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DCP Protocol #: WF-97415

Local Protocol #: WF-97415

Understanding and Predicting Breast Cancer Events after Treatment (UPBEAT)

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Participating Organizations:

WAKE / Wake Forest NCORP Research Base
ECOG-ACRIN / ECOG-ACRIN Cancer Research Group

Protocol Amendment #: 2
Protocol Version Date: 03/09/2018

Protocol History:

Initial	Version 02/13/2017	Not Activated
Amendment 1	Version 03/23/2017	Activated 05/17/2017

<table>
<tr><th colspan="3">CONTACT INFORMATION</th></tr>
<tr><th>For regulatory requirements:</th><th>For patient enrollments:</th><th>For study data submission:</th></tr>
<tr><td>Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal.
Regulatory Submission Portal:
(Sign in at www.ctsu.org,
and select the Regulatory Submission sub-tab under the Regulatory tab.)

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support.

Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.</td><td>Please refer to the patient enrollment sections (Sections 7.2, 7.3 and 7.4) of the protocol for detailed instructions.</td><td>Data forms will be submitted to the WF NCORP Research Base according to Section 11.1 of the protocol.

For assistance, please contact the NCORP Research Base Data Management Center at:
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-or-
(336) 713-5086.

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Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.</td></tr>
<tr><td colspan="3">The most current version of the study protocol and all supporting documents must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password.</td></tr>
<tr><td colspan="3"><u>For clinical questions (i.e. patient eligibility or treatment-related),</u> please contact the WF NCORP Research Base site coordinator at (336) 713-6519.</td></tr>
<tr><td colspan="3"><u>For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission)</u> contact the CTSU Help Desk by phone or e-mail:
CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.</td></tr>
<tr><td colspan="3">The CTSU Website is located at https://www.ctsu.org.</td></tr>
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SCHEMA

Understanding and Predicting Breast Cancer Events after Treatment (UPBEAT)

Study Population
840 Stage I-III female breast cancer patients scheduled to receive adjuvant or neo-adjuvant chemo (+ or - radiation therapy) and 160 healthy cancer free women for statistical comparisons

↓

Baseline data collection
MRI, Labs, Demographic, Medical Chart Review*, Current Medications, Physical Assessments, Self-Administered Questionnaires, Neurocognitive Tests, 6-Minute Walk, Disability Measures, SPPB,45% will undergo a treadmill or stationary bike cardiopulmonary exercise test (CPET) Serum and Plasma Samples*, DNA Lab*

↓

1 month blood collection
Serum and Plasma Samples*

↓

3 month data collection
MRI*, Labs, Physical Assessments, Self-Administered Questionnaires, Neurocognitive Tests, 6-Minute Walk, Disability Measures, SPPB, Serum and Plasma Samples*, DNA Lab*

↓

12 month data collection
Labs, Physical Assessments, Self-Administered Questionnaires, Neurocognitive Tests, 6-Minute Walk, Disability Measures, SPPB, Serum and Plasma Samples*

↓

2 year data collection
MRI, Labs, Physical Assessments, Self-Administered Questionnaires, Neurocognitive Tests, 6-Minute Walk, Disability Measures, SPPB
45% will undergo a treadmill or stationary bike cardiopulmonary exercise test (CPET)
Serum and Plasma Samples*

↓

3-11 yearly data collection
Cardiac event assessment

* Indicated for Chemotherapy Group only

Stratification: equal # of females < 52 years of age vs. ≥ 52 years of age
Anthracycline vs. non-Anthracycline

Study Sample: n=1000

Brief Eligibility Criteria: 840 Females aged ≥ 18 years old who are scheduled to receive adjuvant or neo-adjuvant chemotherapy for Stage I-III breast cancer (including inflammatory and newly diagnosed, or locally recurrent but not metastatic breast cancer being treated with curative intent) and 160 healthy cancer free women for statistical comparisons.

Study Duration: 24 months per subject with an additional follow-up for 7-9 years (total of 9-11 years)

Table of Contents

1. OBJECTIVES

Objective 1: To determine the incidence and time course of alterations in LV and aortic function, exercise capacity and fatigue in women treated for Stage I-III breast cancer. How are these changes interrelated, and how do they impact 7-year occurrence of CV events (myocardial infarction, cardiac death, and symptomatic HF) upon receipt of anthracycline or non-anthracycline-based chemotherapy?

Objective 2: To determine which baseline demographic, behavioral, and psychosocial CV risk factors are associated with the development of LV/aortic dysfunction, impaired exercise capacity, symptomatic fatigue, disability, and CV events.

Objective 3: To determine if Adj-C (with/without XRT) associated changes in serum biomarkers or other CV risk factors precede the onset of LV/aortic dysfunction, exercise intolerance, symptomatic fatigue, disability, or CV events.

Objective 4: To determine if existing American Heart Association (AHA) or other risk factor models forecast the future development of CV events in women that received Adj-C for breast cancer relative to historical forecasts in women not receiving Adj-C. Do models using other metrics further improve one's ability to predict CV events after Adj-C?[1-25]

2. BACKGROUND

Early detection and adjuvant therapies for breast cancer have improved 5-year cancer-related survival rates to over 80**%** for women with Stage I-III breast cancer. Unfortunately, many breast cancer survivors are at high risk for long-term left ventricular (LV) dysfunction, exercise intolerance, progressive fatigue, and cardiovascular (CV) events—including heart failure (HF) —possibly because of their initially life-saving chemotherapy.[1-12] The emergence of these CV events among breast cancer survivors threatens to offset the remarkable gains in cancer-related survival achieved through concurrent management.

Although some treatments (e.g. anthracyclines) are clearly associated with acute HF,[6-12] the problem of cardiovascular complications following chemotherapy for breast cancer goes far beyond anthracyclines alone. In addition, other agents such as trastuzumab, taxanes, and cyclophosphamide may also promote cardiovascular injury. Much more information is needed concerning the etiology, natural history, and factors that potentiate onset of CV complications after non- or anthracycline-based neo- or adjuvant chemotherapy (Adj-C) in women with breast cancer. For instance, do pre-existing or dynamic changes in CV risk factors during Adj-C, or receipt of concomitant treatments (e.g. radiation) influence onset or persistence of LV dysfunction and exercise intolerance? Do different doses of these medications influence fatigue? How do these non- or anthracycline induced effects differ from one another or normal aging? Do cancer therapy-mediated changes in aortic stiffness (versus direct myocardial toxicity) contribute to exercise intolerance or HF? Do existing CV risk equations adequately predict CV events in cancer survivors, and can they be incorporated into adjuvant and neo-adjuvant treatment decisions? Will relatively rapid MRI scans that do not utilize ionizing radiation facilitate management of these patients? Answers to these questions are urgently needed if we are to restore optimal LV function, exercise capacity, and quality of life, as well as preventing CV events in breast cancer survivors.

In two prospective pilot cohort studies,[13,14] we found that CV injury and LV dysfunction a) begin early (1–3 months) after initiating Adj-C, b) persist for at least 6 months after Adj-C, and c) occur concurrently with limitations in daily activities. Based on these preliminary observations, we now propose the first large-scale epidemiologic cohort study to characterize the onset, natural history, and correlates of

subclinical CV dysfunction, exercise intolerance, fatigue, and CV events in women with breast cancer treated with Adj-C via the aforementioned objectives.

Impact: The results of this research will define relationships between Adj-C and CV dysfunction, exercise intolerance, fatigue, an CV events in breast cancer survivors. Our results will inform risk prediction algorithms and the optimal design of future studies to establish guidelines for reducing exercise intolerance, fatigue, and CV events, thus improving overall survival for women treated for Stage I-III breast cancer.

As shown in Figure 1, our primary outcomes will help define the relationships between cancer therapy, CV injury, exercise intolerance and fatigue, while also accounting for the relative contributions of age, menopause status, race/ethnicity, radiation therapy, and psychosocial and behavioral risk factors. In addition, we will assess pre-existing cardiac risk factors (hypertension, smoking, diabetes, coronary artery disease, dynamic changes in body mass index, blood pressure, serum lipids and fasting glucose, physical activity, and chemo- and immunotherapy).

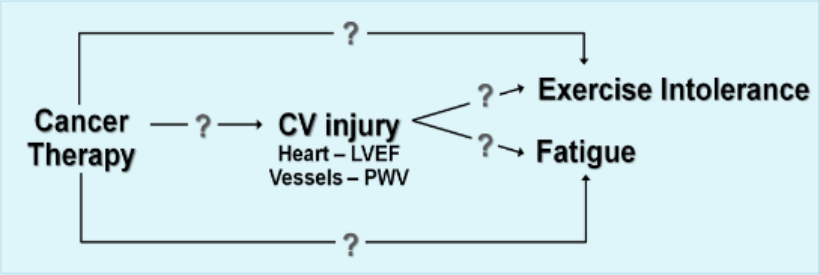

Figure 1. Mechanisms defined by this study. This study will address the relationships between cancer therapy, CV injury, exercise intolerance and fatigue as primary outcomes. Relative contributions of age, menopause status, race/ethnicity, and radiation therapy, as well as psychosocial and behavioral risk factors will be analyzed as covariates.

Based on our strong preliminary data (Section 2.1), we hypothesize that during receipt of either anthracycline or non-anthracycline-based Adj-C, subclinical CV injury occurs early and is associated with short-term (3 months) and with long-term (2 year) development of exercise intolerance and progressive fatigue. Unraveling the sequence of events in breast cancer survivors that promotes conditions associated with CV events could provide the insight necessary to develop therapies to prevent these events after Adj-C. To address this hypothesis, we propose to conduct the first collaborative effort among oncologists (through the NCI) and CV scientists (through the NHLBI) to address prospectively the relationship between Adj-C and CV injury, exercise intolerance and fatigue in women treated for breast cancer.

This study is significant in that it addresses:

I. A prevalent clinical problem. CHF and CV events (34,000 annually in the US) are the primary causes of morbidity and mortality after Adj-C for breast cancer.[7]

II. A shortcoming in the billing code and cross-sectional data is that they are unable to characterize the natural history of subclinical CV disease, exercise intolerance and fatigue in breast cancer survivors using methods previously established and well-validated in other large population-based NHLBI-funded studies (e.g., The Multi-Ethnic Study of Atherosclerosis [MESA] or the Jackson Heart Study [JHS]).[22]

III. The relationship between Adj-C administration, subclinical injury, exercise capacity, and fatigue, controlling for the influence of associated CV comorbidities and psychosocial and behavioral risk factors on this relationship.

IV. NHLBI and NCI mandates including: a) the NHLBI goal to improve understanding of the mechanisms of CV disease, thereby enabling better prevention, diagnosis and treatment;[23] and b) the NCI's goal to determine actionable strategies that reduce the burden of morbidity and mortality in cancer survivors.[24]

2.1. Preliminary Data

During the last 10 years, WFUHS investigators have published nearly 30 peer-reviewed articles[13,25,26,29-55] about the evolution of CV disease, exercise intolerance and fatigue in middle-aged and older individuals, including those receiving chemotherapy. We have demonstrated our capabilities to:

I. Recruit and retain patients receiving treatment for cancer into longitudinal cohort studies involving serial CV MRI assessments of subclinical outcomes;

II. Identify relationships between pulse wave velocity (PWV) and Adj-C administration in cancer survivors;

III. Quantify longitudinal changes in MRI measures of LV volumes, strain, LVEF, and aortic PWV as well as self-reported measures of fatigue; and

IV. Effectively measure differences in peak and submaximal exercise capacity in large multicenter studies.

Publications related to these developments are provided below. These pilot studies were funded by NIH grants R33CA121296 and R01HL076438, and a grant from the Susan G. Komen Foundation.

- MRI assessments of signal intensity within the LV indicate myocellular injury in animals and are abnormal in patients receiving Adj-C. *Circ Cardiovasc Imaging. 2010;3:550-8.*[56] *J Am Coll Cardiol. 2011;57:E782.*[57]

- Within 3 months, Anth-bC increases PWV, a predictor of CV events. *J Clin Oncol. 2010;28:166-72.*[13]

- Six months after Anth-bC, PWV increases; LVEF and myocardial strain diminish; and limitations in daily activities occur and persist 3 months after cessation of Adj-C. *Circulation. 2010;122 Supplement:A12766*[14] *and JACC Imaging 2012 (accepted for publication).*[58]

- Six months after non-anthracycline based chemotherapy, PWV increases, and LVEF diminishes comparable to changes seen with Anth-bC. We measured LV volumes, EF, and PWV at baseline and then 6 months after receipt of non-anthracycline-based chemotherapy for breast cancer. Women receiving non-anthracycline Adj-C also experienced statistically significant and clinically relevant aortic stiffening and diminished LV performance ($p < 0.01$ for both).

