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Protocol title: Preventing Anthracycline Cardiovascular Toxicity with Statins (PREVENT)

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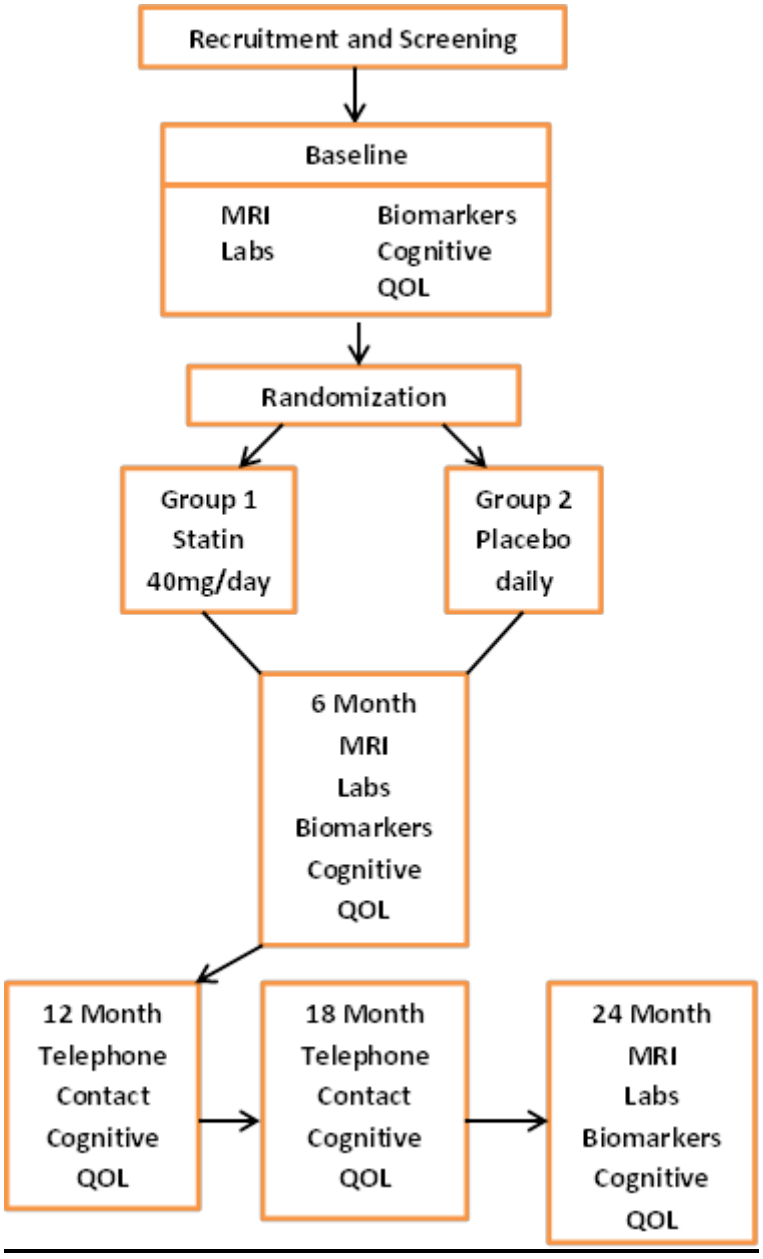
SCHEMA

Preventing Anthracycline Cardiovascular Toxicity with Statins (PREVENT)



Stratification Factors: Doxorubicin equivalent dose: < 240mg/m2 vs. ≥ 240mg/m2
Epirubicin equivalent dose: ≤450 mg//2vs >450 mg/m2
Age: 30 to ≤ 55 years old vs. > 55 years old

Study Sample: N=250
Study Duration: 24 months per subject

Brief Eligibility Criteria:

- Newly diagnosed Stage I-III (including inflammatory) female breast cancer
- Scheduled to receive adjuvant chemotherapy with an anthracycline
- Age: 30–80 years of age
- LVEF ≥ 50% (most recent within last 5 years)

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1. OBJECTIVES

The overall goal of this proposal is to determine if atorvastatin administration attenuates deterioration in left ventricular ejection fraction (LVEF) in women receiving adjuvant anthracycline-based adjuvant therapy (Anthracycline-based adjuvant therapy) for breast cancer. Left ventricular (LV) dysfunction precedes the development of congestive heart failure (CHF), a major contributor to cardiovascular (CV) events, and the 2nd leading cause of morbidity and mortality for 7-year survivors treated with adjuvant chemotherapy for breast cancer.[1-7] This heightened incidence of CV events threatens to offset cancer-related survival and raise healthcare expenditures for breast cancer survivors. An intervention that reduces LV dysfunction and CHF would be *significant*.[1-7]

Anthracycline-based adjuvant therapy is an important component of therapy for those with advanced disease;[8] for breast cancer alone, 5-year survival exceeds 70% after Anthracycline-based adjuvant therapy.[9] The cytotoxic anti-tumor effects from Anthracycline-based adjuvant therapy are related to its interactions with the enzyme topoisomerase-IIα, production of double-strand DNA breaks, and the generation of intracellular cytotoxic free radicals.[10] Unfortunately, in cardiomyocytes, these cytotoxic free radicals promote oxidative and nitrosative stress that, in combination with other Anthracycline-based adjuvant therapy effects (inflammation and neurohormonal activation), promote LV dysfunction, myocardial replacement fibrosis, CHF, and CV events.[11] Primary prevention strategies that reduce Anthracycline-based adjuvant therapy mediated myocellular oxidative/nitrosative stress could diminish LV dysfunction and the subsequent CHF often experienced by women after Anthracycline-based adjuvant therapy. No such strategies currently exist.

In the secondary prevention setting, the notion that modulating Anthracycline-based adjuvant therapy mediated oxidative/nitrosative stress can preserve LV function has been applied to women with poor resting LV function in need of Anthracycline-based adjuvant therapy.[12,13] In these women, intravenous pre-treatment with dexrazoxane, a free iron binding agent that reduces the generation of reactive oxygen and consequent nitrogen species, decreases further LV dysfunction and CV events.[12,13] This therapy is not utilized for primary prevention because of its expense, associated side effects, and the potential concern it may reduce anthracycline-mediated tumor regression.

Several lines of evidence suggest that generic, inexpensive, oral 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) may prevent cardiomyocyte injury during and after Anthracycline-based adjuvant therapy.[14] This class of drugs is used commonly to treat hypercholesterolemia, but also reduces oxidative/nitrosative stress, inflammatory cytokines, and circulating neurohormones.[15,16] In animal models, statins diminish LV dysfunction and oxidative/nitrosative stress after Anthracycline-based adjuvant therapy.[14] In our pilot studies, we find that LV injury occurs early (in the first 6 months of Anthracycline-based adjuvant therapy administration), and LV performance is preserved in women receiving Anthracycline-based adjuvant therapy when statins are co-administered for pre-existing CV disease during these 6 months.[19] Unlike some cardioprotective medications, statins do not promote hypotension, and recent data indicate that breast cancer recurrence does not increase among 18,000 5-year survivors receiving statins during and after treatment for breast cancer.[18]

Given the propensity of statins to ameliorate LV dysfunction and reduce CHF and other CV events, we seek to conduct a clinical trial to determine if statin therapy attenuates the early onset of LV dysfunction in women treated with Anthracycline-based adjuvant therapy for breast cancer. Accordingly, we propose the following.

Primary Objectives

Specific Aim 1: To determine if atorvastatin administration preserves LVEF 24 months after initiation of Anthracycline-based adjuvant therapy for adjuvant treatment of breast cancer.

Specific Aim 2: To determine if baseline to 6-month differences in LVEF predict baseline to 24-month differences in LVEF after Anthracycline-based adjuvant therapy and concomitant atorvastatin therapy.

To achieve these aims, we will perform a double-blind, placebo-controlled, randomized clinical trial of 0 or 40 mg of atorvastatin/day in 250 women scheduled to receive Anthracycline-based adjuvant therapy for treatment of adjuvant breast cancer. We will use innovative noninvasive magnetic resonance imaging (MRI) procedures to accurately measure LVEF. In addition, we will measure LV volumes, myocardial strain, fibrosis, aortic pulse wave velocity (PWV) and wall thickness, all factors that can influence LVEF by altering LV pre-load, after-load, and contractility.[19,20] Advanced serum biomarkers will be measured that assess for the presence of oxidative/nitrosative stress, systemic inflammation and circulating neurohormones that also may influence LVEF.

This study will test a new clinical paradigm to manage breast cancer: primary prevention of Anthracycline-based adjuvant therapy-related LV dysfunction using pre-treatment with low-cost statins. In addition, this trial will be the first systematic collection of data regarding the mechanism(s) and time course by which LV dysfunction and subsequent CHF evolve in women given Anthracycline-based adjuvant therapy for adjuvant breast cancer. These data will be useful to physicians trying to determine the optimal cardiac protection strategies when administering adjuvant chemotherapeutic regimens to their breast cancer patients. The objective of this research is to use inexpensive medications to preserve CV health and thereby improve overall survival in the growing number of breast cancer patients.

Secondary Objectives

Specific Aim 1:

To document the effect of atorvastatin on cognitive function using a battery of neurocognitive tests (HVLT, Rey-Osterreith Figure, COWA, Trail-making Parts A and B, Digit Span and Grooved Pegboard) in breast cancer patients receiving an anthracycline.

Specific Aim 2:

To document the effect of atorvastatin on self-reported quality of life using validated questionnaires (PROMIS including: General form, Cog Concerns, Cog Abilities, Fatigue, Pain intensity and interference, Sleep Disturbance, Physical Functioning and Social Functioning) in breast cancer patients receiving an anthracycline.

2. BACKGROUND

This study is significant in that it addresses:

1) A prevalent clinical problem. CHF and CV events (34,000 annual events in the US) together are the 2nd most common cause of morbidity and mortality in women who receive adjuvant treatment for breast cancer.[21] Administration of Anthracycline-based adjuvant therapy is a major factor related to development of CHF in this population.[22]
2) A therapeutic intervention to counter the mechanism(s) by which Anthracycline-based adjuvant therapy injures the heart. To date, no therapies are routinely given to breast cancer survivors to prevent oxidative and nitrosative stress, systemic inflammation, LV replacement fibrosis, and neurohormonal activation–all initiated on receipt of Anthracycline-based adjuvant therapy. Identifying a therapeutic intervention to address underlying mechanisms of Anthracycline-based adjuvant therapy-related LV dysfunction would be a significant clinical innovation.

