surgery **outside** a Baylor hospital will have a third research biopsy at least 1 week following the last chemotherapy dose and prior to surgery. Core biopsies will be obtained prior to treatment initiation for whole exome sequencing and expression analysis and for characterization of the tumor immune microenvironment. After preoperative treatment, patients will undergo definitive surgery, generally with mastectomy, and if available, fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and residual FFPE breast cancer tissue will be collected for assessment of the immune microenvironment and for whole exome sequencing to identify	Addition of one biopsy time point, for patients who will have their definitive surgery outside a Baylor hospital.

Protocol Section	Original Text	Revised Text	Rationale
		cancer-associated mutations in the residual, chemotherapy-refractory cancer.	
Section 3 – Study Design Schema	(Figure)	(Figure)	Figure of study design schema was updated to reflect the changes in the protocol.
Section 4.4 – Patient Withdrawal from the study Section 7 – Table 1 Section 9.4.9 – Removal due to Adverse Events	5. Treatment is interrupted for more than 4 weeks for any reason (Note: Delays do not count scheduled weeks of rest) NOTE: If any patient requires more than one dose reduction of AC….	---------- NOTE: The treating physician may adjust doses based on individual patient histories to optimize dose delivery, per standard of care. If any patient requires….	Modifications to the protocol to have patients remain on study even if their treatment is interrupted for more than 4 weeks. Clarification that the treating physician may modify dosing of chemotherapy per standard of care, dependent on an individual's patient history (also added as a footnote in Table 1).
Section 6.1.1.5 – Vaccine administration and vaccine schedule	To reconcile the DC vaccine use and disposal, the procedure will be to ship the vial of 1.5 mL of prepared final Vaccine Product to the clinic and following vaccination of the patient, the unused portion of the Vaccine Product will be returned to the GMP QC laboratory for reconciliation and testing. (2 Figures)	To reconcile the DC vaccine use and disposal, the procedure will be to ship the vial of 1.5 mL of prepared final Vaccine Product to the clinic and following vaccination of the patient, the unused portion of the Vaccine Product will be disposed of at the clinic by BIIR or Clinical Research Staff coordinating the study visit. (2 Figures)	Modified the reconciliation procedure of the DC vaccines disposal. Modification of DC vaccination schedules to reflect the addition of a 3rd biopsy for those patients who will have their definitive surgery outside a Baylor hospital.

Protocol Section	Original Text	Revised Text	Rationale
Section 7.1 – Chemotherapy treatment delay	1. Treatment may be delayed no more than 4 weeks for any reason (Note: Delays do not count scheduled weeks of rest). 2. Patients who are off study treatment for more than 4 weeks for any reason will be considered withdrawn from the study.	----------	Modifications to the protocol to have patients remain on study even if their treatment is interrupted for more than 4 weeks.
Section 7.2 – Chemotherapy dose modification for toxicity	There are no chemotherapy dose escalations on this study.	----------	Clarification that the treating physician may modify dosing of chemotherapy per standard of care, dependent on an individual's patient history.
Section 7.3 – Hematologic toxicity	Treatment may be delayed for up to 4 weeks beyond a scheduled treatment to allow sufficient time for recovery.	Treatment may be delayed to allow sufficient time for recovery.	Modifications to the protocol to have patients remain on study even if their treatment is interrupted for more than 4 weeks.
Section 8.6 - Procedures for Vaccine Treatment Visits	1. An abbreviated medical history (note changed from baseline) 2. A limited physical assessment focusing on the affected breast, including vital signs and body weight.	----------	Modification to the procedures that are performed during vaccine treatment visits. Medical history and physical assessment are not necessary at these visits.
Section 8.11 - Biopsies	• Fresh tissue and FFPE breast tissue from definitive surgery will be collected at the time of surgery and through the pathology department, respectively.	• A minimum of four*14-gauge needle biopsy cores will be collected at least 1 week following the last chemotherapy dose and prior to surgery (2 cores frozen, and 2 cores in RNAlater®), for those patients who will have their definitive surgery **outside** a Baylor	Addition of one biopsy time point, for patients who will have their definitive surgery outside a Baylor hospital.