- Our research team can accomplish measurements of cardiac and vascular function with MRI, peak VO_2 and 6-minute walk, and fatigue across multiple centers participating in longitudinal cohort studies or clinical trials.[25,26,38,39,45,46,52] Our exercise testing core lab faculty possesses the expertise to help retain participants in these studies. All of the methods are similar to those utilized in other NHLBI-funded studies.

3. SUMMARY OF STUDY PLAN

We propose a cohort study in 840 women aged ≥ 18 years old scheduled to receive chemotherapy for Stage I-III breast cancer and a comparison population of 160 women without cancer (1,000 total). We will recruit equal numbers of women aged < 52 vs. ≥ 52. At baseline, we will collect innovative MRI measures of CV function (LV and aorta), measurements of submaximal (6-minute walk) and, on 45% of

the cohort, maximal (peak VO_2) exercise capacity (CPET), questionnaire data to assess fatigue and behavioral and psychosocial risk factors, and biomarkers. At sites with the capacity and capability to perform the maximal (peak VO2) exercise capacity test, a random sample of participants will be assigned to the CPET sub-study and receive maximal (peakVO_2) exercise capacity testing. The probability of receiving this testing may vary over time depending on the number of participants accrued to the sub-study. Study measurements will be repeated at the 3 month, 12 month, and 24 month visits as described in Section 7. This study will assess the relevance of pre-existing factors such as age, black/white race, hypertension, smoking (yes/no), diabetes, coronary artery disease, menopause status, CV medications, and physical activity on the study outcomes. Also, this study will assess dynamic change in modifiable CV risk factors (including BMI, blood pressure, serum lipids, serum glucose, physical activity, psychosocial factors, lifestyle behaviors), and the cancer treatment including chemotherapy, radiation therapy, immunotherapy, and surgery.

4. PARTICIPANT SELECTION

4.1. Chemotherapy Group

4.1.1. Inclusion Criteria

- Stage I-III female breast cancer (including inflammatory and newly diagnosed, or locally recurrent but not metastatic breast cancer being treated with curative intent)
- $\geq$ 18 years old
- Scheduled to receive chemotherapy
- Able to hold breath for 10 seconds
- ECOG performance status 0 – 2*
- Able to walk at least 2 blocks without chest pain, dyspnea, shortness of breath or fainting
- Able to exercise on a treadmill or stationary cycle
- Participants in other ongoing clinical trials are eligible for this study

4.1.2. Exclusion Criteria

- Those with ferromagnetic cerebral aneurysm clips or other intraorbital/intracranial metal; pacemakers, defibrillators, functioning neurostimulator devices, non-compatible MRI tissue expanders or breast implants, or other implanted non-compatible MRI devices
- If previously measured, known LVEF < 50%
- Symptomatic claustrophobia
- Unable to provide informed consent
- At the beginning of the study, pregnant women and women who are breast-feeding will not be enrolled
- Severe pulmonary hypertension
- Within the past 6 months:
  - Acute pulmonary embolus
  - Deep vein thrombosis
- Within the past month:
  - Heart attack
  - Unstable or stable angina (cardiac chest pain)
  - Left main coronary artery disease
  - Symptomatic heart failure
  - Uncontrolled hypertension (SBP > 180 mm Hg or DBP > 100mm Hg)
  - Severe valvular heart disease

- Uncontrolled metabolic disease (diabetes with fasting BS >300 mg/dl, thyrotoxicosis, myxedema)
- Aortic aneurism (>45 mm diameter) or aortic dissection
- Uncontrolled slow or fast heart rhythm causing symptoms or hemodynamic compromise
- Hypertrophic obstructive cardiomyopathy

4.2. Control (Healthy) Group

4.2.1. Inclusion Criteria

- Healthy female without known coronary artery disease
- ≥ 18 years old
- Able to hold breath for 10 seconds
- ECOG performance status 0 – 1*
- Able to walk at least 2 blocks without chest pain, dyspnea, shortness of breath or fainting
- Able to exercise on a treadmill or stationary cycle
- No history of cancer
- Never received chemotherapy, radiation therapy, immunotherapy, or had breast surgery

4.2.2. Exclusion Criteria

- Inflammatory conditions, such as rheumatoid arthritis, systemic lupus or inflammatory bowel disease
- Overt coronary artery disease or heart failure
- Those with ferromagnetic cerebral aneurysm clips or other intraorbital/intracranial metal; pacemakers, defibrillators, functioning neurostimulator devices or other implanted non-compatible MRI devices
- If previously measured, known LVEF < 50%
- Symptomatic claustrophobia
- Unable to provide informed consent
- At the beginning of the study, pregnant women and women who are breast-feeding will not be enrolled.
- Severe pulmonary hypertension
- Within the past 6 months:
  - Acute pulmonary embolus
  - Deep vein thrombosis
- Within the past month:
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  - Unstable or stable angina (cardiac chest pain)
  - Left main coronary artery disease
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  - Uncontrolled metabolic disease (diabetes with fasting BS >300 mg/dl, thyrotoxicosis, myxedema)
  - Aortic aneurism (>45 mm diameter) or aortic dissection
  - Uncontrolled slow or fast heart rhythm causing symptoms or hemodynamic compromise
  - Hypertrophic obstructive cardiomyopathy

4.3. Eastern Cooperative Oncology Group (ECOG) Performance Status

*Eastern Cooperative Oncology Group (ECOG) Performance Status – scale used to describe a patient's level of functioning in terms of their ability to care for themselves, daily activity and physical ability (walking, working, etc.).

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 a light or sedentary nature (e.g., light house work, office work)
2 = Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.

4.4. Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial.

	Race/Ethnicity					
Gender	**White, not of Hispanic Origin**	**Black, not of Hispanic Origin**	**Hispanic**	**Asian or Pacific Islander**	**Unknown**	**Total**
Male	0	0	0	0	0	0
Female	620	250	100	30	0	1,000
Total	620	250	100	30	0	1,000

4.5. Recruitment and Retention Plan

Our recruitment strategies are discussed in detail and are led by Drs. Karen Winkfield, Shannon Mihalko, and a comprehensive team with experience recruiting many participants to longitudinal cohort studies of CV disease and breast cancer. Three recruitment strategies will be used: a) community-wide media-based strategies, b) strategies targeted at enriched cohorts identified through presentation to Cancer Centers or, if available, multidisciplinary breast cancer clinics within the respective sites, and c) strategies focused on minority recruitment.

Our study design will emphasize recruitment of minorities, with a particular goal to recruit 25% African-American women, and to meet ethnic distributions within the catchment areas of our enrolling sites. To achieve these goals, we have already reviewed our protocol and recruitment materials with the Wake Forest NCORP Research Base Minority Recruitment Committee and the Comprehensive Cancer Center's Cancer Health Equity Advisory Committee in order to solicit suggestions to enhance the appeal of our study to minority participants.

Throughout the study, we will monitor minority recruitment rates at monthly meetings of our Recruitment Committee, which includes members of our study team and members of the Wake Forest NCORP Research Base. This Committee will also provide feedback to the NCORP sites quarterly via teleconference. During these calls, we will encourage sharing of ideas between sites with strong minority recruitment and our other sites. Black women, aged 35-80 years, account for 10% to 28% of the populations of these regions. In recruitment to our pilot and prior similar NCORP studies of breast cancer, we have achieved 25% enrollment of black women. To achieve our minority recruitment goal of 25% black women, additional strategies have been specifically designed to identify and recruit black women.

Minority recruitment efforts will be directed by Dr. Karen Winkfield. She serves as Director of the Office of Health Equity and is a Radiation Oncologist within the Comprehensive Cancer Center at Wake Forest School of Medicine. She also leads the Wake Forest NCORP Research Base minority and diversity efforts and is a member of the Research Base Executive Steering Committee.

In addition, other individuals identified within the specific sites are available to assist with minority recruitment efforts. The strategies to be employed include focused recruitment from clinics and private practices with a large population of black patients and endorsement from local black physicians, advertisements in newspapers or on radio stations with a predominately black audience, networking with municipal agencies, private services, and social organizations such as The Urban League and local black sororities, and by working with local black church leaders.

In the event that our initial recruitment monitoring indicates that we will not meet our 25% African-American recruitment goal, we will initiate a leadership survey in the black communities in our respective recruitment areas. Briefly, a leadership survey is a systematic method of informing the leaders in a specific community about a program and eliciting their individual and collective support. The survey is a step-by-step process whereby individual meetings are arranged with well-known black leaders, such as aldermen and ministers. Each person is told about the project, asked his or her opinion concerning the efficacy of the project, potential problems, and asked what steps should be taken to insure success of the program. Each leader is then asked to name additional "leaders" to be contacted (with the term "leader" meaning anyone within the community to whom others turn to for help, advice, support, or comfort). The interview process is continued until no new names are submitted or until it is felt that no new information is being gathered.

This process serves several functions within the community:

1. Alerts the leaders of the program and insures that they know what is happening and will, therefore, not be caught by surprise if questioned about it.
2. Notifies the study team of any potential problems that may arise, especially leaders who are negative about the program.
3. Allows members of the study team to meet face-to-face with influential members of the community and get to know them.

It also leads to diffusion of the message, which, in this case, will be advanced notice of the need for women to participate in the program.

The community analysis previously conducted for similar studies have been updated in the Winston-Salem/Forsyth County area and similar analyses are planned to be performed, if necessary, in other NCORP sites.

Our experience suggests that this combination is synergistic and will be successful. Within the recruitment areas of the participating sites, there are over 2,300 women eligible for the study each year. Approximately 95% of these individuals are ≥ 18 years of age. To meet our targeted recruitment, we require that only 17% (1 in 6) of the women approached agree to participate. Recruitment sites were selected based on their ability to a) obtain high quality MRI and exercise testing data, b) access to breast cancer patients with different hormone statuses, anthracycline use, and racial/ethnic and age-related diversity, c) ability to obtain MRI, exercise testing, and questionnaire-related information quickly upon enrollment before chemotherapy begins; and d) a high level of interaction among on-site investigators interested in this project. Each site underwent a thorough interview, including imaging, cardiovascular, and oncologic investigators and staff from each site. The ability to perform all primary outcome measures, presence of the proper equipment, and experience with collecting these data previously was

also confirmed. In addition, each site underwent an administrative review confirming their access to eligible participants, willingness to refer, and ability to meet expected annual enrollment goals.

Enrollment will be through the Wake Forest NCORP Research Base community cancer centers. We need to enroll a total of 1000 participants, 840 participants with cancer and 160 without cancer. We propose to accomplish this recruitment in 2.5 years (10 3-month periods). Therefore, we need, on average, 100 patients per recruitment period (84 with cancer, 16 without). Since we anticipate slower accrual at the start of the study as sites come on board and become familiar with the protocol, we propose that in the 1st period (3-months) we will enroll 20 patients, in the 2nd period we will enroll 50 patients, and in the next 4 periods (1 year) we will target an enrollment of 100 patients per period. In the last 4 periods (1-year) we anticipate enrolling 133 patients in each quarter. This plan will result in 70 patients being enrolled during the first 6 months of the study, an additional 400 patients enrolled in the next year, and 530 patients enrolled during the last year of the study. Within each period the ratio of cancer patients to control patients will be roughly 6:1 (with more cancer patients enrolled during the early periods). With 2,300 women eligible for enrollment per year, we expect that we will be able to meet these target accrual goals. However, the study team will actively monitor accrual and recommend modifications to the recruitment strategy if necessary; if accrual is < 50% of what is expected after 2 months or is < 50% of expected target after 18 months, we will recommend new programs and policies.

To maximize retention, we will use four proven strategies: 1) recruit participants who do not anticipate leaving the area during the study; 2) use cognitive/behavioral management strategies to structure a positive testing environment (site study staff will regularly review the participants' data related to follow-up attendance and identify any who need additional reminders, cues to action and/or in-person meetings); 3) provide a $25 gift card per participant per visit (maximum limit of $100.00); and 4) use telephone interviews if in-person visits are not feasible. Adherence to scheduled clinic visits and the corresponding time windows will be systematically monitored by the Recruitment and Retention Committee. Collectively, these strategies will serve to maximize retention and to promote compliance with all testing procedures and data collection.

5. MEASUREMENTS ACQUIRED

5.1. MRI Variables

MRI investigators from each active site will participate via conference call in a review of the protocol. Then, two practice scans or anonymized scans (clinical or prior research that have used this protocol) will be submitted to the WFUHS MRI Reading Center (directed by Dr. Hundley and managed by Mrs. Kimberly Lane). Scans will be reviewed for adherence to the study protocol. Comments will be returned to the respective enrollment sites and will not be allowed to enroll participants until each site has performed two scans without protocol violations. Dr. Hundley and/or staff will visit sites as needed to verify and resolve any potential questions related to the study protocol.