3) The concept of pre-treatment and primary prevention of myocellular injury in breast cancer survivors. Existing clinical strategies for Anthracycline-based adjuvant therapy-related CHF are based on post-event clinical management. This study will test a new clinical paradigm**:** primary prevention of Anthracycline-based adjuvant therapy-related LV dysfunction using low-cost statin pre-treatment.
4) The evolution of CHF and other subclinical CV disease in breast cancer survivors. The studies planned in this proposal will record the evolution of subclinical processes observed in other large epidemiologic investigations of CHF and CV disease. These data will provide important new information on how cancer survivors develop LV dysfunction that often precedes adverse CV events.
5) CV healthcare expenditures in women with breast cancer. Over $800,000,000 per year in the US is spent providing CV-related care for women experiencing LV dysfunction and CHF after treatment for breast cancer.[3] We will test the effectiveness of a generic, cost-efficient (2 to 5 cents/day), widely available medication that may prevent LV dysfunction in women receiving Anthracycline-based adjuvant therapy for breast cancer.
6) NHLBI and NCI mandates including: a) the NHLBI strategic plan goal to improve understanding of the clinical mechanisms of CV disease and thereby enable better prevention, diagnosis and treatment; and b) the NCI's commitment to determine actionable strategies to reduce the burden of morbidity and mortality in patients with cancer.

In this section, we document the significant background data/information substantiating these statements.

Today, CV events including primarily CHF are the 2nd leading cause of morbidity and mortality in 5-year survivors of breast cancer.[1-7] Because both the number of long-term survivors and the use of complex cancer treatment regimens are increasing, the prevalence of CHF and CV events is growing particularly in women receiving Anthracycline-based adjuvant therapy. The long-term consequences of CHF and CV events after breast cancer are staggering:

- In 236,000 breast cancer survivors treated and followed between 1975 and 2002, CV event rates increased by 25%, offsetting a 15% decrease in cancer-related mortality.[3,6]
- Using the Surveillance, Epidemiology and End Results (SEER) database of 66- to 80-year-old women (n=7,724) treated for adjuvant breast cancer, the incidence of CHF ranged from 29% to 38% depending on the adjuvant treatment regimen received.[23]
- In a study of 63,566 women with breast cancer aged >65 years, CV disease was the leading cause of death (15.9%) followed by breast cancer (15.1%) in 5-year survivors; and, of those women who died as a result of CV disease, only 25% had existing comorbid CV disease at their breast cancer diagnosis.[23] Similar findings were reported in women below the age of 65 years.[24]
- In 179 breast cancer survivors experiencing a stroke (from a cohort of 11,045), events were 2.5 fold higher in those receiving versus not receiving chemotherapy containing primarily Anthracycline-based adjuvant therapy.[22,25]

These findings suggest breast cancer survivors with no prior history of CV disease exhibit high rates of CV events, and a relationship exists between breast cancer therapy and survivors' subsequent CV events.

2.1. Anthracycline-based adjuvant therapy's cytotoxic tumor effects also injure cardiomyocytes by promoting peroxynitrite o($ONOO^-$) induced stress.

Anthracyclines interact with the enzyme topoisomerase-IIα and produce double-strand DNA breaks that inhibit tumor cell DNA synthesis, transcription, and replication.[31] This class of agents also generates oxygen- and nitrogen-derived free radicals that are highly cytotoxic for malignant cells.[32]

Generation of oxygen- and nitrogen-derived free radicals can be harmful to cardiomyocytes. In healthy cardiomyocytes, nitric oxide (NO) is beneficial (green, Fig. 1); however, after Anthracycline-based adjuvant therapy, the increased bioavailability of NO and superoxide (O_2^-) promotes preferential production of $ONOO^-$ that induces myocellular injury (red, Fig. 1).[32] Unlike most other organs, the heart has few free radical scavengers, so it is particularly susceptible to injury from free radical production.[32]

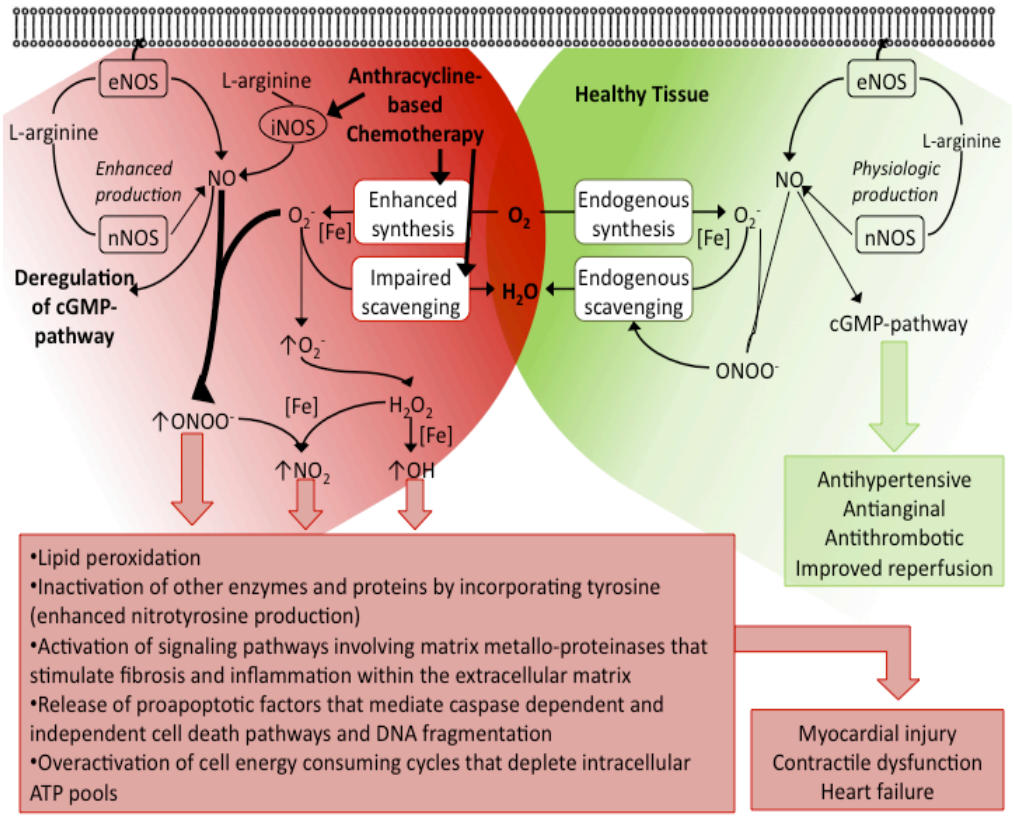


Figure 1: Mechanisms by which anthracycline chemotherapy causes cardiotoxicity. Doxorubicin increases oxidative and nitrosative stress, leading to increased peroxynitrite (ONOO). Peroxynitrite reacts further in both the cytoplasm and the mitochondria, leading to myocardial injury, contractile dysfunction, endothelial dysfunction and heart failure.

Several studies have linked the production of peroxynitrite (Fig. 1) after anthracycline exposure to both cellular myocyte (tension) and global (catheter-based measures of derivatives of pressure and time [dP/dt], LVEF, stroke volume, and cardiac output) abnormalities of LV myocardial performance.[32-34] In C57BL/6J mice receiving single intraperitoneal doses (up to 20 mg/kg) of doxorubicin (DOX, an anthracycline), myocardial apoptosis, levels of inducible nitric oxide synthase (iNOS) expression, mitochondrial superoxide generation, 3-nitrotyrosine (NT) formation (a downstream byproduct of elevated peroxynitrite), myocardial dysfunction, catalase, and glutathione peroxidase were all elevated.[32] These elevations were attenuated by peroxynitrite scavengers.[32] DOX-induced cell death and nitrotyrosine formation were also attenuated by iNOS inhibitors or in iNOS knockout mice.[32] *These studies indicate that Anthracycline-based adjuvant therapy promotes oxidative and nitrosative stress, thus initiating a cascade of events mediated by peroxynitrite formation that injures myocytes and promotes overall LV dysfunction*.

2.2 Statin pre-treatment reduces nitrotyrosine production (related to elevated peroxynitrite formation; red, Figure 1) and subsequent LV dysfunction during and after Anthracycline-based adjuvant therapy.

Five days after a single intraperitoneal injection of DOX at 20 mg/kg, LV function (measured as a change in dP/dt) was reduced in mice receiving DOX alone but not in mice receiving placebo or DOX + fluvastatin (Fig. 2B; red vs. blue and green bars).[14] DOX animals had increased myocellular lipid peroxidation and nitrotyrosine formation (a byproduct of elevated levels of peroxynitrite; Fig. 2A, red bar), increased tumor necrosis alpha (TNFα, a marker of systemic inflammation), and myocellular apoptosis ($p<0.05$ for all).[14] Mice given fluvastatin had lower cardiac expression of nitrotyrosine, an absence of LV dysfunction (Fig. 2B; green

bar), and no increases in markers of systemic inflammation (TNFα) or myocellular apoptosis.[14]

In a recent study of 40 patients scheduled to receive Anthracycline-based adjuvant therapy, those randomized to receive atorvastatin (40 mg per day before and during Anthracycline-based adjuvant therapy) had no decrease in LVEF at 6 months, but those randomized to receive Anthracycline-based adjuvant therapy without statins had an 8% reduction in LVEF (Table 1).[17] In other patients at high risk for CV events not receiving Anthracycline-based adjuvant therapy, pre-treatment with statin therapy before coronary artery interventions elective surgery reduces subsequent CV events by reducing oxidative stress, inflammatory cytokines, and circulating neurohormones.[38-40] *These data suggest that statin pre-treatment may attenuate LV dysfunction upon receipt of Anthracycline-based adjuvant therapy*.