Protocol Section	Original Text	Revised Text	Rationale
		hospital. • Fresh tissue (for patients who have their definitive surgery **within** a Baylor hospital) and FFPE breast tissue from definitive surgery will be collected at the time of surgery and through the pathology department, respectively.	
Section 11.4 – Statistical Methods	An interim safety analysis will be performed after 4 and 9 patients have completed 4 vaccines. Accrual will be put on hold if 2 of 4 or >4 of 9 patients experience the following:	An interim safety analysis will be performed after 10 patients have been enrolled, and accrual will be held at that time. If 2 of 4 or >4 of 9 patients experience the following, then accrual will be put on hold:	Modification of the safety interim analysis, which changed the number of patients included, and also will hold accrual to the trial when the number is reached.
Section 14.2.1 – General guidelines	• Prior to study initiation, the protocol must have the approval of the FDA and of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). • 100% of all patients entered in the study will be monitored. • Each patient enrolled will be monitored 100%. • The first monitoring visit will be scheduled to occur within a month upon the registration of the first patient and continue every 4 to 6 weeks until all data has been monitored for every patient.	• Prior to implementation, the study must have the approval of the FDA and of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). • 100% of all patients entered in the study will be monitored. • Each patient enrolled will be monitored for 100% of the elements. • The first monitoring visit will be scheduled to comply with the monitoring plan upon the registration of the first patient and will continue as described in the	Removed redundant statements and clarified that monitoring should follow the established monitoring plan.

Protocol Section	Original Text	Revised Text	Rationale
	• Prior to implementation, the study must have the approval of the FDA and of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC).	monitoring plan until all data has been monitored for every patient.	
Section 14.2.2 – Study Monitoring Visits	A Monitoring Visit Report will be submitted to the Sponsor of the IND and a follow-up letter outlining observations and outstanding issues noted during the visit will be submitted to the investigator at each clinical site within 15 days following the monitoring visit.	A Monitoring Visit Report will be submitted to the Sponsor of the IND and a follow-up letter outlining observations and outstanding issues noted during the visit will be submitted to the investigator at each clinical site following the monitoring visit.	Removal of 15 day time limit, to comply with the established monitoring plan.
Appendix II - Schedule of Assessments	Group 1 Group 2	LA TNBC ER+/HER2-	Language updated to match protocol.

Minor grammatical errors or misspellings were corrected as needed.

Appendix X 013-154 Amendment 5 List of Changes

Protocol Section	Original Text	Revised Text	Rationale
Title Page	----------	Amendment 5, Date: March 21, 2016	Updated amendment version and date to reflect the changes in the protocol.
Table of Contents, In-Text Tables, and Abbreviations	----------	Corresponding headings and pages have been changed to reflect modifications as a result of Amendment # 5.	Updated protocol
Synopsis – Inclusion criteria Section 4.2 – Inclusion criteria	9. Eligible for treatment with paclitaxel, doxorubicin, cyclophosphamide, and carboplatin.	9. Eligible for treatment with paclitaxel, doxorubicin, cyclophosphamide, carboplatin, and capecitabine.	Updated inclusion criterion due to the addition of capecitabine for patients who do not achieve a pCR.
Synopsis – Medication and Doses Section 3 – Study Design Section 7 – Treatment Plan Section 7.4 – Radiation therapy Appendix II – Schedule of Assessments	----------	TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (cycle length per physician discretion). It will be the physician's discretion to begin capecitabine treatment either during radiation or after radiation is complete.	Modified treatment plan/study design due to the addition of capecitabine for TNBC patients who do not achieve a pCR.
Section 1.8 – Addition of capecitabine as adjuvant chemotherapy for TNBC	----------	**1.8.1 Capecitabine** Capecitabine is a prodrug that is enzymatically converted to 5-fluorouracil in the tumor where it inhibits DNA synthesis and slows growth of tumor tissue. Capecitabine	Modified background information due to the addition of capecitabine for TNBC patients who do not achieve a pCR.