The primary cardiac and vascular outcome measures include LVEF and PWV. A binary LVEF drop by 10% or absolute value < 50% was selected as our primary cardiac outcome because clinically it has been used for > 30 years to guide the administration of potentially cardiotoxic chemotherapy. Also, we will measure LVEF, LV end diastolic volume, LV end systolic volume, myocardial strain and strain rate, and mass according to published standards and treat them as certain variables.[64-66] Since vascular stiffness contributes to exercise capacity,[67] we will measure PWV because it does not rely on a measurement of central aortic pressure, is highly reproducible, and it can be very accurately measured with MRI (as opposed to ultrasound) because it does not require external marking of central landmarks.[66-68] Finally, we will acquire T1 and T2 maps of the LV myocardium that have been shown in other studies to be associated with myocardial injury.[69,70]

The MRI protocol is short (≤ 10-15 mins.) and does not utilize contrast, both of which reduce participant burden.[64,71-75] MRI is selected as the primary assessment of cardiac and vascular function because of a) its reliability (> 98% acquired valuable subjects within the JHS), b) its reproducibility (< 2% for repeated measures in pilot data from cancer participants), c) its accuracy (ability to detect informative changes indicative of prognosis regarding both the cardiac and vascular systems), d) its translational capability (existing large NHLBI efforts to assess CV disease from which cross-sectional comparisons with the data set will be made), and e) its recent recognition as superior to echocardiography for identifying cardiac dysfunction after chemotherapy.[76] Our research group and Dr. Hundley (PI) have extensive experience developing and using these MRI techniques in single-center or multi-center efforts, including the Jackson Heart Study (Dr. Hundley is Co-PI for the core lab) and the MESA study.[64,71,72,74,75] All MRI scans will be performed after a formal evaluation of the physical performance parameters of the CMR scanner. Each MRI assessment will be blinded to patient identifiers, the corresponding MRI exam, and prior MRI exams of the same measure (a blinded, unpaired read). A complete double reading on 15% of the CMR cases will be performed for quality control.

5.1.1. Quality Assurance Scans

Quality assurance (QA) scans for T1 mapping and ECV will be performed every six months according to study recommendations using a phantom specifically designed for such purposes [REF2]. T1 maps of the phantom will be acquired using the same scan parameters as used for human subjects. Detailed scan procedures are provided by the phantom manufacturer (Resonance Health, Claremont, Australia) and are supplied to each site for their guidance in executing the QA scans. Images are uploaded for analysis to the core lab in the same manner as subject images. A fully automated analysis program has been developed which determines the mean T1 value in each of the tubes in the phantom and uploads the results to Redcap for monitoring of changes over time. Feedback will be provided to sites on an ongoing basis to ensure T1 maps are accurate.

REF1: Messroghli et al. J Cardiovasc Magn Reson. 2017 19:75

REF2: Captur et al. J Cardiovasc Magn Reson. 2016 18:58

5.1.2. Additional Considerations

We will not administer gadolinium contrast to assess LV fibrosis because serum creatinine rose in 20% of our pilot subjects after chemotherapy (incurring an increased risk of nephrogenic systemic fibrosis with gadolinium).

5.2. Maximal (Peak VO_2) and Submaximal (6-minute walk exercise capacity outcomes)

Both maximal (peak $V0_2$)[52,77-79] and submaximal (6-minute walk) exercise capacity will be measured. Peak $V0_2$ is the primary exercise outcome because it is one of the most objective measures of exercise capacity, integrates the physiologic response to exercise, and provides information related to exercise and fatigue.[52,77-79] It provides an objective threshold of disability and a metric that forecasts adverse cardiac prognosis, and can be used to modify medical regimens to reduce the likelihood of morbidity and mortality.[52,77-79] It is feasible in large multi-center studies, as it is reproducible and standardized. It is semi-automated and relatively inexpensive to perform. For this study, Drs. Brubaker, Kitzman, and Mihalko will implement the cardiopulmonary exercise testing at all sites.

Our team has extensive experience with using Peak $V0_2$ as a primary measure.[26,45,46,50,52,77-83] Among 3 studies for which our team serves as the core lab, the reproducibility and variance of the measure in

elderly individuals (> 65 years) or those with evidence of congestive HF ranges from 7% to 12% in repeated measures.[52,77-79] In addition, we will measure the 6-minute walk, a measurement that is relatively easy to administer, is feasible, and accurately assesses submaximal exercise capacity. Similar to Peak $V0_2$, the 6-minute walk is an independent predictor of CV mortality. Because it is commonly performed, we can compare data from this cohort with other large NHLBI-sponsored multi-center initiatives. Our team, including Dr. Mihalko, has utilized the 6-minute walk as an outcome measure in studies of exercise interventions in individuals treated for cancer.[30]

5.3. Fatigue and Health Status

The Functional Assessment of Cancer Therapy Fatigue scale (FACT-F) will be used to assess fatigue.[84] The FACT-F scale is a 13-item scale used in many studies to assess cancer-related fatigue. Respondents rate the degree to which each item applies in the past 7 days using a 5-point scale. Scores range from 0–52, with higher scores indicating greater fatigue. Although initially developed as part of the FACT cancer scales, the scale is also appropriate for a general population. A recent review noted the FACT-F had robust psychometric data and easy administration.[85]

The RAND MOS 36-item Short Form Health Survey (SF-36) will also be used as a general measure of health status.[86] The SF-36 is perhaps the most widely used measure of health status and includes domains of vitality and physical function. The SF-36 consists of 36 items measuring the following 8 domains: physical function, role limitations due to physical health problems, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional health problems, and mental health. These 8 domains also provide two summary scores. Sedentary lifestyle behaviors will be assessed by the PACE Adult Sedentary Behaviors Survey.

5.4. Behavioral and Psychosocial Risk Factors

The following psychosocial and behavioral covariates will be assessed as CV risk factors that might mediate the relationship between cancer therapy and CV outcomes.[87] Depression will be measured by the 10-item Center for Epidemiological Studies Depression Scale (CESD-10), a screening questionnaire assessing depressive symptoms during the last week.[88] During the assessment if the patient is identified as depressed, the site study staff will assess the severity of the depression and report to the treating physician at the site to further evaluate if medication and/or counseling is needed.

Perceived Stress will be measured by Cohen's 4-item Perceived Stress Scale (PSS), a summed scale asking how often over the prior two weeks four aspects of stress were experienced (1=never to 5=very often).[90] 14 items (True or False) from the Cook-Medley Hostility Scale questionnaire will be used to assess the effect of hostility associated with cardiovascular risk factors.

The RAND Social Support Scale[91] will be used to measure social support and assesses four aspects: emotional support, tangible support, informational support, and appraisal support. Self-reported physical activity using the Godin Leisure-Time Exercise Questionnaire (LTEQ) will be our main process measure of physical activity participation. The LTEQ asks participants how often they participated in physical activity for more than 30 minutes within a typical 7-day
period, at three different intensity levels (strenuous, moderate, and mild).[92] Active smoking will be ascertained from the American Thoracic Society questions. Finally, sociodemographic data will include marital/partner status, education, employment, financial strain, and race/ethnicity.

The PROMIS short form 8a measure of sleep disturbance, consisting of 8 items will be used to assess the time course and risk factors associated with sleep disturbance and fatigue.

Two PROMIS measures of self-assessed cognition: the Applied Cognitive Abilities Short Form 8a and the Applied Cognition General Concerns Short Form 8a measure different aspects of cognitive functioning since fatigue can have a direct impact on cognitive functioning and cognitive functioning may impact exercise capacity.

The Walking Efficacy for Duration scale (McAuley and Mihalko) is comprised of six items and will be included as a measure of exercise capacity. Self-efficacy for walking is highly related to physical activity participation in women with breast cancer. Participants will rate their confidence in their ability to walk at a moderate pace for 10-60 minutes on a scale from 0% to 100%.

5.5. Neurocognitive Tests

COWA and Trailmaking Tests A & B will be administered by study staff according to instructions provided in the Neurocognitive Tests booklets. Study staff must be certified by the Wake Forest NCORP Research Base Site Coordinator team in order to administer these tests.

Controlled Oral Word Association Test (COWA): The COWA (Benton & Hamsher, 1976) measures speed of mental processing, verbal fluency, and executive function. Subjects are asked to name as many words as possible all beginning with a specified letter. A total of three trials are administered, each with a different letter (C-F-L). The score on the COWA is the total number of words named across the three trials minus repetitions. The COWA has two equivalent forms (C-F-L and P-R-W) that will reduce practice effects. Internal consistency reliability (alpha=0.83) and test-retest reliability (r = 0.74*)* are excellent (Ruff et al., 1996).

Trail Making Test, Parts A & B (TMT-A, TMT-B): Part A of the TMT(Reitan, 1958) measures attention and visual motor skills and processing speed and requires subjects to connect 25 numbered circles in the proper sequence (1-2-3-…) as quickly as possible. TMT-B is similar except subjects are required to connect dots in an alternating numerical and alphabetical sequence (1-A-2-B-…). TMT-B with its added complexity and set shifting requirements is a widely used measure of executive function. The score for TMT-A and TMT-B is the total time in seconds required to complete the task. Scores can also be generated for number of errors and number of circles correctly connected. The TMT has excellent reliability and validity (Reitan, 1992).

5.6. Disability Measures

Range of Motion: Range of motion at the shoulder joint will be assessed with shoulder flexion and shoulder abduction using a goniometer. To assess flexion, the participant will be asked to stand tall and will be cued to raise and hold the arm as straight as possible to the point of tension; palms should face medially toward the body, raising arm straight out in front as high as possible without pain. To assess abduction, the participant will again be asked to stand tall with arms down to the side; this time, the palms face away from the body and are raised straight out to the side as high as possible without pain. For both flexion and abduction, two trials should be conducted alternating each arm with at least 30 seconds between trials for the same arm.

Grip Strength: Grip strength is a relatively easy test to perform with established normative data. Grip strength is assessed using an isometric handgrip dynamometer while the participant is seated with the head facing straight ahead. The elbow should be bent at a 90 degree angle and the wrist should be at the mid-prone position. The participant should exert maximally and quickly (about 1 second) and two trials should be made alternately with each hand, with at least 30 seconds between trials for the same hand. The tester should record the force in kilograms.

5.7. Expanded Short Physical Performance Battery (SPPB)

Standing balance: For the test of standing balance, participants will be asked to maintain balance for up to 30 seconds in three positions characterized by a progressive narrowing of the base support: heel of one foot beside the big toe of the other foot (semi-tandem position), heel of one foot in front of and touching the toes of the other foot (tandem position), and single leg stand**.**

Gait Speed Test: The gait speed test will assess the participant's ability to walk 4 meters. Participants will be instructed to start at a marked walking course with toes touching the start line and when cued to start, will begin walking at their usual speed. The time to walk from the starting line to the end of the 4-meter walk will be recorded.

Chair Stands: The repeated chair stand test will be performed using a straight-backed chair placed with its back against a wall. Participants will be first asked to stand from a sitting position without using their arms. If they can perform the task, they will then be asked to stand up and sit down five times as quickly as possible. The time to complete the task will be recorded.

5.8. Serum and Blood Derived Biomarkers

Cardiac biomarkers, including serum troponins and N-terminal pro-B-type natriuretic peptide (NT pro-BNP), have emerged as clinically relevant methods to appreciate cardiac injury associated with chemotherapy.[93,94] In this study, early assessments of serum troponin commensurate with acquisition strategies used in other cohort studies will be acquired to determine associations between troponin levels during therapy and the onset of cardiac and vascular dysfunction, exercise capacity, and fatigue. Serum N-terminal pro-BNP as a predictor of exercise intolerance and CRP to assess whether systemic inflammation persists long after the administration of chemotherapy or radiation therapy will be obtained.[95]

5.9. Optional DNA Testing

DNA tests may include searches for combinations of genetic nucleotide polymorphisms that are associated with susceptibility to a fall in LVEF in patients receiving chemotherapy. At present, planned testing is not specified and thus not included in our specific aims.

5.10. Assessments of Therapy

All of the site staff and coordinators will be oriented into methods to collect the types, routes of administration, and amounts of the various therapies (chemotherapy, immunotherapy and radiation) for cancer treatment. These will be entered monthly into a central database managed and operated through the Wake Forest NCORP Research Base.

At the conclusion of the administration of chemotherapy and radiation therapy (RT) for each participant, a rectification of data entered into the relational database will occur with the participant's medical records from the treating facilities. Discrepancies will be resolved through communications with the site-specific staff and the treating oncologists. Dr. Heidi Klepin, who has expertise in the implementation of cohort studies of breast cancer patients as well as multiple treatment regimens, will supervise this aspect of the study.

For individuals receiving adjuvant RT, treatment records, CT treatment planning data (DICOM) and dose data (DICOM-RT) files will be electronically submitted to the WF NCORP Research Base Data Management Center. Training for each of the staff will be provided by Dr. Winkfield at site request and

then as needed to insure compliance and accuracy. A validated cardiac atlas will be used for identification of cardiac structures and dose to each of these areas will be determined.[96] A multi-modality deformable registration program (MIM software) will be used to align treatment planning data to the cardiac MRIs from each participant. Due to the variety of radiation delivery methods employed at each site for left-sided breast cancer treatment and the natural comparators of right-sided breast cancer patients (who should have minimal cardiac radiation), we expect to see a range of doses to cardiac structures (0-60 Gy). We will then be able to analyze whether there is a radiation treatment effect, and if so, determine a dose-response relationship.