Table 1: Comparison of Echocardiographic Parameters: Baseline vs. Follow-Up Values in Pilot Study

LVEF (%)	Statin Group (n=20)	Control Group (n=20)	p Value
Baseline	61±8	63±7	
After 6 months	63±9	55±10	
Mean change	+1±4	-8±8	<0.001

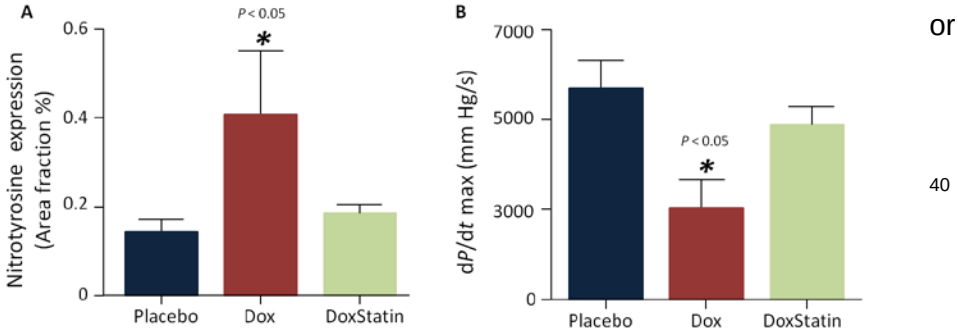


Figure 2: (A; left panel) Cardiac nitrotyrosine expression and **(B; right panel)** systolic LV function in 3 groups treated with placebo, doxorubicin, and a combination of fluvastatin and DOX. *Columns*, mean; *bars*, SE.[14]

2.3. Statins are well-tolerated and do not interfere with cancer therapy response rates or breast cancer recurrence after treatment for stage I-III breast cancer.

Degreef et al. found that statins were highly tolerated and contributed to lowering of adverse serum lipid levels during cancer treatment.[41] Statins plus classical cytotoxic agents in breast cancer cell lines often enhance anti-cancer effects.[42] Statins appear to stimulate apoptotic cell death in breast cancer cells and inhibit cancer cell proliferation by arresting the cell cycle at the G1S phase and inducing apoptosis.[43] Statins did not increase breast cancer recurrence in a population-based prospective cohort study of 18,769 women diagnosed with stage I-III invasive breast carcinoma.[18]

2.4. Preliminary Data

Six months after Anthracycline-based adjuvant therapy, PWV increases and LVEF and myocardial strain diminish.

Aortic stiffness increases upon receipt of anthracycline chemotherapy. J Clin Oncol. 2010;28(1):166-172.[19] Early and persistent evidence of subclinical cardiovascular injury after receipt of anthracycline chemotherapy. Circulation. 2010;122(21) Supplement:A12766[47] Supported by NIH-R33CA12196

We measured LV volumes, EF and circumferential strain; aortic PWV (a measure of aortic stiffness that contributes to LV after-load; and serum troponin I (TnI); before and 1, 3, and 6 months after initiating 3-month courses of low to moderately dosed Anthracycline-based adjuvant therapy in 51 individuals with breast cancer, leukemia or lymphoma. Participants averaged 52±2 (range 19 to 81) years in age; 65% were women, and 20% were black. LV end systolic volume (LVESV), aortic PWV, and serum TnI increased, and LVEF and circumferential strain decreased after Anthracycline-based adjuvant therapy (Fig. 3, in color). These findings persisted after adjustment for age, gender, race (white-black), doxorubicin (DOX) equivalent chemotherapy dose, CV co-morbidities, cancer diagnosis (breast vs. not breast), and resting cardiac or vascular function (p=0.032 to 0.0001 for all).[47] Change in LV or aortic function parameters was not DOX equivalent dose-related (r=0.01 to 0.05; p=0.85-0.92 for both). In a separate study, we substantiated early decreases in LVEF and increases in PWV after

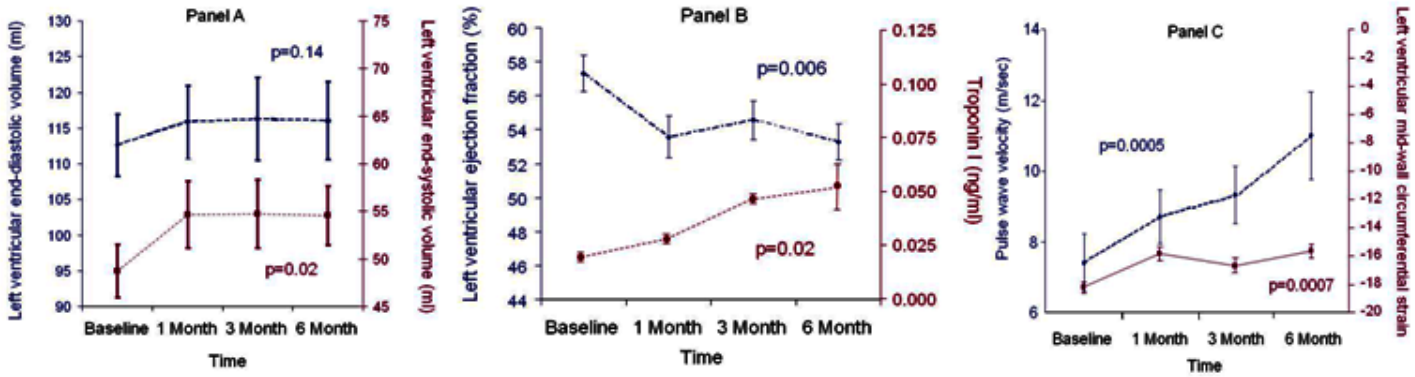


Figure 3: Time-dependent changes in left ventricular (LV) end diastolic volume (left y-axis; panel A) and LV end systolic volume (right y-axis; panel A); in LV ejection fraction (LVEF) (left y-axis; panel B); and mid-wall circumferential strain (right y-axis; panel C). The mean ± the standard error are shown. LV end systolic volumes increase and LVEF and myocardial strain decrease (less negative is a decrease), all indicating a subclinical deterioration in LV systolic performance. These subclinical abnormalities of LV and aortic function are associated with 2-4 fold increases in CV events.[47]

Anthracycline-based adjuvant therapy compared to a control population after accounting for age, sex, cardiac output, cancer diagnosis, cardioactive medications, and underlying clinical conditions known to influence PWV, such as hypertension or diabetes (p<0.0001).[19] The 3- to 6-month increase in PWV was similar in effect to aging the vascular system by 15 years. *Cardiac dysfunction occurs early after relatively low to moderate doses of Anthracycline-based adjuvant therapy, and persists 6 months after initiation (3 months after completion) of Anthracycline-based adjuvant therapy.*

In a retrospective study from our institution, statin therapy appears to preserve LVEF after exposure to Anthracycline-based adjuvant therapy. ***Preservation of left ventricular ejection fraction with statins during receipt of anthracycline-based adjuvant therapy. J Am Coll Cardiol 2012;59:E986.***[51] In 50 participants (33 women, 17 men; aged 48±14 years), we performed blinded MRI measurements of LVEF prior to and 6 months after initiation of Anthracycline-based adjuvant therapy for patients with breast cancer, leukemia, or lymphoma. 14 individuals received a statin for guideline-based indications and 36 did not. Statin therapy was 38±31 mg (range 5-80 mg) atorvastatin (n=6) or simvastatin (n=8). The mean cumulative anthracycline dose and DOX equivalent doses ranged from 30-450 mg/m^2: 193±95 mg/m^2 and 201±99 mg/m^2 in statin and non-statin users, respectively. At baseline, the LVEF was 57±5% and 57±9% for those taking and not taking a statin, respectively. In a multivariable model accounting for age, gender, co-morbidities (DM, HTN, HLD), and the dose of anthracycline, the LVEF decline after statin use was -0.5% compared to -7.8% in non-statin users (Fig. 4; p=0.0005). Moreover, in those receiving 40-80 mg/day of a statin, LVEF increased 2%; it decreased 3% after low (5-20 mg/day) dose statin (p<0.03).

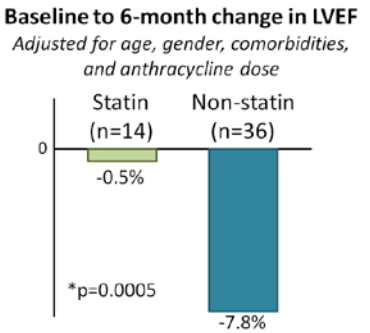


Figure 4: In a multivariable model adjusted for age, gender, co-morbidities, and the anthracycline dose, those who received statin had an LVEF decline of -0.5% compared to the non-statin group at -7.8%.

These data indicate that patients on statins for other guideline-based indications may be protected against deterioration in LVEF after Anthracycline-based adjuvant therapy.

3. SUMMARY OF STUDY PLAN

3.1. Overview of Study and Study Design

We propose a double-blind, placebo-controlled randomized clinical trial of 40 mg of atorvastatin (Lipitor®) or placebo per day in 250 women age 30 to 80 years old scheduled to receive Anthracycline-based adjuvant therapy for Stage I-III including inflammatory Breast Cancer. Innovative noninvasive MRI measures of LV volumes, EF, myocardial strain, PWV, arterial wall thickness, and other serum markers plus key patient reported outcomes including cognition and quality of life measures will be collected at baseline, 6 and 24 months after initiating treatment with Anthracycline-based adjuvant therapy. In addition, participants will be contacted by telephone to assess medication compliance/toxicities and Quality of Life at 12 and 18 months.

3.2. We will use the established CCOP structure of the Comprehensive Cancer Center of the Wake Forest University Research Base (CCCWFURB). We will recruit from CCOP sites with appropriate MRI facilities that treat modest numbers of breast cancer patients with adjuvant Anthracycline-based adjuvant therapy. Importantly, the PI has visited and conducted interviews with all participating CCOP sites to verify their MRI and breast cancer recruitment capabilities.