Protocol Section	Original Text	Revised Text	Rationale
		is approved in both colorectal and breast cancer. Capecitabine demonstrated single-agent activity in subjects with MBC with an ORR of about 20% in subjects whose disease had progressed during or following anthracycline and taxane-based therapy.[52] Capecitabine is also indicated as a combination treatment with docetaxel in early-line treatment for MBC and in combination with ixabepilone or lapatinib as second-line treatment after failure of prior anthracycline and taxane-containing chemotherapy.[52] The recommended, approved dose of capecitabine is 1250 mg/m^2 daily BID for 14 days, followed by 7 days without treatment. This dose/schedule necessitates dose interruptions or reductions in approximately 30% of patients, and in clinical trials, approximately 17% of patients discontinued the drug due to toxicities (primarily hand-foot syndrome, diarrhea and stomatitis).[53,54] **1.8.2 Adjuvant Capecitabine**	

Protocol Section	Original Text	Revised Text	Rationale
		Improves DFS in TNBC Lee and colleagues recently published results from a 5 year follow up of a Phase III trial of adjuvant capecitabine in breast cancer patients with HER2-negative pathological residual invasive disease after neoadjuvant chemotherapy. Oral capecitabine was administered for 8 cycles at 1250 mg/m^2 PO BID after standard neoadjuvant chemotherapy that contained an anthracycline and/or a taxane, when patients did not have a complete pathologic response. Overall, disease-free survival (DFS) rate and overall survival increased with capecitabine as compared to standard therapy (DFS: 74.1% vs 67.6% and 89.2% vs 83.9%, respectively). Specifically in patients with triple-negative breast cancer that were non-pathologic complete responders following surgery and neoadjuvant treatment, there was a 42% reduction in the risk of recurrence with the addition of adjuvant capecitabine.Since women with TNBC who do **not** achieve a pCR have an increased risk of recurrence,	

Protocol Section	Original Text	Revised Text	Rationale
		decreased overall survival, and post-recurrence survival, the addition of capecitabine provides an additional benefit and option for this population.	
Section 3 – Study Design Section 8.10 – Blood samples for immunomonitoring	Blood samples for immunomonitoring studies will be obtained at baseline, prior to each DC vaccination, prior to surgery, prior to radiation, 2 weeks after the last DC vaccination, and at each follow up appointment. (Old study design flow chart)	Blood samples for immunomonitoring studies will be obtained at baseline, prior to each DC vaccination, prior to surgery, prior to radiation, 2 weeks ± 3 days after the last DC vaccination, and at each follow up appointment. (New study design flow chart)	Addition of a 3 day window to the timing of the blood collection 2 weeks after the last vaccination. Updated figure to match Amendment 5 changes.
Section 5.1 – Prohibited treatments	----------	• Administration of coumarin-derived anticoagulants and phenytoin during treatment with capecitabine	Addition of prohibited treatments, due to the addition of capecitabine in Amendment 5.
Section 6.1.1.5 – Vaccine administration and vaccine schedule	----------	TNBC patients who are non-pathologic complete responders and/or have positive lymph nodes following neoadjuvant treatment and surgery will receive capecitabine for 6-8 cycles (number of cycles per physician discretion). It will be the physician's discretion to begin capecitabine treatment either during radiation or after radiation is complete. These adjuvant treatments will not alter the	Modified treatment plan due to the addition of capecitabine for TNBC patients who do not achieve a pCR.

Protocol Section	Original Text	Revised Text	Rationale
		vaccine schedule delineated above.	
Section 6.2 – Non investigational products	----------	• For TNBC non-pathologic complete responders: capecitabine treatment to begin either during radiation or after radiation is complete.	Modified treatment plan due to the addition of capecitabine for TNBC patients who do not achieve a pCR.
Section 6.5.4 – Capecitabine	----------	Entire section	Included the drug-drug interactions for capecitabine, due to Amendment 5.
Section 7 – Treatment plan	Table 1: ----------	Table 1: Capecitabine/1000 mg/m^2/Cycles 6-8, Days 1-14, q21 days/PO	Included the treatment schema for capecitabine, due to Amendment 5.
Section 7.2 – Chemotherapy dose modification for toxicity	----------	Capecitabine dose reductions are permitted per standard of care, drug package insert, or physician's standard practice.	Included the dose reduction information for capecitabine, due to Amendment 5.
Section 8.6 – Procedures for vaccine treatment visits	1. Assessment of PS on the ECOG scale (Appendix III)	1. Assessment of PS on the ECOG scale (Appendix III), only during visits prior to definitive surgery. No assessment of ECOG is required at vaccine visits post-surgery.	Modification of assessing ECOG status for vaccine visits.
Section 8.7 – Procedures for end of treatment visit (EOT)	…within 24-48 hours… ECOG performance status scale (Appendix III)	…within 14 days ± 2 days… ----------	Modification of EOT assessment window to allow for more flexibility to the patient. Removal of ECOG assessment for EOT

Protocol Section	Original Text	Revised Text	Rationale
			visit.
Section 8.8 – Procedures for follow up visits	ECOG performance status scale (Appendix III)	----------	Removal of ECOG assessment for follow up visits.
References	----------	New references: Xeloda PI, 2015 Mackean et al, 1998 O'Shaughnessy et al, 2001 Lee et al, 2015	Additional references to support the addition of capecitabine to the treatment regimen in the protocol.