6. DEVICE AND GIFT CARD INFORMATION

6.1. Availability and Distribution

Grip strength will be assessed using an isometric handgrip dynamometer. Range of motion will be assessed using a goniometer. A measuring wheel will be used to measure the distance walked during the 6-minute walk test. A stopwatch will be used for each timed test. The Wake Forest NCORP Research Base Coordinating Center will distribute devices to sites upon receipt of local IRB notification. Upon completion of the last participant, sites will return devices to Wake Forest NCORP RB.

Gift cards will be administered to each site for distribution to study participants upon their completion of all evaluations/tests at baseline, 3, 12 and 24 months.

7. STUDY EVALUATIONS AND PROCEDURES

7.1. Study Parameter Tables

7.1.1. Chemotherapy Group

Evaluations/ Tests	Pre-Study Evaluation	Enrollment/ Baseline	1 Month (±7 days)	3 Month (±30 days)	12 Month (±60 days)	24 Month (±60 days)	Years 3-11 (J) (±60 days)
Confirm Eligibility	X						
Decline Form (A)	X						
Informed Consent	X						
Enrollment Form		X					
Data Collection Form		X	X	X	X	X	
Chemotherapy Treatment Form				X	X	X	
Radiation Intake Form		Complete form for the first radiation treatment					
Radiation Summary Form		Complete form once radiation treatment has been completed					
Medical Chart Review – Chemotherapy Group		X					
Physical Assessment (B)		X		X	X	X	
CV Medication Review Form		X		X	X	X	
Other Medications Form		X		X	X	X	
Study Lab Chemistry (C)(D)		X		X	X	X	
Serum and Plasma Samples (E)		X	X	X		X	
DNA Lab (F)		X		X			
The KC Cardiomyopathy Questionnaire (KCCQ-12)		X				X	X
Self-Administered Questionnaire (booklet) (G)		X		X	X	X	
Neurocognitive Tests (booklet) (G)		X		X	X	X	
6-Minute Walk, Disability Measures, SPPB (booklet) (G)		X		X	X	X	
MRI		X		X (I)		X	
CPET (H)		X				X	
Cardiac Event Form and Phone Interview							X

(A) Complete for all subjects who were eligible when screened and approached but declined participation.
(B) Height, weight, heart rate, blood pressure and BMI.
(C) Hematocrit, glucose, creatinine, lipid profile (LDL Cholesterol, HDL Cholesterol, Total Cholesterol, Triglycerides) and C-reactive protein.
(D) Samples will be sent to Lab Corp.
(E) Only Chemotherapy Group participants will have serum and plasma samples collected. Samples will be sent to the WF NCORP Research Base lab.
(F) DNA samples will be collected from participants in the Chemotherapy Group who have given consent to store blood for future DNA testing. Samples will be sent to the Center of Genomics and Personalized Medicine of Wake Forest School of Medicine, Winston-Salem, North Carolina.
(G) Questionnaires and tests in each booklet are listed in Section 7.8.1, 7.8.2, and 7.8.3.
(H) Only participants randomly assigned to CTEP after electronic enrollment has been submitted.
(I) Only participants in the Chemotherapy Group will receive an MRI at the 3 Month time point.
(J) All study participants will be followed annually from the 3 year time point through the 11 year time point or until enrollment closed, whichever occurs first.

7.1.2. Control (Healthy) Group

Evaluations/ Tests	Pre-Study Evaluation	Enrollment/ Baseline	3 Month (±30 days)	12 Month (±60 days)	24 Month (±60 days)	Years 3-11[G] (±60 days)
Confirm Eligibility	X					
Decline Form [A]	X					
Informed Consent	X					
Enrollment Form		X				
Data Collection Form		X	X	X	X	
Physical Assessment [B]		X	X	X	X	
CV Medication Review Form		X	X	X	X	
Other Medications Form		X	X	X	X	
Study Lab Chemistry [C][D]		X			X	
The KC Cardiomyopathy Questionnaire (KCCQ-12)		X			X	X
Self-Administered Questionnaire (booklet) [E]		X	X	X	X	
Neurocognitive Tests (booklet) [E]		X	X	X	X	
6-Minute Walk, Disability Measures, SPPB (booklet) [E]		X	X	X	X	
MRI		X			X	
CPET [F]		X			X	
Cardiac Event Form and Phone Interview						X

(A) Complete for all subjects who were eligible when screened and approached but declined participation.
(B) Height, weight, heart rate, blood pressure and BMI.
(C) Hematocrit, glucose, creatinine, lipid profile (LDL Cholesterol, HDL Cholesterol, Total Cholesterol, Triglycerides) and C-reactive protein.
(D) Samples will be sent to Lab Corp.
(E) Questionnaires and tests in each booklet are listed in Section 7.8.1, 7.8.2, and 7.8.3.
(F) Only participants randomly assigned to CTEP after electronic enrollment has been submitted.
(G) All study participants will be followed annually from the 3 year time point through the 11 year time point or until enrollment closed, whichever occurs first.

7.2. Pre-Study Evaluation and Consent

7.2.1. Chemotherapy Group

At each participating NCORP site, medical charts will be screened to determine potential eligibility by physicians (including residents or fellows, if applicable), research nurses, or site study staff. NCORP sites should follow the requirements of their institutional policy regarding contacting patients (i.e: if partial HIPAA waiver is required). Patients identified as potentially eligible will then be asked to consider joining the study. while in-clinic or from their physician informing them about the study, and indicating that a research nurse/assistant will be calling to tell them more about the study. Patients who meet the Chemotherapy Group eligibility criteria and agree to participate must sign informed consent prior to enrollment and scheduling baseline study activities. Consent must be obtained within 30 days before baseline study activities.

7.2.2. Control (Healthy) Group

Participants in the Chemotherapy Group will be asked if they are aware of friends or family members that may want to participate in the study Control (Healthy) Group. In addition, CIRB approved recruitment materials will be made available for use at participating sites and at healthcare delivery systems that also have cancer centers that are enrolling individuals with breast cancer. It is from these two sources that individuals from similar communities will be enrolled. Individuals who meet the Control (Healthy) Group eligibility criteria and agree to participate must sign informed consent prior to enrollment and scheduling baseline study activities. Consent must be obtained within 30 days before baseline study activities.

7.3. Participant Enrollment

Electronic enrollment must be completed within 7 days of obtaining consent.

NCORP site staff will electronically enroll their study participants in the Wake Forest NCORP Research Base database website, CCRBIS, at https://ccrbis.phs.wakehealth.edu.

- Log in to the database website using your CCRBIS username and password.
- In the drop-down menu next to Enroll Patient/Patient Info select 97415, then click Enroll Patient/Patient Info.
- Click on Enroll New Patient.
- Complete the Eligibility Checklist/Enrollment Form then click Submit.
- Following successful submission, a confirmation page will appear with the assigned PID, print this page for your records.
- Submit a copy of the signed informed consent form to the Wake Forest NCORP Research Base by fax at (336) 713-6476 or mail to:

Attn: UPBEAT Site Coordinator
WF NCORP Research BaseWake Forest Baptist Medical Center
Building 525@Vine, 4th floor (Box 573152)
Medical Center Boulevard
Winston-Salem, NC 27157

If you have questions related to the participant enrollment process or require assistance with enrollment, please contact the Wake Forest NCORP Research Base between 8:00am and 5:00pm EST, Monday through Friday at (336) 713-3172 or (336) 713-5086 or by email at NCORP@wakehealth.edu.

7.4. Baseline Evaluations

All study participants will undergo a baseline physical assessment (to obtain height, weight, heart rate, blood pressure and BMI), review of current medications with site study staff, MRI exam, laboratory tests (study lab chemistry), KCCQ-12, complete self-administered questionnaires, neurocognitive tests, 6-minute walk, disability measures, and exercise tests (Expanded SPPB).

Only participants randomly assigned to have a treadmill or stationary bike cardiopulmonary exercise test (CPET) will complete CPET at baseline. Site staff will be notified if a participant has been randomly assigned to perform CPET after the electronic Eligibility and Enrollment Form has been completed.

Only participants in the Chemotherapy Group will have a medical chart review and serum/plasma samples collected and shipped to the Wake Forest NCORP Biospecimen Lab at baseline. Chemotherapy Group participants who have given consent to DNA testing will also have DNA blood samples collected and shipped to the Wake Forest NCORP Biospecimen Lab at baseline.

Due to the scheduling complexity of protocol requirements for participants in the Chemotherapy Group, baseline activities may be performed at the discretion of the site within 30 days before the start of the first chemotherapy treatment. Baseline activities performed greater than 30 days before the start of the first chemotherapy treatment must be repeated.

All baseline activities for the Control (Healthy) Group must be completed within 30 days of obtaining consent.

7.5. Evaluations During Study

7.5.1. Chemotherapy Group

All study visits for the Chemotherapy Group will be scheduled based on the date of the participant's first chemotherapy treatment.

1 Month Visit:

Serum and plasma samples will be collected and shipped to the WF NCORP Research Base lab at the 1 month visit.

3 Month, 12 Month and 24 Month Visits:

At the 3 month, 12 month and 24 month visits, the Chemotherapy Group participants will undergo a physical assessment (to obtain height, weight, heart rate, blood pressure and BMI), review current medications with site study staff, complete self-administered questionnaires, neurocognitive tests, 6-minute walk, disability measures, and exercise tests (Expanded SPPB).

Study Lab Chemistry – laboratory tests will be collected and shipped to LabCorp at the 3 month, 12 month and 24 month visits.

Serum and plasma samples will be collected at 3 month, and 24 month visits and shipped to the Wake Forest NCORP Biospecimen Lab.

A DNA Lab blood sample will be collected from Chemotherapy Group participants who have given consent to DNA testing and will be shipped to the Wake Forest NCORP Biospecimen Lab.

The KCCQ-12 will be administered only at the 24 month visit.

An MRI exam will be performed at the 3 and 24 month visits.

Participants who are randomly assigned to have a treadmill or stationary bike cardiopulmonary exercise test (CPET) will complete CPET only at the 24 month visit.

7.5.2. Control (Healthy) Group

–

All study visits for the Control (Healthy) Group will be scheduled based on the date baseline activities are initiated.

3 Month, 12 Month and 24 Month Visits

At the 3 month, 12 month and 24 month visits, the Control (Healthy) Group participants will undergo a physical assessment (to obtain height, weight, heart rate, blood pressure and BMI), review current medications with site study staff, complete self-administered questionnaires, neurocognitive tests, 6-minute walk, disability measures, and exercise tests (Expanded SPPB).

Study Lab Chemistry – laboratory tests will be collected and shipped to LabCorp at the 24 Month visit.

The KCCQ-12 will be administered at the 24 month visit.

An MRI exam will only be performed at the 24 month visits.

Participants who are randomly assigned to have a treadmill or stationary bike cardiopulmonary exercise test (CPET) will complete CPET at the 24 month visit.

7.6. Evaluations at Completion of Study – Years 3 through 11

All study participants will be followed annually from the 3 year time point through the 11 year time point or until the study is closed, whichever occurs first. Annual follow-up assessments for participants in the Chemotherapy Group will be scheduled based on the date of chemotherapy initiation. Annual follow-up assessments for participants in the Control (Healthy) Group will be scheduled based on the date baseline activities are initiated. Site staff must contact the study PI for permission to schedule follow-up visits outside of the protocol designated study window.

Years 3-11 annual follow-up assessments for cardiovascular events will include the KCCQ-12 and structured phone interviews assessing cardiac outcomes and potential cardiovascular adverse events.

7.7. Laboratory Samples and Analyses

Please refer to Appendix B for detailed specimen collection and shipping instructions.

7.7.1. Study Lab Chemistry

Participants in both the Chemotherapy Group and Control (Healthy) Group will have blood samples in the total amount of approximately 8 teaspoons withdrawn from a vein (Chemotherapy Group: baseline, 3 month, 12 month and 24 month visits; Control (Healthy) Group: baseline and 24 month visits). The following labs will be obtained: hematocrit, glucose, creatinine, lipid profile (LDL Cholesterol, HDL Cholesterol, Total Cholesterol, Triglycerides) and C-reactive protein. These labs will be sent to LabCorp for analysis.