3.3. MRI was selected to provide the endpoints of left ventricular volumes, LVEF, myocardial strain and PWV because of it's:

- Reliability (>90% of the valuable subjects in the Jackson Heart Study for which Dr. Hundley serves as the Co-PI of the core lab and the preliminary cancer studies mentioned in Section C.1.),
- Reproducibility (<2% discordance for repeated measures in the pilot data from the cancer participants),
- Accuracy (its ability to detect informative changes indicative of both CV disease and forecasting prognosis),
- Translational capability (its association with large NHLBI efforts to assess CV disease and thereby make additional comparisons), and
- Versatility (in that with a single imaging modality one cannot only collect information regarding the measurement of LVEF but also the factors such as pre-load and after-load that can also impact the measurement of LVEF).

Importantly, all sites have been verified as to the performance of these procedures via onsite interviews and case image recovery.

Ultrasound and radioisotope studies can be used to assess cardiac function. These measures, however, exhibit high variance, are operator-dependent, and are difficult to obtain in individuals with unfavorable body habitus. Radioisotope studies require exposure to ionizing radiation. Catheter-based assessments of left ventricular and vascular structure or function can be acquired but these techniques are associated with the risks of an interventional procedure. Thus, we selected MRI for this study.

4. PARTICIPANT SELECTION

4.1. Inclusion Criteria

4.1.1 Newly diagnosed Stage I-III female breast cancer (including inflammatory breast cancer)
4.1.2 Scheduled to receive adjuvant chemotherapy with an Anthracycline (doxorubicin and epirubicin
4.1.3 30 to 80 years of age
4.1.4 LVEF $\geq$ 50% (Most recent within the last 5 years)
4.1.5 Prior chemotherapy regimen not containing anthracyclines is allowed
4.1.6 Able to hold breathe for 15 seconds
4.1.7 Prior cancers allowed if no evidence of disease in last 5 years
4.1.8 ECOG 0 or 1

Participants must have required labs (within 1 month prior to registration) as defined below:

X Fasting serum Fasting Lipid Profile: Total Cholesterol/HDL/LDL
(**LDL levels prior to chemotherapy must be $\leq$140 mg/dl)**
X Alanine aminotransferase level (ALT) $\leq$ 2x the ULN
X Creatinine level $\leq$ 2.0
X Total bilirubin $\leq$ 2.0
X TSH $\leq$ 1.5 times ULN
X Creatinine kinase $\geq$ 3x the ULN
X Fasting Glucose
X Fasting Hemoglobin A1C
X CBC, platelet and diff
X Fasting Triglycerides

4.2 Exclusion Criteria

4.2.1 Prior use of lipid-lowering therapy within the last 6 months
4.2.2 Current postmenopausal hormone-replacement therapy
4.2.3 Uncontrolled hypertension (systolic blood pressure >190 mm Hg or diastolic blood pressure >100 mm Hg)
4.2.4 **Scheduled to receive neoadjuvant chemotherapy with an anthracycline**
4.2.5 No active liver disease allowed
4.2.6 Uncontrolled hypothyroidism
4.2.7 Recent history (within past 3 years) of alcohol or drug abuse, inflammatory conditions such as lupus or inflammatory bowel disease, use of immunosuppressant agents, or another medical condition that might compromise safety or the successful completion of the study.
4.2.8 Patients with ferromagnetic cerebral aneurysm clips or other intraorbital/intracranial metal; pacemakers, defibrillators, functioning neurostimulator devices or other implanted electronic devices.
4.2.9 Unstable angina; significant ventricular arrhythmias (>20 PVCs/min due to gating difficulty) atrial fibrillation with uncontrolled ventricular response; coronary artery disease; acute myocardial infarction within 28 days
4.2.10 Females < 18 years old

4.2.11 Current use of CYP 3A4 inhibitors. These include Clarithromycin, HIV protease inhibitors, Itraconazole, grapefruit juice, Cyclosporine, Rifampin or Digoxin
4.2.12 Current or history of hepatic dysfunction
4.2.13 Unable to provide informed consent
4.2.14 Claustrophobia
4.2.15 Planning to move within 24 months of trial enrollment
4.2.16 Pregnant or breast–feeding

4.3. Inclusion of Women and Minorities

Table 2: Race/Ethnicity

Gender	White	Black or African-American	Hispanic or Latino	Asian or Pacific Islander	Unknown	Total
Male	0	0	0	0	0	0
Female	200	46	2	2	0	250
Total	200	46	2	2	0	250

4.4. Recruitment and Retention Plan

4.4.1 In the past 2-3 years, we have enrolled 43% (1 in 2-3) of cancer patients into a similar study protocol with a similar level of participant burden. 570 women are eligible for enrollment among the 6 sites. Using a conservative projection that 17% (1 in 6) women will consent to the study, we expect 97 women to be enrolled annually. We anticipate 20% of our cohort will not drop out, so we expect 200 women to complete the study.
If enrollment is unexpectedly low, we can add additional CCOP sites in which study coordinators, imaging expertise, and patient populations are readily available.

4.4.2. At each site, specific recruitment plans may include the following according to the site's institutional policy: screening clinic charts; tumor registry data; referral sources such as patient advocate groups, retirement communities, churches, support organizations, community organizations, newspapers, radio; or patient recruitment posters and recruitment letters.

4.4.3. The research PI or designee at each WFU Research Base CCOP, which may include the clinic physician, resident, research nurse or research assistant, will review cancer registry and medical chart information to identify patients eligible for this protocol. Patients identified using these methods will be asked to join the study during their next clinic visit/consultation.

Accrual is expected to be 8 patients per month. Targeted accrual should be met in approximately 2.5 years for the 5 year study. A maximum of 250 patients will be enrolled on this trial. Patients will be followed for 24 months. After 24 months (the last visit), the patient is no longer followed and data is no longer collected from the patient.

5. AGENT ADMINISTRATION

5.1. Dose Regimen and Dose Groups:

Group 1 (one 40 mg atorvastatin tablet each morning by mouth for 24 months) and Group 2 (one placebo tablet each morning by mouth for 24 months).

5.2. Lipitor/Placebo Administration

Patients will be 1:1 randomized to receive the study agent, (one 40 mg atorvastatin tablet daily by mouth for 24 months) or a placebo (one tablet daily by mouth for 24 months).
Either agent will be taken once any time of day, with or without food, 7 days per week, on an outpatient basis, for a total of 24 months. The atorvastatin pills and matching placebo pills used for this study will be provided free of charge to the patient. Missed doses do not need to be made up. However, they should be recorded in the medication diary. Patients who miss doses, regardless of the number of missed doses, will remain on study.

5.3. Run-in Procedures. N/A

5.4 Contraindications

Active liver disease, which may include unexplained persistent elevations in hepatic transaminase levels, or hypersensitivity to any component of this medication.

5.5. Concomitant Medications

Atorvastatin is metabolized by cytochrome P450 3A4. Concomitant administration of this statin with strong inhibitors of CYP 3A4 can lead to increases in plasma concentrations of atorvastatin. The extent of interaction and potentiation of effects depend on the variability of effect on CYP 3A4. These include Clarithromycin, HIV protease inhibitors, Itraconazole, grapefruit juice, Cyclosporine, Rifampin or Digoxin.

5.6 Dose Modification

Dose modification is only allowed for Myalgias.

5.6.1 Dose Modification for Myalgias

Grade 1 Myalgias – If needed patient may take NSAIDs/analgesics at the discretion of the treating physician. Frequently this type of skeletal pain is not relieved with these medications.

Grade 2 or 3 Myalgias - Study medication should be stopped for 14 days. If myalgias improve to a Grade 1 or patient has no further pain study medication may be restarted. If myalgias return to a Grade 2 or 3 the study medication should be discontinued. Patient will remain on study and be followed as usual.

Any patient with suspected rhabdomyolysis/compartment syndrome of lower leg, rupture of tendon or Grade 3 Myalgias should stop the study medication immediately. The treating physician should assess the patient and order CPK isoenzymes and/or other labs as deemed necessary. Patient will remain on study and be followed as usual.

5.7 Adherence

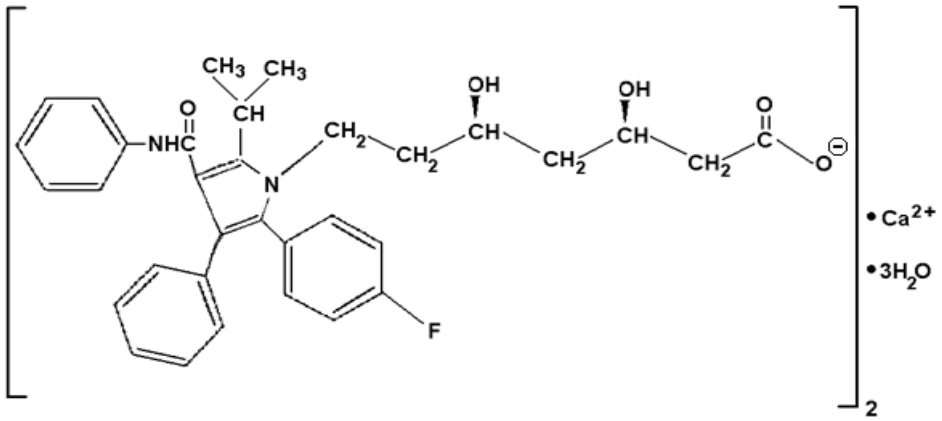
Adherence will be estimated as: 1) the proportion of expected pills taken while on treatment and 2) the proportion of the total number of pills that could be taken if the participant completed the study. We will calculate and report the mean adherence across all individuals as well as the proportion of patients who were 75% adherent (using both definitions of adherence). The primary analyses will include all randomized participants, regardless of adherence. Secondary analyses will be done using participants who were at least 75% adherent, separately using each definition.

To determine patients' adherence with the study agent, a medication diary will be utilized. It will be administered by staff at each enrollment site. At 6, 12, 18 and 24 months medication diaries will be collected to verify medication usage. In addition, at 6 and 24 months any remaining bottle(s) of study agent will be collected from the patient and a pill count will be performed to verify the patient-recorded information in their medication diary.

6. PHARMACEUTICAL INFORMATION

6.1 Atorvastatin (Lipitor): The dosing for this study will be a daily dose of 40mg for 24 months. Lipitor can be administered as a single dose at any time of the day, with or without food. Pharmaceutical tests of atorvastatin suggests aggressive dosing of HMG-CoA reductase inhibitors (statins) reduce lipid levels and cardiovascular mortality and morbidity to a significantly greater extent than standard statin doses, according to the PROVE IT and REVERSAL trials. However, in our study, dosage has been set at 40mg daily in consideration of breast cancer chemotherapy treatment.