Minor grammatical errors or misspellings were corrected as needed.

Appendix XI 013-154 Amendment 6 List of Changes

Protocol Section	Original Text	Revised Text	Rationale
Title Page	----------	Amendment 6, Date: October 13, 2016	Updated amendment version and date to reflect the changes in the protocol.
Table of Contents, In-Text Tables, and Abbreviations	----------	Corresponding headings and pages have been changed to reflect modifications as a result of Amendment # 6.	Updated protocol
Section 3 – Study Design	Blood samples for immunomonitoring studies will be obtained at baseline, prior to each DC vaccination, prior to surgery, prior to radiation, 2 weeks ± 3 days after the last DC vaccination, and at each follow up appointment.	Blood samples for immunomonitoring studies will be obtained at baseline, prior to each DC vaccination, prior to surgery, prior to radiation, 2 weeks ± 3 days after the last DC vaccination, 6 months and 1 year after the last DC vaccination.	Updating the amount of blood collected for patients during the follow up visits; reduction from every 3 months for 3 years to once at 6 months and 1 year after the last vaccination.
Section 4.4 – Patient Withdrawal from the study	---------- If the patient is withdrawn for any reason, the end of study assessments must be completed.	…(“off study”)… If the patient is withdrawn for any reason, the end of treatment assessments must be completed (see Section 8.7).	Clarification for withdrawn from the study, which can also be labeled “off study”. Clarification that assessments are end of treatment assessments, referencing the appropriate section in the protocol.
Section 8.5 – Procedures during study drug treatment	----------	10. Immunomonitoring studies: Obtain approximately 30 mL of whole blood prior to all vaccinations; prior to surgery; and prior to radiation (see Section 8.10).	Addition of an assessment to the proper section (it is not an additional or new assessment).

Protocol Section	Original Text	Revised Text	Rationale
Section 8.7 – Procedures for end of treatment visit (EOT)	… (End of Treatment Visit)… ----------	… (End of Treatment or Off Treatment Visit)… **NOTE**: End of treatment, or off treatment, is NOT considered off study or withdrawn from the study. 6. Immunomonitoring studies: obtain approximately 40 mL of whole blood 2 weeks ± 3 days after the last DC vaccination (see Section 8.10).	Clarification for "end of treatment", which can also be labeled "off treatment". Clarifying that end of treatment does not equal off study/withdrawal from the study. Addition of an assessment to the proper section (it is not an additional or new assessment).
Section 8.8 – Procedures for follow up visits	6. Obtain approximately 30 mL of whole blood for immune response to vaccine.	6. Obtain approximately 30 mL of whole blood for immune response to vaccine at 6 months and 1 year after the last DC vaccination.	Modification of the blood collection. Blood was previously collected at every 3 months for 3 years during the follow up phase of the protocol, but is modified to only 2 blood collections total during follow up.
Section 8.10 – Blood samples for immunomonitoring	• 30 mL at the time of each follow-up appointment	• 30 mL at 6 months and 1 year after the last DC vaccination.	Modification of the blood collection. Blood was previously collected at every 3 months for 3 years during the follow up phase of the protocol, but is modified to only 2 blood collections total during follow up.
Appendix II - Schedule of Assessments	For 40 mL blood draws, 4 CPT tubes and 2 tempus tubes will be used for collection. For 30 mL blood draws, 3 CPT tubes and 2 tempus tubes will be used for collection.	For 40 mL blood draws, 4 CPT tubes and 2 tempus tubes will be used for collection. For 30 mL blood draws, 3 CPT tubes and 2 tempus tubes will be used for collection. For blood draws	Language updated to match the protocol amendment.

Protocol Section	Original Text	Revised Text	Rationale
		occurring during follow-up, 30 mL will only be collected at 6 months and 1 year after the last DC vaccination.	

Minor grammatical errors or misspellings were corrected as needed.