7.7.2. Serum and Plasma Samples – Chemotherapy Group

Participants in the Chemotherapy Group will have additional blood drawn in the total amount of approximately 11 teaspoons at baseline, 1 month, 3 month, and 24 month visits. Serum and plasma samples will be separated into aliquots at the site, frozen then shipped to the Wake Forest NCORP Biospecimen Lab. Samples will be shipped and stored with a unique identifier and will not include any information protected by HIPAA regulations. Biomarkers Troponin-I and N-terminal pro-B-type

Natriuretic Peptide (NT-pro BNP) will be processed in batches at the Hypertension & Vascular Research Center and Dr. Mark Lively's Lab at Wake Forest BioTech Place. Once biomarker processing is complete, the remaining samples will be stored and later used for assessment of markers that will become evident in the future as forecasters of cardiovascular health.

7.7.3. DNA Lab – Chemotherapy Group

At the baseline visit and 3 month visit, additional blood draws totaling approximately 3 teaspoons at each visit will be collected from participants in the Chemotherapy Group who have given consent to store blood for future DNA testing. Blood samples will be de-identified by using only the patient's protocol identification number (PID) and will not include any information protected by HIPAA regulations. This lab sample will be shipped to the Wake Forest NCORP Biospecimen Lab and processed at Dr. Timothy Howard's laboratory at the Center of Genomics and Personalized Medicine of Wake Forest School of Medicine, Winston-Salem, North Carolina.

7.8. Questionnaires, Neurocognitive Tests and 6-Minute Walk/Disability Measures/SPPB

Comorbidities, family history, smoking status, fatigue, psychosocial and behavioral factors, and physical activity will be assessed with a battery of questionnaires that have been widely used in other cancer-related studies. The battery of questionnaires will be self-administered by study participants and reviewed by the study interviewer. Study booklets titled "Self-Administered Questionnaires" are provided for each study time point to capture the battery of questionnaires. Neurocognitive Tests will be administered by study staff, and are provided in booklets titled "Neurocognitive Tests" for each time point. The 6-Minute Walk, disability measures and Expanded SPPB will be administered by site staff; these measures are provided in booklets for each time point. Visually impaired subjects will need a study team member to read the questionnaires to them. A note to file should be created and a witness is required to verify the questionnaire was read verbatim.

The self-administered questionnaires (approximately 30-40 minutes), neurocognitive tests (approximately 10-15 minutes), and 6-minute walk/disability measures/SPPB (approximately 20-30 minutes) will be obtained at baseline, 3 months, 12 months and 24 months.

7.8.1. Self-Administered Questionnaires Booklet

DOMAIN ASSESSED	SECTION LETTER IN BOOKLET		INSTRUMENT
	Baseline	*3, 12 and 24 Month*	
Comorbidities	A	A	Study specific questionnaire
Family History	B	(n/a)	Study specific questions
Smoking and Tobacco Use	C	B	Study specific questions
Limitations in Daily Activity	D	C	RAND MOS Short Form Health Survey (SF-36)
Fatigue	E	D	Functional Assessment of Cancer Therapy - Fatigue Scale (FACT-Fatigue) (Version 4)
Depression	F	E	Center for Epidemiological Studies Depression Scale (CESD-10)
Social Support	G	F	MOS Social Support
Perceived Stress	H	G	Cohen's Perceived Stress Scale (PSS)
Sleep Disturbance	I	H	PROMIS Item Band v1.0 - Sleep Disturbance - Short Form 8a
Cognition	J	I	PROMIS v1.0-Applied Cognition-Abilities-Short Form 8a
Cognition	K	J	PROMIS v1.0-Applied Cognition-General Concerns-Short Form 8a
Self-Efficacy for Walking	L	K	FORM 16: Self-Efficacy for Walking
Hostility	M	(n/a)	Cook-Medley Hostility Scale (only collected at Baseline)
Physical Activity	N	L	Godin Leisure-Time Exercise Questionnaire
Sedentary Behavior and Physical Function	O, P	M, N	PACE Adult Sedentary Behaviors Survey

7.8.2. Neurocognitive Tests Booklet

DOMAIN ASSESSED	INSTRUMENT
Attention, visual motor skills, processing speed and executive function	Trail Making Tests – Part A & B [Timed Test]
Speed of mental processing, verbal fluency, and executive function	COWA

7.8.3. 6-Minute Walk, Disability Measures, and Expanded-SPPB Booklet

DOMAIN ASSESSED	INSTRUMENT
Walking Distance	6-Minute Walk Test
Flexibility for Normal Activities	Range of Motion Test at the Shoulder
Overall Body Strength	Grip Strength Test
Balance Ability	Balance Tests
Gait Speed	4 Meter Walk (Gait Speed Test, Narrow Walk Test)

Leg Strength	Chair Stand Test

7.9. MRI Exams

At the baseline, 3 month (Chemotherapy Group participants ONLY), and 24 month visits each participant will undergo an MRI exam. The exam will measure left ventricular volumes, ejection fraction, myocardial strain/strain rate, mass, mapping, aortic pulse wave velocity, and aortic wall thickness. The exam will take 10-15 minutes to complete.

Chemotherapy Group participants must have the Baseline MRI exam within 30 days before initiation of chemotherapy.

Within weeks of receipt of the MRI exams, the presence or absence of severe declines in left ventricular ejection fraction, left ventricular wall motion abnormalities, the presence of a severe left ventricular hypertrophy, or unanticipated enlargement of the diameter of the ascending aorta will be reported to site personnel.

7.9.1. Reproductive Risks

At the beginning of the study, pregnant women and women who are breast-feeding will not be enrolled in the study as clinically these women do not receive anthracycline based or other forms of chemotherapy due to possible harm to the developing fetus as a result of the cancer treatment. Later in the study, MRI scans may be performed and recent studies show the risk of an adverse condition or outcome for the fetus or mother is no different than the general population when MRI contrast is not used during the MRI scan. In this study, MRI contrast will not be used so the added risk of the MRI is not expected. It is important to note that adverse conditions, such as spontaneous abortion, occur more prominently in the first trimester of pregnancy, but this occurrence has not been related to receipt of an MRI scan performed without the use of contrast. In addition, the study requires for some individuals a maximal exercise test. Maximal exercise if the participant is pregnant or thinks she may be pregnant has been found to be of similar risk as that experienced by the general public. However, if the participant notices a lack of balance while walking or other condition related to a pregnancy that develops during the study and do not wish to exercise vigorously on a treadmill due to the pregnancy, the participant may continue to participate in the study and receive the follow-up exercise test within 9 months of the 24 month visit.

7.9.2. MRI Sedation

In the event a participant is mildly claustrophobic (extreme discomfort or fear of small spaces) they may be offered an intravenous injection of an anti-anxiety medication (benzodiazepine) to make the participant drowsy, relaxed and comfortable during the MRI scan. This is at the discretion of the site physician. Because anti-anxiety medications may decrease alertness and cause lightheadedness or dizziness, the participant must have another adult drive them home from the clinic if they are given sedation. Benzodiazepines are a standard class of drugs that can be given for this indication without presenting a requirement for additional monitoring.

Possible risks and side effects of frequently used anti-anxiety medications may include:

Most Common Events may occur in about 2% of patients.
- dizziness
- headache

Less Common

- unusually fast/slow/irregular heartbeat,
- fainting,
- confusion,
- mental/mood changes,
- trouble breathing,
- muscle twitching and involuntary movements,

Rare but Serious Events
- throat discomfort,
- skin rash and hives.

7.10. MRI and CPET Invoicing

MRI invoices should be e-mailed to Kimberly Lane at klane@wakehealth.edu **or** mailed to Wake Forest Baptist Health, Medical Center Blvd, Winston-Salem, NC 27157 Attention: Kimberly Lane/Cardiology (encrypted e-mail is required).

7.11. Off Study Criteria

Participants may go "off-study" for the following reasons: lost to follow-up, medical contraindication, withdrawn consent, or death.

8. PROTOCOL SPECIFIC TRAINING REQUIREMENTS

8.1. MRI Training

The study PI presented the MRI protocol for review and discussion with the MRI technologists and associated study coordinators during the Investigator meeting held at the 2015 Wake Forest NCORP RB Annual Meeting.

Prior to the start of participant enrollment into the study, Wake Forest University Medical Center cardiology imaging specialists will observe and continue MRI training at each of the study locations.

Each site will be required to submit 2 satisfactorily performed cardiac MRI exams to the Image Coordinating Center at Wake Forest before accruing to the study.

8.2. CPET Training

Prior to enrolling their first participant, each participating center will be required to complete the training process by submitting relevant data from one cardiopulmonary exercise (CPX) test conducted on a healthy volunteer and other background information to the CPX core laboratory for review. The CPX core laboratory (directed by Drs. Kitzman and Brubaker) will evaluate the data and determine if further steps are required before enrolling participants.

8.3. Questionnaires, Neurocognitive Tests and 6-Minute Walk/Disability Measures/Expanded-SPPB

According to processes used in other NCORP RB studies, staff will be trained as needed in the administration of questionnaires, neurocognitive tests, the 6-minute walk, and disability measures. Each

questionnaire will be self-administered and then reviewed by a study interviewer. Results will be documented on the appropriate CRF and sent to the Research Base Data Management Center. These forms will then be uploaded into an electronic database from which further statistical analyses can be performed.

9. SPECIMEN MANAGEMENT

Materials for proper specimen collection and transport are supplied by LabCorp and the Wake Forest NCORP Biospecimen Lab. Each site will receive Study Lab Chemistry supplies in bulk when their LabCorp account is established. Each site will also receive the additional serum and plasma samples and DNA sample kits from the Wake Forest NCORP Biospecimen Lab after submitting the Specimen Collection and Shipping Kit Request Form. Sites must comply with Shipping and Handling Instructions compliant with International Air Transport Association (IATA) Dangerous Goods Regulations. For detailed instructions please refer to Appendix B.

NOTE: Since LabCorp will be testing specimens associated with this study, specimens should be collected in containers provided by LabCorp. For the Chemotherapy Group additional serum and plasma samples and optional DNA testing samples, kits provided by the Wake Forest NCORP Biospecimen Lab must be used.

Only participants in the Chemotherapy Group who have given consent to store blood for future DNA testing. Samples for future DNA testing should not be drawn and sent to the Wake Forest NCORP Biospecimen Lab until consent for DNA testing has been verified.

Only researchers approved by Dr. Gregory Hundley (the principal investigator at Wake Forest School of Medicine) will receive the Chemotherapy Group additional serum and plasma aliquotted samples. Assays will always be performed with coded samples and conducted following Good Laboratory Practice (GLP) guidelines.

See Appendix B – Lab Guidelines for detailed specimen collection and shipping instructions.

10. REPORTING ADVERSE EVENTS

This is an observational study with limited possibility of an adverse event; for this reason, only Grade 4 and Grade 5 Serious Adverse Events that are definitely, probably, or possibly related to the study are to be reported (i.e. MRI sedation, exercise intolerance).

Reporting begins after the informed consent is signed. Serious Adverse Events occurring within 30 days of study completion must be reported via FDA Form 3500 (MedWatch).

10.1. Protocol Specific Reporting for Serious Adverse Events (SAEs)

DEFINITION: ICH Guideline E2A and Fed. Reg. 62, Oct. 7, 1997 define serious adverse events as those events which meet any of the following criteria.

- Results in death.
- Is life threatening (Note: the term life-threatening 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

- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Is a congenital abnormality/birth defect.
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.
- **Grades 4 and 5 unexpected (unsolicited) SAEs that meet the above definition for SAEs and/or regardless of attribution (i.e. regardless of whether they are related to this study intervention or not) should be reported to the RB DMC using the FDA Form 3500 (MedWatch).**
- Site staff and/or Principal Investigators will report to the RB Data Management Center within 24 hours of discovering the details of all unexpected severe, life-threatening (grade 4) and/or fatal adverse events (grade 5) if there is reasonable suspicion that the event was definitely, probably, or possibly related to the study intervention.

Otherwise, the MedWatch should be sent to the RB DMC by fax or email within 10 working days of discovering the details of the SAE.

Data Elements to Include on the MedWatch are:

- SAE reported date
- CTCAE Term (v4.03)
- Event onset date and event ended date
- Severity grade (use table provided in Section 10.2 below)
- Attribution to study intervention (relatedness)
- Action taken with the study participant and intervention
- Outcome of the event
- Comments

10.2. Guidelines to Determine Grade and Severity of SAEs

Identify the adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

SAEs will be assessed according to the CTCAE grade associated with the SAE term. SAEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v5.0. as stated below.

Grade	Severity	Description
4	Life threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

Activities of Daily Living (ADL)
***** Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
****** Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

The Research Base Grant PI, Safety and Toxicity Review Committee and/or Study Chair will take appropriate action to inform the membership and statistical personnel of any protocol modifications and/or precautionary measures, if this is warranted.

The Wake Forest NCORP Research Base is responsible for communicating SAEs to the FDA, the drug sponsor, WF IRB, the WF Safety and Toxicity Review Committee (STRC) and/or other regulatory agencies as appropriate per agency reporting requirements.