Pharmacological Description: Lipitor is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Atorvastatin calcium is [R-(R*, R*)]-2-(4-fluorophenyl)-ß, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. The empirical formula of atorvastatin calcium is (C33H34 FN2O5)2Ca•3H2O and its molecular weight is 1209.42. Its structural formula is:



Atorvastatin calcium is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile; slightly soluble in ethanol; and freely soluble in methanol. Lipitor tablets for oral administration contain 10, 20, 40, or 80 mg atorvastatin and the following inactive ingredients: calcium carbonate, USP; candelilla wax, FCC; croscarmellose sodium, NF; hydroxypropyl cellulose, NF; lactose monohydrate, NF; magnesium stearate, NF; microcrystalline cellulose, NF; Opadry White YS-1-7040 (hypromellose, polyethylene glycol, talc, titanium dioxide); polysorbate 80, NF; simethicone emulsion.

6.2 Reported Adverse Events and Potential Risks

Common Events

- **Gastrointestinal:** Abdominal pain (In pre-marketing and open extension studies with a median follow-up period of 18 months, abdominal pain was reported in 7.3% of enrolled patients [n=2423]), constipation (In pre-marketing and open extension studies with a median follow-up period of 18 months, constipation was reported in 6.6% of enrolled patients [n=2423]), nausea

(In pre-marketing and open extension studies with a median follow-up period of 18 months, nausea was reported in 5.4% of enrolled patients [n=2423])

- **Neurologic:** Headache (In pre-marketing and open extension studies with a median follow-up period of 18 months, headache was reported in 7.4% of all patients enrolled [n=2423])
- **Respiratory:** Upper respiratory infection (9%)
- **Muscle Pain (Myalgia):** Soreness usually in upper arms and shoulders or hip and thighs about 6%.

Serious Events

- **Hepatic:** Cholestatic hepatitis, increased liver enzymes (1%)
- **Musculoskeletal:** Compartment syndrome of lower leg, disorder of muscle (20 to 40 mg/day, 0.02% to 0.08%; 80 mg/day, 0.53% to 0.9%), rhabdomyolysis, rupture of tendon

6.3 Drug Availability and Description

Atorvastatin will be manufactured and supplied by Greenstone. The study agent will be directly supplied to Biologics, Inc. who will fill pill bottles and create labeled prescriptions.

Biologics, Inc. will provide procurement, over encapsulation and placebo manufacture services.

Atorvastatin - supplied as 40mg capsules packaged in 90-count bottles. Biologics will procure the active supply from wholesaler for use in this study. The active supply will then be over encapsulated to maintain the blind of the study. Each bottle of over encapsulated Atorvastatin will be 180-ct. Biologics anticipates making two purchases of active supply, one prior to the start of the study and one at year two, to accommodate the five year length of the trial and expirations of the product. Prior to study launch, Biologics will procure and over encapsulate approximately 54,720 tablets. At year two, Biologics will procure and over encapsulate approximately 54,720 tablets to complete the study.

Placebo – matching placebo supplied as capsules packaged in 90-count bottles. Biologics will facilitate the manufacturing of a matching placebo to maintain the blind of the study. Each bottle of matching Placebo will be 180-ct. Biologics anticipates the manufacture of two batches of the matching placebo, one prior to the start of the study and one at year two, to accommodate the five year length of the trial and expirations of the product.
Prior to study launch, Biologics will facilitate the manufacture of approximately 54,720 placebo capsules. At year two, Biologics will facilitate the manufacture of approximately 54,720 placebo capsules to complete the study.

Shipment 1
12 Month Patient Specific Supply

- Biologics will ship 2 bottles of study drug to the study site
- Months 1-12: 2 bottles (180-ct)

At approximately month 11, Biologics will contact the site to prepare the subsequent shipment of study drug for the patient to complete months 13-24.

Shipment 2
12 Month Patient Specific Supply

- Biologics will ship 2 bottles of study drug to the study site
- Months 13-24: 2 bottles (180-ct)

6.4 Agent Ordering and Distribution

Biologics, Inc. will provide with atorvastatin 40mg pills and matching placebo and will distribute study drug/placebo directly to participating sites (WF exempt). The contact at the Biologics, Inc. is:

Karl Buer
120 Weston Oaks Court
Cary, NC 27513
(919) 459.4991

6.5. Agent Accountability

Each participating CCOP site will maintain a careful record of the inventory and disposition of the study agents received (atorvastatin or placebo pills) using the NCI Drug Accountability Record Form (DARF). This will include adequate records of receipt, dispensing, quantities, dates and final disposition of study agent.

6.6 Packaging and Labels

Summary

The study will remain open approximately 5 years or until all 250 patients have been accrued and can complete the study. Biologics, Inc. will send to the participating site the 24-month supply for each patient randomized to the study.

Packaging

- All study drugs will include a patient specific label adhered to the bottles. Biologics will place the bottles in a Ziploc bag and include a patient specific label on the outside. Biologics will also include the study number on the label to avoid confusion at the sites.
- Each shipment includes a label on the Ziploc bags with the following information (as needed):
- The Study Number
- The Patient study ID number
- The Patient's name or initials
- The number of capsules
- Administration instructions (i.e. 'Administer as Directed per Protocol')
- Drug identification (Atorvastatin 40mg OR Placebo)
- Expiration Date
- Dosing instructions (Administer as Directed per Protocol)
- Storage instructions ("Store at controlled room temperature, 20-25°C (68-77°F)")
- Date dispensed
- IND caution statement and/or local regulatory statements
- Emergency contact instructions

For all drug shipments from Biologics, Inc., a packing slip will be enclosed that includes the date and quantity of drug provided patient name/initials, study ID number, drug identification and expiration date.

The Biologics, Inc. will process and ship "same day" of patient randomization if received before 2:00 p.m. EST Monday through Friday. Orders received after 2:00 pm EST Monday through Friday will be processed and shipped the next business morning.

All drug orders are shipped via *FedEx for Priority Overnight* delivery. Study drug is shipped in boxes designed to maintain temperature stability.

Once study drug is received at the clinical trial site, the designated site coordinator validates contents of package match information provided on packing slip with study medication received. The inventory and disposition of atorvastatin or placebo pills must be recorded using the NCI Drug Accountability Record Form (DARF).

6.7. Storage

Atorvastatin pills are supplied as 40mg pills in 180 count bottles and stored at 20-25°C (68-77°F).

6.8. Registration Process

An IRB letter of approval and an IRB approved consent form must be received by the Research Base Protocol Information Office, Attn: Site Coordinator, prior to patient registration. Fax: (336) 716-6275. An "Eligibility Checklist / Registration Form" will be used to complete the on-line registration.

Online Registration

Instructions for on-line registration are as follows:

"Log on to the CCCWFU Research Base registration web site at https://ccrbis.phs.wfubmc.edu/. Enter your user name and password (which may be obtained by contacting June Fletcher-Steede at jsteede@wakehealth.edu.). *In the 'Patient Registration and Protocol Information' table, click the 'Register Patient/Patient Info', with the corresponding protocol number found in the drop down box to the right. Fill in the eligibility criteria forms using the drop down boxes.* Once the patient information has been entered online, print a copy of the eligibility checklist/registration form for your records. Press the submit button; a confirmation page will appear. Print this confirmation sheet for your records. The CCCWFU On-line Protocol Registration/Eligibility form, initial flow sheet, signed consent, histology reports, scan reports and lab reports (as required in protocol) should be faxed to (336) 713-6476 or mailed to Data Management:

Research Base Data Management Center
Department of Radiation Oncology
1st Floor Cancer Center
WFUBMC
Medical Center Boulevard
Winston-Salem, NC 27157

These forms should be retained in the patient's study file. These forms will be evaluated during an institutional NCI/CCCWFU CCOP Research Base site member audit. If you have questions related to the registration process or require assistance with registration, please contact the CCCWFU CCOP Research Base Data Management Center between 8:30am and 4:00pm EST, Monday through Friday at (336) 713-3172 or 713-6507.

6.9. Unblinding Methods

In the event a patient on this study develops a toxicity (adverse event or severe adverse event) for which the patient's physician or other health care professional feels that it is in the patient's best interest to know what drug they are taking (active study drug(s) or placebo), the following procedure should be followed:

- Step 1: The patient's physician or a designated health care professional should call the Wake Forest University Baptist Medical Center Physician Access Line at (336) 716-7654 and ask that Dr. Ed Shaw, Director of the CCCWFU CCOP Research Base, be contacted immediately either in his office, by pager, or at home. In the event Dr. Shaw cannot be reached, the PAL operator should contact Dr. Glenn Lesser, Director, Symptom Treatment Protocols, in his office, by pager,

or at home. If neither Dr. Shaw nor Dr. Lesser can be reached, the PAL operator should contact Gina Enevold, GNP, Research Base Administrator, either in her office, by pager, or at home.

- Step 2: Once contact has been made, the patient's physician or health care professional should explain the reason for the request to unblind the treatment arm that the patient is on. If the

  Research Base representative feels that the toxicity (AE/SAE) is possibly, probably or definitely related to the study drug, then the next step will be followed.

- Step 3: The responsible Research Base representative will call the pharmacist @ Biologics, Inc. (phone: 1-800-850-4306). There is an "on-call" service provided 24 hours a day, seven days a week for the Chemical Drug Trials unblinding service. The Biologics pharmacist may contact the patients' physician and/or health care professional directly with the unblinding information. Written documentations of the unblinding process will be sent to the Research Base Principal Investigator by Biologics, Inc.

- Step 4: In the event that the patient's treatment is unblinded, that patient will be taken off study with no further study follow-up. Appropriate procedures for grading toxicities, assigning causality, and reporting severe adverse events (if applicable), should be followed for each protocol for all Phase II and Phase III Clinical Trials. The event will be reviewed by the CCCWFU Clinical Research Oversight Committee and reviewed by the CCOP Research Base Data Safety and Monitoring Board.

Record the details of the adverse event and/or unblinding in the site source documentation and complete appropriate AE forms.

Unblinding Study Participants at Study Completion
Study participants may be unblinded at the conclusion of the study if **all** patient specific data for the requesting site are completed and submitted to the Data Management Center (DMC) and all of the primary study outcomes have been determined and the final data set is cleaned and prepared for analysis.. Site members can obtain unblinding information by sending an email request to the CCCWFU CCOP Administrator or Data Management Supervisor with a list of PID #s.
After confirming with the DMC that patient specific data for all patients at the requesting site have been received, completed and entered into the RB database, the Research Pharmacy will be notified. An email from the Research Pharmacy containing the unblinding information will be sent directly to the requesting site.

6.10. Agent Destruction/Disposal

Unused drug should be destroyed on site following site institutional policies and procedures.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

At each participating Research Base site, medical charts will be screened to determine potential eligibility by physicians (including residents or fellows, if applicable), research nurses, or clinical research associates. Patients identified as potentially eligible will then be asked to consider joining the study. Patients meeting initial eligibility criteria and who agree to participate in the study will sign informed consent and then undergo a baseline history and physical exam, laboratory tests and quality of life tests. At baseline, 6, and 24 months, the entire battery of neurocognitive tests and questionnaires will be administered by a trained and certified examiner. At 12 and 18 months participants will be contacted by phone to assess toxicities and medication compliance. Quality of Life Questionnaires will be completed via mail. Patients will be instructed in the self-administration

of the study agent. Patients will take one pill daily. Potential side effects and risks are described in Section 6.2. Adverse event reporting is described in Section 10. Study outcomes will be assessed at baseline, after 6 months and at the end of 24 month.

7.2. Baseline Testing/Pre-study Evaluation

Subjects will be checked for height, weight, waist circumference, heart rate and blood pressure. Body Mass Index (BMI) will be calculated at data center. Patient physical exam including peripheral edema, smoking status and family history will be collected.

Serum and blood derived biomarkers - A blood sample of approximately 4 tablespoons from a vein in the arm/central line for lab analysis. Blood samples will be stored with a unique identifier and will not include any information protected by HIPAA regulations.

Statins exert pleiotropic effects (modulation of systemic inflammation and the neurohormonal axis) that in other models of heart failure influence LV function,[57,58] and have been modulated in pilot animal studies.[14] In this study, we will determine the relationship between Anthracycline-based adjuvant therapy administration, statin use and biomarker evidence of systemic inflammation[59] and neurohormonal axis activation.[60] We will associate these relationships with changes in both LV and vascular function.

MRI Variables - MRI exams will be accomplished according to previously published techniques described in Appendix II. Our research group and Dr. Hundley (PI) has extensive experience developing and utilizing these 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.

LVEF is the primary outcome because it is the most widely clinically implemented assessment of LV performance, and is used to guide therapeutic intervention. LVEF has limitations as it can vary dependent on LV pre-load or after-load. To account for these limitations we are utilizing MRI to measure LVEDV (pre-load) and PWV (after-load). Also, we will perform advanced assessments of LV contractility including myocardial strain which is utilized in other NHLBI studies (such as MESA). This will allow us to measure systolic performance independent of LV pre-load and enable cross-sectional comparisons of our data with other NHLBI-funded cohorts.

Qualified study participants will be randomly assigned in a 1.1 ratio to receive 40mg atorvastatin or placebo pill daily. Dispensation of pill numbers will be uniform for both study groups.

7.2.a. Neurocognitive function
Cognition and quality of life

Once eligibility has been established the neurocognitive test battery and the study questionnaires will be completed. Individual components of the full battery are described below. Tests have been selected to represent a range of cognitive abilities which have been reported in the literature to be affected by chemotherapy including attention, verbal and figural memory, working memory, executive functions, speed of mental processing, verbal fluency and psychomotor performance. All cognitive testing will be performed by a trained and certified research assistant blinded to treatment assignment. The CCCWFU Research Base has conducted training workshops for administering this cognitive test battery for our on-going trials of donepezil among irradiated brain patients, and maintains a stringent certification process (Section 8.2).

Each cognitive test has adequate psychometric properties and has been used in cancer research including large national and international clinical trials (Ahles & Saykin, 2002; Mehta et al., 2002; Meyers et al., 2004). The entire battery takes 45 minutes to administer. It will be administered with the questionnaires which takes 10 minutes at baseline, 6 months and 24 months.

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).

Hopkins Verbal Learning Test-Revised (HVLT-R)

The HVLT-R measures verbal learning and memory. It consists of a 12-item word list which is read to subjects on three successive learning trials. Free recall scores are recorded for each learning trial. After a 20-minute interval during which subjects complete other non-interfering tasks and questionnaires they are asked to recall the target words. Lastly, a yes/no recognition task is then presented in which subjects are asked to identify all target words by responding "yes," and to reject 12 non-target words by responding "no." The HVLT has six equivalent alternate forms (Brandt, 1991) to minimize confounding by practice effects. Test-retest reliability of the HVLT is quite good (0.74). The test is brief, taking only 10 minutes to administer, and it is well-tolerated by compromised (geriatric and dementia) populations. Scores for immediate recall (total of three trials), delayed recall (total number of words recalled after 20 minutes), and recognition (total number of words correctly identified) will be the variables derived from the HVLT-R.

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).

Rey-Osterrieth Complex Figure-Modified (ROCFm)

ROCFm (Fastenau et al., 1999) is a test of visuomotor skills and non-verbal memory. First, subjects are asked to copy a complex figure then draw the figure from memory. Several minutes later during which subjects are completing other non-interfering tests and questionnaires they are asked to redraw the figure from memory. The score for each drawing is the sum of figure elements recalled using standardized scoring criteria.

Digit Span Test (DST)

The DST (Wechsler, 1981) assesses attention and working memory. It requires respondents to repeat back gradually increasing spans of numbers. Eight series of two spans of each length are presented and repeated forwards and seven other series are repeated backwards. A total score is the sum of the longest span forwards and backwards.

Grooved Pegboard (GP)

The GP (Trites, 1977) task measures motor performance and dexterity. The respondent inserts 25 small pegs with a key tip into 25 randomly positioned slots, first with the dominant hand and then with the non-dominant hand. The score is the total number of pegs correctly inserted in the time allotted. Pegboards will be provided on loan by the WFU Research Base. A pegboard will be shipped to the site upon request. Send requests to June Fletcher-Steede at jsteede@wakehealth.edu. Pegboards must be returned to WFU Research Base at completion of the study.

Self-Report Questionnaires:

The Patient Reported Outcomes Measurement Information System (PROMIS) is a collection of well-validated, brief, fee-free instruments assessing key patient reported outcomes related to this study. Each PROMIS scale has excellent psychometric properties (reliability and validity) and are being used in many clinical trials and observational studies. For this study, we will include the short forms for cognitive concerns (8 items) and cognitive abilities (8 items), fatigue (7 items), mood (depressed, anxious, angry; 25 items), pain intensity and interference (9 items), sleep disturbance (8 items) and physical (10 items) and social (4 items) functioning. The total administration time for these questionnaires is 10 minutes.

7.3. Evaluations During Study Intervention

Study participants in both statin and placebo groups will have an Cardiac MRI, and blood drawn for a cardio profile, lipids, CK, ALT, glucose, Hemoglobin A1C, CBC, platelet, diff, TSH and neurohormonal biomarkers collected at 6 months. Biomarkers studied include ultrasensitive troponin I (for myocellular injury); C-reactive protein, interleuken-6, tumor necrosis factor-a (systemic inflammation); and rennin and aldosterone (circulating neurohormones).In addition, neurocognitive test battery and Quality of Life Questionnaires will be obtained. At 12 and 18 months participants will be contacted by phone to assess toxicities and medication compliance. Quality of Life Questionnaires will be completed via mail.

7.4. Evaluations at Completion of Study Intervention

All study participants will have an Cardiac MRI and blood drawn for a cardio profile, Lipids, CK, ALT, glucose, Hemoglobin A1C, CBC, platelet, diff, TSH and neurohormonal biomarkers noted in 7.3. Neurocognitive testing and Quality of Life Questionnaires will be obtained. Follow-up assessments will include laboratory evaluations, pill counts, and structured interviews assessing outcomes and potential adverse events.

7.5. Post-intervention Follow-up Period. N/A

7.6 Methods for Clinical Procedures

Processes for obtaining the MRI and lab sample data are provided in Appendices 9 and 10.

7.7. Study Parameters Table

****Study medication must be started 2 days prior to chemotherapy****

Baseline evaluations must be within 1 month of registration.

Evaluation/Procedure	Baseline	6 Months	12 Months	18 Months	24 Months	Description
Informed Consent	X					
Physical Exam	X					Height, weight, Blood Pressure, Heart Rate, Peripheral Edema
Cardiac/Vascular MRI	X	X			X	LV Volumes, Ejection Fraction, Strain, Fibrosis, Wall Thickness, Pulse Wave Velocity
LVEF	X					Must not be recent in the last 5 years and $\geq$ 50%
General Labs Including Fasting Labs and Non-fasting Labs	X	X			X	**Fasting**: LDL Cholesterol, HDL Cholesterol, Total Cholesterol, Triglycerides, Glucose, Hemoglobin A1C. **Non-Fasting:** TSH, ALT, Serum BHCG within 10 days prior registration for women of child-bearing potential.
Biomarker Labs	X	X			X	Creatinine, Creatinine Kinase, Hematocrit, Serum and Plasma, Biomarkers (C-reactive Protein, Interleukin-6, TNFα Renin, Aldosterone, Ultra Sensitive Troponin-I, Nitrotyrosine). Stored labs for future use if applicable.
Baseline Booklet (Includes QOL's)	X					**Cognitive Function:** Memory, attention, language, executive function, processing speed. (Baseline, 6 & 12 months) **Quality of Life:** Cognitive symptoms, pain, fatigue, mood, physical and social functioning, sleep
6 Month Booklet (Includes QOL's)		X				
12 Month Booklet (Includes QOL's)			X			
18 Month Booklet (Includes QOL's)				X		
24 Month Booklet (Includes QOL's)					X	
QOL Booklets			X	X		**12** and **18** month booklets may be collected via mail
Flow Sheet/Addenda	X	X	X	X	X	
TAS (Toxicity Assessment Sheet)	X	X	X	X	X	If **"No"** toxicities noted on telephone contact form at **12** and **18** months, TAS does not need to be submitted.
Medication Diaries		X	X	X	X	**12** and **18** month diaries may be collected via mail.
Phone Contact Form			X	X		
Early Withdrawal Form for Treatment/Consent		Submit this form when a participant withdraws from active treatment or withdraws consent prior to study completion.				