Institutions must comply with their individual Institutional Review Board (IRB) policy regarding submission of documentation of adverse events. All MedWatch reports should be sent to the local IRB in accordance with the local IRB policies.

10.3. Follow-up of SAEs

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the MedWatch form in the appropriate format. Follow-up information should be sent to the RB Data Management Center as soon as available.

SAEs (Grade 4 and/or Grade 5) for this protocol should be followed for those related to the study intervention. Documentation should include:

- PID
- Date of SAE
- Description of the event
- Relationship of the SAE to the study intervention
- Severity
- Intervention/Resolution

11. STUDY MONITORING

11.1. Data Management Schedule

The signed consent form must be submitted to the Wake Forest NCORP Research Base within 14 days from the consent date. All assessments and case report forms must be completed within the designated study visit windows (Sections 7.1.1 and 7.1.2), then data submitted to the Wake Forest NCORP Research Base within 14 days from the date assessments are completed.

If you have questions please contact the Wake Forest NCORP Research Base between 8:00am and 5:00pm EST, Monday through Friday at (336) 713-3172 or (336) 713-5086.

11.2. Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF).

11.3. Source Documents

Source documents are the original signed and dated records of participant information (e.g., the medical record, shadow chart) which may include electronic documents containing all the information related to a participant's protocol participation. Source documents are used to verify the integrity of the study data, to verify participant eligibility, and to verify that mandatory protocol procedures were followed. An

investigator and other designated staff are required to prepare and maintain adequate and accurate documentation that records all observations and other data pertinent to the investigation for each individual participating in the study. All data recorded in the research record (including data recorded on CRFs) must originate in the participant's medical record, study record, or other official document sources.

Source documents substantiate CRF information. All participant case records (e.g., flow sheets, clinical records, and physician notes, correspondence) must adhere to the following standards:

- Clearly labeled in accordance with HIPAA practices so that they can be associated with a particular participant or PID;
- Legibly written in ink;
- Signed and dated in a real time basis by health care practitioner evaluating or treating the participant;
- Correction liquid or tape must not be used in source documents or on CRFs; and
- Corrections are made by drawing a single line through the error. Do not obliterate the original entry. Insert the correct information, initial, and date the entry.

All laboratory reports, pathology reports, X-rays, exercise tests, imaging studies, and scans must have:

- Complete identifying information (name and address of the organization performing, analyzing, and/or reporting the results of the test); and
- Range of normal values for each result listed.

11.4. Data Safety Monitoring Board

The Research Base Data Safety Monitoring Board includes members demonstrating experience and expertise in oncology, biological sciences, and ethics and meets every six months to review all ongoing Wake Forest NCORP Research Base studies. Since this is an observational study, review will focus mainly on patient accrual, retention, and data completeness.

11.5. Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.), as well as IRB records and other regulatory documentation, will be retained by the Investigator in a secure storage facility in compliance with HIPAA, OHRP, FDA regulations and guidance, and NCI/DCP requirements unless the standard at the site is more stringent.

11.6. CDUS Reporting

The Wake Forest NCORP Research Base will submit quarterly reports to DCP/CTEP by electronic means using the Clinical Data Update System (CDUS).

12. STATISTICAL CONSIDERATIONS

We will recruit 1,000 women, 840 with breast cancer (CAN) (420 to receive non-anthracycline Adj-C) and 420 to receive anthracycline-based Adj-C (Adj-C) and 160 women without cancer (NoC). In this design we plan to enroll an equal number of pre- and post-menopausal women, and at least 25% of patients in each group will be African-American (AA). There are 2 primary types of statistical analyses to address the study questions. The first (Part 1) includes testing hypotheses concerning between group

(i.e. CAN vs NoC) and within group (i.e. longitudinal changes within Adj-C group) comparisons (Aims 1-3). Part 2 includes developing predictive equations to determine if baseline or early post-chemo patient-level characteristics can predict future CV dysfunction, events, and exercise intolerance/fatigue (Aim 4).

12.1. Part 1, Objectives 1 and 2—Continuous Outcomes

Within-group and between-group comparisons will be made using longitudinal mixed models to compare within and across the 3 groups for outcomes (i.e. PWV or fatigue) measured on a continuous scale. These mixed models will include fixed effects for group (AAdj-C, Adj-C, NoC), baseline (pre-chemo) assessment of the outcome of interest (i.e. PWV) to adjust for potential risk-factor profile differences between groups and the time point at which the measurements are made relative to the baseline pre-MRI assessment. Additional patient-level risk factors (both measured at baseline and at follow-up) will also be considered as fixed effects in this model. We can write this model as follows:

$$Y_{ijk} = \mu + \alpha_k + \beta_j + \alpha\beta_{jk} + \gamma_i + \Delta_i + \lambda_{ij} + \varepsilon_{i(k)},$$

where Y_{ijk} is the outcome measured on the i^{th} woman in the j^{th} time period in the k^{th} group; μ is the grand mean;
α_k is the fixed effect for group k (1 [AAdj-C], 2 [Adj-C], or 3 [NoC]); β_j is the fixed effect for time (0, 3 mo, 12 mo, or 24mo – depending on the measurement taken); $\alpha\beta_{jk}$ is the group by time interaction effect; γ_i is the baseline assessment of the outcome of interest, Δ_i is the fixed effect for baseline, non-time varying covariates (i.e. race) for each woman, λ_{ij} is the fixed effect for time-varying covariates for each woman (e.g., blood pressure at visit) and $\varepsilon_{i(k)}$ is the error term for the i^{th} woman nested within group. Of primary interest in many of our hypotheses will be the main effect terms for group and the group x time interaction ($\alpha\beta$) terms. Here we can examine whether there is a difference in the rate of change in the outcomes. We hypothesize that there may be a difference in slopes (interaction) during different time periods when comparing groups (i.e. NoC group may differ across the full time span when compared to CAN groups; however, the 2 CAN groups may differ only after 12 months). We can test these hypotheses by constructing appropriate contrasts. In these models, time can be modeled as continuous or categorical to test whether the time effect is linear (time/continuous) or non-linear. To examine within-group changes, the above model will be fit separately for each group.

Individual level covariates can take on two forms: baseline characteristics and time-varying characteristics. Most covariates will be included in the model for adjustment purposes, being included as both main effects and as potential interactions with group membership depending on the hypotheses. Thus, we can examine whether different characteristics modify the relationship between groups and the outcomes of interest. The inclusion of age and race as covariates allows us to examine and control for their overall predictive value for the outcomes of interest. There will be at least 25% African-American women in each group and age will also be balanced between groups (Cancer vs NoC). In addition, we will examine the potential effects of adjuvant vs neo-adjuvant therapy, radiation (XRT) therapy, and trastuzumab therapy.

We anticipate that 20% of CAN participants will receive trastuzumab, 67% XRT, and 80% neo-adjuvant therapy.

12.2. Part 1, Objective 3—Models

In addition, we will examine in sequential models whether cardiac or vascular measures mediate the association between groups and outcomes. For example, we will use mediation models[121,123] to test whether Adj-C is associated with a drop in LVEF, which in turn is associated with an increase in fatigue. In brief, a series of mixed models that are formed using the model we described above are fit regressing: 1) outcome on exposure; 2) mediator on exposure; and 3) outcome on both mediator

and exposure. A change in the estimate of the exposure effect from Model 1 to 3 is evidence of mediation. For example, we will estimate the indirect effect of cancer group (NoC vs. CAN) on fatigue that is mediated through falling LVEF, and the direct effect that is not explained by dropping LVEF. Based on Figure 1, there are several potential pathways that we can examine. These include examining direct effects on cardiac/vascular injury, exercise capacity (ECap)/fatigue (Fatg) and CV events as well as potential indirect (mediated) effects on ECap/Fatg and CV events (by cardiac/vascular injury) or indirect (mediated) effects on CV events by cardiac/vascular injury and ECap/Fatg.

We will use bootstrap methods for significance testing of the mediation effect and for calculating confidence limits for the mediated effect.[117] Additional covariates can be incorporated into these models (age, race, etc.). This mediation modeling will allow us to better understand the specific contribution of Adj-C on diminished ECap, fatigue, or CV events. For example, we can examine the specific impact on two known components of ECap —heart function (measured by LVEF) and vascular stiffness (measured by PWV). Absence of mediation will suggest that other non-cardiovascular factors (e.g. psychosocial factors, etc.) may be responsible for the variability in ECap.

The potential mediator data will be measured at baseline and at 3, 12, and 24 months. Thus, we can examine the temporal relationship among these measures. We can examine whether baseline or change in PWV is more predictive of future ECap (or Fatg). We will also determine whether LVEF/PWV changes occur prior to or simultaneously with changes in ECap (or Fatg). Finally, in addition to these models that will examine LVEF and PWV singularly, we will form models that incorporate both potential mediators at the same time for predicting ECap or Fatg. Some variables in these models may be correlated with each other; therefore, appropriate steps will be taken to identify and reduce potential multi-collinearity.

Diagnostics:
Prior to performing analyses, we will determine if the assumptions of the models are satisfied. Diagnostics and residual plots will be reviewed. If assumptions are violated, transformations will be considered where the order in choosing a transformation will be to satisfy the: 1) linearity, 2) homogeneity of variance, and 3) normality assumption.

Binary Outcomes:
If the outcome measures are binary (i.e. a 10% or greater drop in LVEF at a particular time point), then we will use a Generalize Estimating Equations (GEE) approach for data analysis, which accounts for the repeated assessments of the patients in modeling.

Time to Event Outcomes (Objectives 1, 2, 3):
With the NCORP funding, we will have follow-up available to assess hospitalizations and cardiovascular (CV) events (7-9.5 years of follow-up allowing for all patients to have at least 7 years follow-up and those enrolled at the beginning to have 9+ years follow-up). We can compare overall event rates or the time to events between groups using multiple logistic regression models at fixed points in time (i.e. compare event rates 7 years post-baseline MRI assessment) or survival analysis models, respectively. For the survival analyses, in addition to examining overall unadjusted time-to-event data using Kaplan Meier survival curves, we will fit Cox-proportional hazards regression models to allow for inclusion of patient level covariates. We can also incorporate time-varying covariates into these models for measures that are available at multiple time points.

12.3. Part 2 (Objective 4) Predictive Equations

We will determine whether we can prospectively identify women at higher risk for potential CV complications based on their baseline or early post-chemo measures. With at least 7 years of follow-up

for hospitalizations and/or CV events, we can dichotomize women into those with and without an event of interest after 7 years. We will then compare overall rates using multiple logistic regression models. We can fit models to predict which pre-chemotherapy characteristics (demographic or clinical) exist more frequently in women with an adverse CV event post-chemotherapy (i.e. drop in EF greater than 10%, an absolute LVEF < 50%, or a CV event).

Furthermore, we can develop models in a hierarchical approach, fitting first the most straight-forward and cost efficient models using baseline measures that are readily available and low in cost. We propose as a first step to use the existing AHA predictive model and then determine whether additional characteristics improve the predictions. Then we can determine whether additional predictive precision is gained by adding more sophisticated and possibly more expensive risk factors to the AHA model. To do this, we will calculate the net reclassification improvement (NRI) to determine whether the addition of other covariates improves the predictive ability of the AHA model.[123]

Next, we will develop de novo predictive equations that do not originate from the AHA model. We will fit two sets of models: one with all 1,000 women enrolled, adjusting for whether they have cancer, and the second is focused on women with cancer only. Several characteristics will be measured at 3 months post-chemotherapy on women with cancer only; thus, we can use these early outcome data in our predictive equations. We will determine whether the baseline and 3-month measures predict the occurrence of outcomes at 2 years (i.e. significant fatigue, significant drop in LVEF) or CV events within 7 years after the baseline assessment. We will examine observed values of the patient characteristics at the two time points and their change in the predictive models. Based on our previous animal model work,[42] we found that the early worsening in LVEF often predicted later sustained worsening in CV measures.

For this approach, we will not have an a priori model to expand; thus, we propose to find the optimal prediction models by using a 100-fold validation procedure. We will randomly select 75% of the available data for model development (testing data) and use the remaining 25% for model validation (validation data) by examining the model's predictive ability. We will repeat this procedure of selecting testing and validation data 100 times with different random selections being made each time.

For each test and validation model, we will examine the C-statistic, which measures the ability of the logistic regression models to discriminate patients with and without the outcome of interest (i.e. a CV event within 7 years). By using this procedure, we will be able to examine the C-statistics across the 100 samples and determine whether certain models consistently show high levels of discrimination. Once this process is completed, we will select the group of risk factors that appeared most consistently in the test and validation models with the highest C-statistics and use this group of variables to fit the final model, using all of the available data. This will be considered the best prediction model.

12.4. Missing Data Considerations

Some women may not complete the study, leading to missing data. We will compare the baseline characteristics of participants who drop out with those who did not to determine if specific covariates should be included in the analyses. If missingness is non-informative, analyses performed using the proposed repeated measures mixed models (with covariates that predict missingness included) approach (PROC MIXED in SAS) will handle the missing data appropriately. However, if there is evidence that the missing data is informative, more sophisticated statistical methods will be applied. Wake Forest University Health Sciences Statistical Department's expertise will be useful in addressing the complexities that may arise if missing data are found not to be missing at random.[119,120,124-126] If this occurs, we will consider modeling the relationship between groups and the outcomes conditioning on the pattern of missing data.

12.5. Power and Sample Size Considerations:

The sample size was chosen for this study to allow for 90% or greater power to address the specific aims for between and within group comparisons of interest. The formula shown below is used to describe the minimum detectable difference between the different groups of interest. In the formula, r^2 is the percent of the variance of the follow-up outcome explained by baseline measurements (i.e. FACT score before chemotherapy), $Z_{1-\alpha/2}$ is the value from the standard normal distribution corresponding to alpha (α=0.025 [2-sided]), allowing for 2 primary comparisons (CAN vs NoC and AAdj-C vs Adj-C), $Z_{1-\beta}$ corresponds to the power (90%), σ^2 is the variance of the outcome of interest (i.e. FACT score), *k* is the ratio of the sample sizes in the two groups (n_1/n_2), and n_1 is the number of participants in one of the groups. For the majority of outcomes, the full cohort will be used for analyses; however, for one outcome of interest (Peak VO_2), a subset of 450 participants will be measured. For the analyses using the full cohort, depending on the comparison of interest, we will have different numbers of women available. When comparing the CAN and NoC groups, we will have 714 women (assuming 85% of the 840 women) in the CAN group and 136 (85% of 160) in the NoC group. The term Δ corresponds to the detectable difference in the mean values of the two groups being compared. Using this formula, we examined the detectable differences for a conservative r^2 value of 0.25 (i.e. a correlation of 0.5 between the baseline and follow-up assessment of the outcome of interest) assuming 90% power and alpha=0.025.

Table 3

90% Power	Estimated	Detectable Difference	
Continuous Outcomes	SD	CAN vs NoC	Adj-C vs Adj-C/Anth
6-minute walk	107.1	31.1	24.4
LVEF (%)	7.7	2.23	1.76
PWV (m/sec)	3.7	1.07	0.84
LV-ESV (ml)	19.7	5.71	4.49
Strain (σ)	2.94	0.85	0.670
FACT-Fatigue	9.8	2.84	2.23
Binary Outcomes	Power	Observed Proportion of Event	
CV EVENTS	90%	7% vs 1.2%	7% vs14%
Drop in LVEF (Yes/No)	90%	9% vs 2%	10% vs 18%
Subsample Outcome (n=375 total, 150 Anth, 150 Nonanth, 75 Controls) after accounting for approximate 15% drop-out			
90% Power	Estimated SD	Detectable Difference	
		CAN vs NoC	Adj-C vs Adj-C/Anth
Peak VO_2 (ml/kg/min)	4.3	1.69	1.51

For the measure of peak VO2 (where a total of 450 participants will be measured), when comparing the CAN and NoC groups, we will have at least 300 women (assuming approximately 85% of the 360 women) in the CAN group and at least 75 women (approximately 85% of 90) in the NoC group.

Table 3 shows detectable differences for the comparisons.

We have 90% power to detect a 2.84 unit difference in the FACT scale, or a 1.69 ml/kg/min difference in peak VO_2 between the CAN and NoC groups. These differences correspond to a detectable effect size of 0.29 standard deviations (SDs) unadjusted for correlation with baseline for the full sample and a detectable effect size of 0.39 standard deviations for the analyses on the subsample with Peak VO2 measures. When we compare the two Cancer Groups (n=357 in each) the detectable effect size is 0.228

SDs for the full sample and 0.351 for the subsample with Peak VO2 measures (n=150 [or more] in each). These analyses can also be used to determine whether there is a time x group interaction. Essentially, if the adjusted outcome measures differ at 2 years (adjusted for baseline levels), this indicates that the rate of change in the measure differed between the groups. When we fit the mixed models, we will include the 3 and 12 month time points to add precision to our estimates of group effects.

For binary outcomes such as a 6% drop in LVEF, there is greater than 80% power to detect a 9% difference in the percent of patients with a drop in LVEF when comparing the participants in the CAN group with the NoC group. We expect very few NoC participants (i.e. less than 5%) will have a 10% drop in LVEF after 2 years, so this calculation should be conservative. When comparing the Adj-C and AAdj-C groups, there is 90% power to detect a difference in the percent of patients with a drop in LVEF equal to 8%, assuming that 10% in the Adj-C group have this outcome and 18% (or more) in the AAdj-C have this outcome.

These tests are based on Fisher's Exact tests with α=0.025 (2-sided test). Based on published data, we anticipate that less than 1.2% of the women without cancer may have a CV event after 8 years of follow-up.[127-129] If at least 7% of the CAN patients have a CV event after 8 years, then we will have 80% power to detect this difference using a Fisher's exact test (with α=0.025 2-sided test). For examining time to events, we anticipate 59 events over the 8 years of follow-up and will have 90% power to detect a HR of 2.5 with alpha = 0.025 (2-sided). As described above, participants will have between 7-9.5 years follow-up beyond the first two years of the study, depending on when they are enrolled, so it is expected that the average follow-up for the survival analyses will be 8 years.

In addition, we have high power to detect within-group differences between cancer participants; there is 90% power to detect a change in the FACT score of 1.9 units or more after 2 years in either the Adj-C or Adj-C groups using a paired t-test with α=0.025 2-sided test. Analyses that control for additional characteristics (e.g. XRT exposure) will be examined in these longitudinal between-or within-group models. The addition of these variables into our models should increase the precision of the outcome measures, thus increasing our statistical power.

For the power calculation of the prediction equation analyses, there will need to be at least one risk factor that can discriminate between the groups (i.e. those with and without a CV event in 8 years). Based on this sample size, as long as a risk factor measured on a continuous scale is 0.39 standard deviations different between patients with and without a CV event, we will have 90% power to detect this effect. In other words, if the FACT score is 3.8 units apart for patients with and without a CV event, then we will have 90% power to identify this variable as a significant predictor in our risk prediction equation.

13. SITE REGISTRATION

All sites must register through the CTSU.

13.1. CTEP Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR)

(https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

Additional information can be found on the CTEP website at < https://ctep.cancer.gov/investigatorResources/default.htm >. For questions, please contact the RCR Help Desk by email at < RCRHelpDesk@nih.gov >.

13.2. CTSU Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

13.2.1. IRB Approval

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRB Manager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

13.2.2. Downloading Site Registration Documents

Site registration forms may be downloaded from the WF-97415 protocol page located on the CTSU members' website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand
- Click on the WAKE link to expand, then select trial protocol #WF-97415
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided.

13.2.3. Requirements For WF-97415 Site Registration

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

13.2.4. Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission
When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

13.2.5. Checking Your Site's Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

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APPENDIX A: **Performance Status Criteria**

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B – Lab Guidelines
(Includes specimens for LabCorp and Wake Forest NCORP Biospecimen Lab)

Section 1 - Schedule for Blood Collection

Baseline Visit

SAMPLES for LabCorp (ALL GROUPS)

Fasting 3 hours prior to lab draw. Ambulatory at least 30 minutes prior to lab draw.

One (1) 8.5 mL Red/Black Speckled SST
One (1) 4.0 mL Lavender Top EDTA

SAMPLES for Wake Forest Biospecimen Lab (CHEMOTHERAPY GROUP ONLY).

Serum and Plasma Samples
Fasting *not* required prior to lab draw.

One (1) 5.0 mL Gold top tube with SST Silica & polymer gel
One (1) 6.0 mL Lavender top, EDTA

Blood for Future DNA Testing
Fasting *not* required prior to lab draw.
Participant must provide authorization on the consent form for blood to be drawn for future DNA testing.

One (1) 8.5 mL Yellow Top ACD

1 Month Visit

SAMPLES for Wake Forest Biospecimen Lab (CHEMOTHERAPY GROUP ONLY).

Serum and Plasma Samples
Fasting *not* required prior to lab draw.

One (1) 5.0 mL Gold top tube with SST Silica & polymer gel
One (1) 6.0 mL Lavender top, EDTA

3 Month Visit

SAMPLES for LabCorp (CHEMOTHERAPY GROUP ONLY)

Fasting 3 hours prior to lab draw. Ambulatory at least 30 minutes prior to lab draw.

One (1) 8.5 mL Red/Black Speckled SST
One (1) 4.0 mL Lavender Top EDTA

SAMPLES for Wake Forest Biospecimen Lab (CHEMOTHERAPY GROUP ONLY).

Serum and Plasma Samples
Fasting *not* required prior to lab draw.

One (1) 5.0 mL Gold top tube with SST Silica & polymer gel
One (1) 6.0 mL Lavender top, EDTA

Blood for Future DNA Testing
Fasting *not* required prior to lab draw.
Participant must provide authorization on the consent form for blood to be drawn for future DNA testing.

One (1) 8.5 mL Yellow Top ACD

12 Month Visit

SAMPLES for LabCorp (CHEMOTHERAPY GROUP ONLY)

Fasting 3 hours prior to lab draw. Ambulatory at least 30 minutes prior to lab draw.

One (1) 8.5 mL Red/Black Speckled SST
One (1) 4.0 mL Lavender Top EDTA

24 Month Visit

SAMPLES for LabCorp (ALL GROUPS)

Fasting 3 hours prior to lab draw. Ambulatory at least 30 minutes prior to lab draw.

One (1) 8.5 mL Red/Black Speckled SST
One (1) 4.0 mL Lavender Top EDTA

SAMPLES for Wake Forest Biospecimen Lab (CHEMOTHERAPY GROUP ONLY).

Serum and Plasma Samples (Chemotherapy Group Only)
Fasting *not* required prior to lab draw.

One (1) 5.0 mL Gold top with SST Silica & polymer gel, clots 30 min
One (1) 6.0 mL Lavender top, EDTA

Section 2 - Study Lab Chemistry:

Collection Guidelines for LabCorp Specimens

Chemotherapy Group: Study lab chemistry serum and plasma samples will be collected and shipped to LabCorp at the baseline, 3 month, 12 month, and 24 month visits.

Control (Healthy) Group: Study lab chemistry serum and plasma samples will be collected and shipped to LabCorp at baseline and 24 month visits.

1. The participant must fast for 3 hours prior to drawing blood samples.
2. Draw blood samples into one 8.5mL Red/Black speckled SST tube and one 4.0 mL Lavender top EDTA tube using standard venipuncture procedures.
3. Invert the Lavender top EDTA tube 8 times immediately after collection.
4. Allow the Red/Black speckled SST tube to stand upright for 30 minutes at room temperature, then centrifuge the sample no later than 45 minutes after collection at 3300g for 10 minutes.
5. Package a completed lab requisition (see next page) with both tubes in the biospecimen bag provided by LabCorp then store the bag at 4°C. LabCorp should be notified immediately for same day sample pick-up.

<table>
<tr><th>Test Name</th><th>Tube Type</th><th>Special Instructions</th><th>Centrifuge</th><th>Specimen Temp</th></tr>
<tr><td>Lipid Panel*</td><td rowspan="4">One – 8.5 mL Red/Black speckled SST tube</td><td rowspan="2">Patient should fast 3 hours prior to collection of Glucose and Lipid Panel</td><td rowspan="4">Speed-3300g
Time - 10 min.</td><td rowspan="4">Ambient</td></tr>
<tr><td>Glucose *</td></tr>
<tr><td>Creatinine</td><td>None</td></tr>
<tr><td>CRP**</td><td>Specify <u>Female</u></td></tr>
<tr><td>Hematocrit</td><td>One - 4.0 mL Lavender Top EDTA tube</td><td>Invert tube gently eight times after venipuncture</td><td>Do Not Centrifuge</td><td>Ambient</td></tr>
</table>

**Fasting 3 hours prior to lab collection. **Patient should be ambulatory x 30 minutes prior to lab collection.*

- All LabCorp specimens should be collected in tubes and bags provided by LabCorp.
- LabCorp couriers will deploy to each site from local LabCorp facilities for pick-up as specimens are drawn.

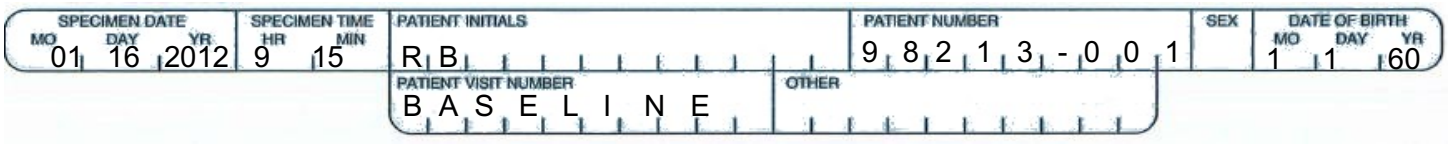
Tube label and lab requisition instructions are provided on the next page.