7.8. Off Treatment Criteria

Participants may stop taking atorvastatin for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, medical contraindication, or interruption of breast cancer chemotherapy due to adverse events or death.

Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. The study is designed to account for drop-outs due to poor health or death; thus, there will be no replacement of subjects that must drop out of the study after enrollment.

7.9 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, concomitant medication, medical contraindication, withdraw consent, or death.

8. PROTOCOL SPECIFIC TRAINING REQUIREMENTS

8.1 MRI Training

- Refer to Appendix II for MRI protocol.
- MRI technologists and associated study coordinators will receive MRI training at the Wake Forest University Medical Center. This 1-2 hour session will include: review of the scan protocol, scanning a scheduled volunteer participant, and ability to ask questions regarding the protocol.
- 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 studies to the image coordinating center at Wake Forest before initiating the study.

8.2 NEUROCOGNITIVE TRAINING

8.2.1 Certification Requirement

Certification for the administration of the neurocognitive battery and questionnaires will include self-study, internet-based training, didactic presentations, role-played administrations, and Q&A and feedback. All training will be supervised by experienced test administrators (Dr. Rapp at WFUSM, June Fletcher-Steede, Site Coordinator or other trained/certified administrator who will be responsible for certifying test administrators.) They will also be responsible for helping staff maintain certification by having regular meetings to discuss the procedures and providing supplemental training as needed.

All assessments will be conducted by trained and certified research personnel. Certification procedure must be completed by staff prior to patient enrollment.

8.2.2 Certification Procedures

All neurocognitive assessments will be conducted by trained and certified research personnel. Training and certification procedures must be completed by research staff prior to patient enrollment. Certification for administration of the neurocognitive battery will include viewing of a training video on the Wake Forest University Health Sciences website, reviewing the content of the protocol-specific test booklets including all aspects of administration and scoring, a didactic presentation of each test and questionnaire to be administered, and role-playing of administrations with Q&A and feedback. All training will be supervised by experienced test administrators (Dr. Stephen Rapp, psychologist and co-PI of this study, June Fletcher-Steede, trained/certified administrator and other trained/certified administrators designated by the CCCWFU CCOP Research Base.)

There are 3 ways to become certified: 1) Training and certification on-site by June Fletcher-Steede (contact Ms. Steede at (336) 716-6733 to schedule a training/certification visit); 2) Training and certification at the annual meeting of the CCCWFU CCOP Research Base, held each fall (contact Michele Bailey, Research Base Administrative Secretary at (336) 716-0891 for date of next meeting; 3) By sending in a recorded tape and administration booklet to June Fletcher-Steede for certification review.

*** Any research staff already trained and certified for CCCWFU Protocol 91105 (Donepezil Study) or 97211 (Chemo Brain Study) will automatically be considered certified for this study. If you have been trained and certified **only** for CCCWFU Protocol 97509 (Armodafinil Study) you will need to be certified in the additional tests that are included in the current protocol prior to registering a patient.

Periodic re-certifications will be required if it has been more than 6 months since the staff member administered the tests. This procedure has been successfully used in our studies of cognitive effects of cancer and its treatments as well as by Dr. Rapp and his team in large scale, multi-site clinical trials and observational studies (e.g., WHIMS, CoSTAR, SPRINT, Look AHEAD, LIFE, MESA).

9. SPECIMEN MANAGEMENT

Storage of Whole Blood for Future Genetic Testing
During the baseline visit only AND if the patient has given consent to store whole blood for future genetic testing, draw an additional 3 tubes of whole blood.
Initially the following biomarkers will be analyzed (**Troponin I, Renin, CRP, Interleukin-6, TNF-α, Aldosterone).**
These future 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. As with the serum samples, this whole blood will be stored for 5 years from its acquisition. Patient identifiers will be removed from the samples.

9.1. Laboratories

Materials for proper specimen collection and transport are supplied by LabCorp.
NOTE: Since LabCorp will be testing all specimens associated with this study, all specimens should be collected in containers provided by LabCorp.

Clearly label each specimen with the patient's protocol identification number and the date and time of collection. Labels will be provided, and should be applied at the top of the specimen tube, just below the tube's top or cap. This places the label away from other labels on the tube and should prevent it from being covered up by bar codes, accession labels, etc. Room is available on the label so that a sequence number, collection time, time description, or the description of a draw site may be written.

LabCorp couriers will deploy to each site from local LabCorp facilties for specimen pick-up as samples are drawn. Note storage times and temperatures in instructions for each test.

9.2 Collection and Handling Procedures

Refer to Appendix 10 for full procedures

10. REPORTING ADVERSE EVENTS

- A list of adverse events/serious adverse events that have occurred or might occur that are related to this study intervention can be found in Section 6.2.

- Adverse Event/Serious Adverse Event reporting begins after the informed consent is signed (or begins at the run-in period of the study or begins at the wash out period of the study).

- Serious Adverse Events occurring within 30 days of study completion must be reported via FDA Form 3500 (MedWatch).

10.1 Protocol Specific Reporting for Adverse Events (AEs)

o DEFINITION: An adverse event (AE) is any untoward medical occurrence in a study participant.

o **Grades 1, 2, and 3 expected (solicited) and unexpected (unsolicited) AEs that meet the above definition for an AE and are ONLY definitely related, possibly related or probably related to this study intervention should be reported to the RB DMC using the Toxicity Assessment Sheet.**

10.2 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 3, 4, and 5 expected (solicited) and 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 Staff 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 10.1.3 below)
- Attribution to study intervention (relatedness)
- Action taken with the study participant and intervention
- Outcome of the event
- Comments

10.3 Guidelines to determine grade and severity of AEs and/or SAEs

Identify the adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. 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

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.03. as stated below.

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
3	Severe	Severe or medically significant but not immediately life-threatening; Hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
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 RB DMC is responsible for communicating AEs/SAEs to the FDA, the drug sponsor, WFU IRB, the WFU 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.

10.4 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 Eligibility checklist/Registration Form should be completed on-line prior to placing the patient on study. Data forms will be submitted to the CCCWFU CCOP Research Base. See Section 10.2.2 for mailing address, or fax to (336) 713-6476 according to the timetable below:

Form	Submission Schedule
Consent Form, PE, MRI, LVEF, General Labs, Biomarker Labs, Baseline Booklet, Flow Sheet/Addenda, TAS (Toxicity Assessment Sheet)	Baseline
MRI, General Labs, Biomarker Labs, 6 month Booklet, Flow Sheet/Addenda, TAS (Toxicity Assessment Sheet), Medication Diaries	6 months
12 month Booklet, QOL Booklets, Flow Sheet/Addenda, TAS (Toxicity Assessment Sheet), Medication Diaries, Phone Contact	12 months
18 month Booklet, QOL Booklets, Flow Sheet/Addenda, TAS (Toxicity Assessment Sheet), Medication Diaries, Phone Contact	18 months
MRI, General Labs, Biomarker Labs, 24 month Booklet, Flow Sheet/Addenda, TAS (Toxicity Assessment Sheet), Medication Diaries	24 months

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, 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; and
- Correction liquid or tape must not be used in source documents or on CRFs.
- 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, imaging study 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 and Safety Monitoring Board

The Data Safety Monitoring Board meets every six months to review all phase II and phase III protocols. The Board includes members demonstrating experience and expertise in oncology, biological sciences, biostatistics and ethics. The DSMB report is generated by the RB statistician. Areas of review may include the following: Date study Opened; Study Objectives; Patient Accrual; Patient Status and Retention; Study Status; Last Contact Status; Patient Compliance; Number of Biopsies/Labs as needed; Patient Characteristics; Summary of Observed Toxicities; Adverse Events; Date, Event briefly described, Relationship to Drug, Arm assigned; Summary of Primary and Secondary Measures.

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 CCCWFU CCOP Research Base Data Management Center will submit quarterly reports to DCP/CTEP by electronic means using the Clinical Data Update System (CDUS)

12. STATISTICAL CONSIDERATIONS

12.1. Study Design/Endpoints

This is a multi-center, double-blind, placebo-controlled clinical trial that randomizes 250 participants into 1 of 2 treatment arms: Group 1 (one 40 mg atorvastatin tablet each morning by mouth for 24 months) or Group 2 (one placebo tablet each morning by mouth for 24 months) with the goal of having at least 200 remaining at the trial's end for final assessments (assuming approximately a 20% loss to follow-up). A permuted block randomization stratified by Anthracycline-based adjuvant therapy dose (<or $\geq$240 mg/m^2 of DOX equivalents; median dose of our pilot studies), and age will ensure treatment group balance is achieved within strata. This will be performed at each study site. As in all our pilot studies and clinical practice, DOX equivalent doses will be assessed to account for receipt of different anthracyclines (doxorubicin, epirubicin, etc.). The primary endpoint in this trial is LVEF, and thus the primary efficacy analysis will examine whether the statin therapy can attenuate the reduction in LVEF in patients with breast cancer who receive Anthracycline-based adjuvant therapy. Secondary endpoints include LVESV, LV end diastolic volume (LVEDV), LV mid-wall circumferential strain, T1-ECV (a measure of replacement fibrosis), PWV, aortic wall thickness, nitrotyrosine levels, CRP and TNF-α, serum lipoprotein levels, and circulating neurohormones.

12.2. Primary Endpoint(s)

Specific Aim 1: To determine if atorvastatin administration preserves LVEF 24 months after receipt of Anthracycline-based adjuvant therapy for adjuvant breast cancer.
Specific Aim 2: To determine if baseline to 6-month differences in LVEF predict baseline to 24-month differences in LVEF after Anthracycline-based adjuvant therapy and concomitant atorvastatin therapy.