LabCorp Specimen Labels:

- Sites should apply labels at the top of the specimen tube, just below the tube's top or cap.
- Place protocol-specific label on each specimen with the following patient information:
  - Initials (First initial. Last initial.)
  - PID (protocol identification number assigned at enrollment)
  - Visit (baseline, 3 month, 12 month or 24 month)
  - DOB Enter 01/01/year of birth
  - Collection date and time (military time)
  - Specify Labs

Example:
Initials: R. B.
PID: 97415-0001
DOB: 01/01/1960
Visit: Baseline
Specimen Date: 1/16/12
Specimen Time: 09:15 (military time)
Lab: _____

Lab Requisitions:



LabCorp will supply sites with lab requisitions. In the upper right corner of the requisition under "Significant clinical information" mark as appropriate "fasting" or "non-fasting". The requisition should be filled out completely with the patient's initials, DOB, sex, PID, collection date and collection time. Once completed the top copy of the form should be inserted into the specimen bag. A copy of the completed requisition should be kept in the participants study record at the site.

*DOB must be entered on all forms by entering (01/01/year of birth) on all LabCorp forms to match PID number.

Section 3: Additional Serum and Plasma Samples and Future DNA Testing Samples - Specific Blood Collection Instructions

Wake Forest NCORP Biospecimen Lab

CHEMOTHERAPY GROUP ONLY

Serum and Plasma Samples: Baseline, 1, 3, and 24 month visits

1. Draw blood using standard venipuncture procedures.
2. Invert Lavender top and Gold top tubes 8 times immediately after collection.
3. Allow tubes to stand upright for 30 minutes at room temperature before centrifuging at 2000 X g for 15 minutes.
4. Using a supplied sterile plastic transfer pipette, carefully transfer the plasma from the Lavender top tubes into five approximately equal aliquots in the pre-labeled PURPLE screw top cryotubes and close each securely.
5. Using a supplied sterile plastic transfer pipette, carefully transfer the serum from the Gold top tube after allowing 30 minutes for clotting into five approximately equal aliquots in the pre-labeled RED screw top cryotubes and close each securely.
6. Freeze the 10 tubes in an upright position. Place the tubes directly into dry ice to freeze quickly if the samples will be shipped the same day. Or you may place them in an upright position in a -20°C freezer until shipped.

Test Name	Tube Type	Special Instructions	Centrifuge	Specimen Temp
Plasma Sample*	6.0 ml Lavender Top	Invert 8 times immediately after collection, centrifuge, pipet into five equal aliquots and freeze before shipping. **	Spin serum & plasma tubes together at 2000g Time - 15 min	Frozen AFTER dividing into aliquots in sample tubes
Serum Sample*	5.0 ml Gold Top with gel	Invert 8 times immediately after collection, clot for 30 min. at room temperature, centrifuge, pipet into five equal aliquots, and freeze before shipping. **		Frozen AFTER dividing into aliquots in sample tubes

* Fasting *NOT* required prior to lab draw

** If plasma and serum samples collected **at the Wake Forest site** can be delivered to the Biospecimen Lab on the same day it is NOT necessary to freeze the samples. Refrigerate prior to delivery.

Blood for Future DNA Testing: Baseline and 3 month visits ONLY

1. Patient must provide authorization on the consent form for blood to be drawn for future DNA testing.
2. Fill tube as much as possible up to 8 mL and Invert several times to insure mixing with anti-coagulant.
3. Blood tube may be stored at room temperature for up to 2 hours before refrigeration.
4. Blood tube can be stored in the refrigerator at 4°C for a few days (e.g. over the weekend) before shipping.
5. DO NOT FREEZE

Test Name	Tube Type	Special Handling to Lab	Centrifuge	Specimen Temp
DNA*	1 ACD Yellow Top 8.5 ml	DO NOT FREEZE	Do not Centrifuge	Room temp for 2 hours before refrigeration

* Fasting *NOT* required prior to lab draw.

Delivery of Blood Samples to Wake Forest NCORP Biospecimen Lab

From Wake Forest School of Medicine Site

After plasma, serum, and blood for DNA specimens have been prepared:

1. Complete the UPBEAT Specimen Log Form, make a copy to include with the samples, and place the original in the patient file. Be SURE to put the patient ID (PID) number and the sample tube IDs on the form. The plasma (purple top) and serum (red top) cryotubes will have the same code number. The Baseline DNA yellow top tube will have a different code number.
2. Do not include individually identifiable health information on the Specimen Log Form. If the PID is not yet available, use patient initials (first initial – last initial) in place of the PID.
3. If plasma and serum samples are to be delivered to the Biospecimen Lab on the day collected, the samples should be refrigerated but NOT frozen. Freeze at -20°C if delivery is delayed to the next day or over the weekend. **Blood for DNA in yellow top tubes should be refrigerated and NEVER frozen**.
4. Contact the NCORP Biospecimen Lab at Wake Forest Biotech Place by phone AND email **to arrange same or next day pick up**. Our lab personnel will bring an insulated container to transfer the samples back to the lab. Try to call as early in the day as possible to let us know there will be samples to pick up:
   - Biospecimen Lab, located in Wake Forest Biotech Place – **Mark Morris or Gulya Kourman: 716-2581**. If no answer, please leave a detailed voice mail message. Our team will get an email notification and return your call as soon as possible.
   - Tumor Tissue Core Lab, located at Wake Forest Baptist Medical Center in the Hanes Building – **Tiffany Walker: 716-3721**
   - Email: 7NCORP@wakehealth.edu

Shipping of Blood Samples to Biospecimen Lab

From NCORP and ECOG-ACRIN Sites

Overview

IMPORTANT NOTE: *Blood samples may be collected on any day of the week and shipped to Wake Forest after the samples have been stored as described in the blood collection instructions. DO NOT ship samples on Friday.*

1) Baseline study blood samples must be obtained only after the patient has provided consent and been enrolled in the UPBEAT study.
2) **Blood specimens collected at the Baseline and 3 month visits in yellow-top DNA tubes must be stored in the refrigerator WITHOUT freezing and placed in the upper compartment of the shipper**.
3) Use the pre-paid shipping label supplied with each specimen shipping kit to ship specimens by Priority Overnight (FedEx) ONLY on Monday through Thursday. **PLEASE do NOT ship on Friday or on the day before a holiday.** Samples collected on Friday may be stored in the refrigerator without freezing over a weekend and shipped on Monday.

Each specimen shipping kit will contain:

1) **Shipping Container with two compartments**: The shipping box has a compartment on top for a silver cushioned zip style bag and an insulated foam container below for frozen samples on dry ice. A pre-labeled UPBEAT Specimen Log Form will be enclosed.
2) **Shipping containers for Baseline and 3 month visit samples are different**: Blood for DNA is collected ONLY at baseline and 3 month visits. Only specially labeled shippers for Baseline and 3 month visit samples will contain yellow top ACD tubes. Be sure to use a shipping kit labeled "For UPBEAT Baseline OR 3 Month Specimens ONLY" for newly enrolled patients.
3) **Upper Specimen Section for ambient temperature samples**: Only shippers for Baseline and 3 month samples will include one insulated silver zip-style bag with one gel insulator pack. The pack will contain one pre-labeled 8 mL Yellow top ACD Vacutainer, a segmented absorbent pouch, and a zip-style biohazard bag. This compartment is for the Yellow top blood tube collected ONLY at the Baseline or 3 month visits.
4) **Lower Frozen Specimen Section:** One white foam insulated container with ten (10) pre-labeled 1.6 mL cryotube vials; two zip-style biohazard bags; two segmented absorbent pouches; one white plastic screw top canister; and four individually wrapped sterile transfer pipettes (two extra). This compartment is ONLY for the frozen serum and plasma samples shipped on dry ice.
5) **Tubes are pre-labeled.** All cryotubes in the kit for serum (purple tops) and plasma (red tops) are pre-labeled with the same sample identifier number for this collection. The Yellow top blood tube is labeled with a different code.
6) **Documents:** One pre-paid return shipping label to be completed by the sender; one re-sealable FedEx shipping label pouch; and one UPBEAT Specimen Log Form.
7) **Sites will provide:** Supplies needed for venipuncture; 4-5 pounds dry ice; and packaging tape.

Shipping Container Packing Instructions

NCORP and ECOG-ACRIN Sites

1) **Upper compartment – for blood sample for DNA isolation in Yellow top tube.**
   a. **Blood for DNA is collected ONLY at the Baseline and 3 month visits. The silver pouch will not be included in shippers for other scheduled visits.**
   b. Record the Yellow top tube ID number on the UPBEAT Specimen Log Form.
   c. Place the filled Yellow top tube inside an absorbent pouch, seal the pouch inside the zip-style biohazard bag, place the biohazard bag between the layers of the included gel pack then close the gel pack and bag inside the cushioned silver pouch.
   d. Place the silver bag on top of the closed insulated foam box after the lower compartment has been packed.
2) **Lower compartment – for serum and plasma on dry ice.**
   a. Record the sample ID number for these samples on the UPBEAT Specimen Log Form. Make a copy of the completed Log Form for the patient record.
   b. Place the five frozen aliquots of plasma into one absorbent pouch and seal the pouch inside a biohazard bag.
   c. Place the five frozen aliquots of serum into a second absorbent pouch and seal the pouch inside a biohazard bag.
   d. Insert both biohazard bags inside the white plastic screw top canister and close its top.
   e. Place the screw top container with the plasma and serum samples in the insulated foam box and add 3 to 4 pounds of dry ice.
   f. Replace the top on the insulated foam box, tape it in place with packing tape, place the packed silver pouch (Baseline and 3 month shipments ONLY) on top with the completed Specimen Log Form, and securely close the cardboard box with packing tape.

SHIPPING INSTRUCTIONS

1) Complete pre-paid shipping label by entering your return address including telephone number.
2) Ship by FedEx priority overnight shipping, specifying that shipment will contain Exempt biospecimens. Ship ONLY Monday through Thursday. Do not ship on Friday or on the day before a holiday.
3) Ship container immediately to the WF NCORP Biospecimen Laboratory using the preaddressed shipping label supplied with the specimen kit.
4) Notify the Biospecimen Lab of the shipment with the shipping number by email to 7NCORP@wakehealth.edu.

UPBEAT Study
Ms. Gulya Kourman
WF NCORP Biospecimen Lab
Wake Forest Biotech Place
575 Patterson Avenue, Suite 240-250
Winston-Salem, NC 27101
Phone: (336) 716-2581

Specimen Collection and Shipping Kit Request Form
All Sites

Contact Name: _____

Site Name: _____

Site Shipping Street Address: _____

(No P.O. Boxes) _____

Site Phone Number: _____

Site Fax Number: _____

Contact Person Email: _____

Number of Kits Requested: _____

The initial IRB Approval letter must be sent to the WF NCORP Research Base Protocol Information Office **before** your first patient can be registered to this study.

Please fax a completed copy of the Specimen Kit Request Form **along with your initial IRB approval letter and consent** to the following:

Attn: UPBEAT Site Coordinator
Fax: (336) 716-6275
WF NCORP Research Base
Wake Forest Baptist Medical Center
Building 525@Vine, 4th Floor (Box 573152)
Medical Center Boulevard
Winston-Salem, NC 27157-3152

To Request Additional Shipping Kits:

Sites should contact Ms. Gulya Kourman by email at 7NCORP@wakehealth.edu. The contact information for the Wake Forest NCORP Biospecimen Laboratory is:

UPBEAT Study
Ms. Gulya Kourman
WF NCORP Biospecimen Lab
Wake Forest BioTech Place
575 Patterson Avenue, Suite 240-250
Winston-Salem, NC 27101
Phone: (336) 716-2581
Email: 7NCORP@wakehealth.edu

UPBEAT Specimen Log Form

A copy of this completed form must be sent with the specimen samples to the

Wake Forest NCORP Biospecimen Laboratory.

Place the original copy in the patient's protocol chart.

PID: _____ Patient Initials: _____

Blood for Future DNA Testing – Sample ID: _____
Collected at Baseline and 3 Month Visits ONLY

	Month	Day	Year		Hour	Min
Date				**Time**		

Date and time blood sample drawn: (Please use military time)

Blood Serum Samples – Sample ID: _____
Patient Visit (Circle one): Baseline, 1 Month, 3 Month, or 24 Month

	Month	Day	Year		Hour	Min
Date				**Time**		

Date and time blood sample drawn: (Please use military time)

Blood Plasma Samples – Sample ID: _____
Patient Visit (Circle one): Baseline, 1 Month, 3 Month, or 24 Month

	Month	Day	Year		Hour	Min
Date				**Time**		

Date and time blood sample drawn: (Please use military time)

FOR BIOSPECIMEN LAB USE ONLY

Date Received	Received by	Receipt Entered in Database?	Information Complete?