12.3. Analysis Plan

The primary hypothesis for Specific Aim 1 is that statin use will protect against a harmful reduction in LVEF measured over 24 months for women with breast cancer who receive Anthracycline-based adjuvant therapy. All randomized patients will be used in the analysis to compare groups ("intent to treat" approach). Since LVEF is measured on a continuous scale and this is a randomized trial, our primary analysis will fit an analysis of covariance (ANCOVA) model that compares the 24-month values of LVEF between groups adjusting for the baseline (pre-treatment) levels of LVEF. This analysis will address the first specific aim.
To address the second primary aim, we will examine whether the early treatment effect of statin use (6 months post randomization) predicts the long-term (24 month) effect of statins. Pilot data (Section 2) suggest that Anthracycline-based adjuvant therapy injury occurs early and concomitant statin use attenuates this early Anthracycline-based adjuvant therapy-mediated injury. We will fit an ANCOVA model that compares the 24-month LVEF values for the treated and control groups and will also include covariates for the baseline LVEF and 6-month change in LVEF values. We will examine the treatment by 6-month LVEF change interaction to determine whether the correlation between early and late changes in LVEF differs by treatment. Thus, this model will have 4 terms of interest: the main effect for treatment, the main effect for the change in LVEF after 6 months, the main effect for baseline pre-treatment LVEF, and the treatment by change in LVEF after 6-month interaction. If the interaction is non-significant, we will examine the model with the 3 main effects. Since there may be limited power to test this interaction, we will use a 0.15 threshold for testing significance of the interaction term in the model.

After primary efficacy analyses, additional analyses of the primary hypotheses will be done to assess relationships between treatment and outcome. We will fit ANCOVA models that consider the 24-month LVEF measure as the outcome, the baseline LVEF measure as a covariate, and other patient-level characteristics (e.g. age, cancer stage, and race) as other covariates in order to control for other baseline characteristics possibly related to LVEF in the model. Interactions between the treatment and covariates will be examined to determine whether the treatment effect is consistent across subgroups (e.g., black/white race by treatment interaction). Finally, we will conduct a repeated measures ANCOVA model that incorporates the intermediate (6-month) LVEF measurement into the model. We anticipate examining different covariance structures. Past experience suggests that a compound symmetry structure is usually appropriate; we will also examine whether an unstructured covariance is needed. We will fit these models using a mixed models approach, where the individual patient will be treated as a random effect in the model and the treatment indicator as patient characteristics (and baseline LVEF) will be considered as fixed effects. A mixed models approach for modeling the longitudinal data will allow for appropriate inference to be made in the presence of missing data (i.e., patient drop-outs) if the missing data are missing at random (MAR). Below, we describe methods to address MAR assumptions. Diagnostics and residual plots will be reviewed to ensure that all assumptions are met. If not, transformations of the outcome data will be considered, where the order of the priority in choosing a transformation will be to satisfy the (1) linearity assumption, (2) homogeneity assumption (homoscedasticity), and (3) normality assumption.

12.4. Potential Analytic Concerns in Primary Efficacy Analyses

Lost to Follow-up: We designed this study to recruit 250 total patients so that 200 are available at the end of the study. Thus, we will need to consider how to analyze data from the 50 patients who may drop out. To examine whether the missing data are either missing completely at random or missing at random, we will compare the baseline characteristics of those who drop out and those who complete the trial. If there is no evidence of informative missing data, analyses will be done using the repeated measures ANCOVA with a mixed models approach (PROC MIXED in SAS). However, if there is evidence that the missing data are informative and treatment-related (e.g. if there are fewer dropouts in the statin group or if missingness is related to other medical outcomes that are strongly associated with the outcome of interest) and not MAR, then we will use more sophisticated statistical methods.[78-80] One possible approach is to model the probability of dropping-out for each participant conditional on her baseline characteristics and treatment assignment. These probabilities would then be used to stratify participants (both completers and drop-outs) before making treatment comparisons. Thus each stratum would contain participants with similar predicted probabilities of trial completion (although only a subset would be completers). The final treatment effect estimates could be made using the completers with weights assigned to them based on the ratio of drop-outs to completers within each stratum. This approach and other methods for handling missing data have been by previously described by Dr. D'Agostino.[78-80]

12.5. Secondary Analyses: Once the primary efficacy analysis is completed, secondary efficacy variables will be examined to provide insight into mechanisms by which statins may influence LVEF. These include: LVESV and strain (reflective of LV contractility), LVEDV (reflective of LV pre-load), PWV and aortic wall thickness (reflective of LV after-load), and LVECV (a measure of myocellular replacement fibrosis). In addition, we will measure a series of subclinical biomarkers, including nitrotyrosine, serum lipids (LDL, TC, HDL), glucose, serum troponin I, CRP, IL-6, TNF-α, renin, and aldosterone at the 6- and 24-month endpoints. For each, we will fit ANCOVA models that will examine the 24-month endpoints adjusted for baseline assessments, followed by ANCOVA models that also include patient-level characteristics. Next, we will fit repeated measures ANCOVA models that incorporate intermediate (6-month) time point assessments. This approach will be repeated for the cognitive and patient reported outcomes. The latter analyses will also include data for the 12 and 18 months follow-ups.

12.6. Power and Sample Size considerations for the primary hypothesis

For the primary hypothesis, sample size and power calculations can be made based on direct comparisons between the expected changes in outcome means in the different treatment groups. These calculations need to account for the proportion of the variance in the primary efficacy of outcome (LVEF) explained by the other terms in the model (i.e., baseline values). To adjust for the proportion of variance explained by the baseline measurement when estimating the variance for the follow-up outcome measure, we used the formula $V_{\text{follow-up adjusted for baseline covariates}} = V_{\text{follow-up}} (1-r^2)]$, where r^2 is the square of the correlation between the baseline and follow-up measure. Our primary efficacy analysis is based on comparing the change in LVEF levels from baseline to 24 months, and r^2 above is the correlation between these two measurements. These power calculations may be conservative, since intermediate assessments of LVEF at 6 months are not included. In other words, the mixed models approach (described in the additional analyses) would likely provide greater power than the ANCOVA model used here, but further assumptions concerning the covariance structure to be used and correlation among intermediate measures would need to be made. Thus, we believe that the power described by the simpler method (ANCOVA) is adequate to address our hypotheses and is expected to be conservative.

The following formula was used to describe the minimum detectable difference for the LVEF between the statin and placebo groups:

$$\text{Detectable difference} = \sigma \frac{\sqrt{2(1-r^2)(Z_{1-\alpha/2}+Z_{1-\beta})^2}}{\sqrt{n}}$$

In the above, r^2 is the percent of the variance of the follow-up outcome explained by the baseline measurement (LVEF), $Z_{1-\alpha/2}$ is the value from the standard normal distribution corresponding to the alpha level chosen (1.96, which corresponds to alpha=0.05 [two sided]), $Z_{1-\beta}$ is the value from the standard normal distribution that corresponds to the power chosen for the study (here 80% and 90%), and n=100 corresponds to the total number of patients studied for the statin group. We chose this sample size (100 per group) for power calculations to allow for up to 50 patients out of the 250 accrued to potentially drop-out. Note that this will somewhat conservative for the mixed models as those with partial data will also provide information. Based on preliminary data examining LVEF levels in comparable patients measured over a 6-month period we found that the standard deviation was approximately 7.7 and the correlation between baseline and 6-months was 0.5. Since we do not know what the correlation will be after 2 years, Table 5 depicts detectable differences for different correlations and power values. In addition, we show detectable differences for key secondary variables for which we have preliminary data (LVEDV, LVESV, and PWV).

Thus, for example, we have 80% power to detect a difference of 2.6% in LVEF between groups if the correlation between the baseline and 24-month LVEF is 0.5. In preliminary observational data, we saw a difference in LVEF between statin users vs. those who were not at 2.5% at 6 months. Thus, effect sizes of the magnitude in Table 5 are plausible and clinically meaningful. 2-6 ml increases in LVESV, or 3-5% drops in LVEF (from a normal resting LVEF) have been associated with adverse CV prognoses in a recent large trial of patients with HF and a preserved resting LVEF.[81] In 483 hypertensive patients aged 50±13 years, aortic PWV measures of 11 m/s were associated with odds ratio of 2.14 for cardiac events (p<0.0001).[82] Other studies that have initiated generic therapies such as ACE inhibitors or beta blockers with antioxidant properties have shown a reduced occurrence of CV events in cancer survivors.[83] Importantly however, it remains to be seen whether early initiation of statin therapy reduces the onset of subclinical CV disease in women with breast cancer.

Table 5: Detectable Differences for Different Power and Correlation Values							
Outcome	Estimated SD	80% Power			90% Power		
		Correlation between baseline and 2-yr measure					
		0.4	0.5	0.6	0.4	0.5	0.6
LVEF (%)	7.7	2.79	2.64	2.44	3.23	3.06	2.82
LV-EDV (ml)	33.1	12.0	11.4	10.5	13.9	13.1	12.1
LV-ESV (ml)	19.7	7.1	6.8	6.2	8.3	7.8	7.2
PWV (m/sec)	3.7	1.34	1.27	1.2	1.6	1.5	1.4

12.7. Reporting and Exclusions

We will measure the level of compliance in randomized patients. If a patient drops out of the study early due to an adverse event that may be related to statin use, this will indicate that they were not compliant, and thus still inform the primary endpoint for our analyses, and their outcome will be considered as "non-compliant". For patients who drop out early due to a non-statin related adverse event or die during the study we will analyze them in the following ways.

First we will conservatively assume that all drop-outs//deaths are non-compliant. Since there is a placebo group in this study we can assume that the rates would be similar between groups and still allow a comparison of compliance rates between groups. Next, we will consider two forms of imputation for secondary analyses. The first will use early compliance data (if available) to determine a compliance rate and that value will be carried forward to the end (i.e., Last observation carried forward) and the second would be to use a multiple imputation procedure to impute final compliance rates for participants based on modeling the compliance rates for those with complete data.

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12.8. Evaluation of Toxicity

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

12.9. Evaluation of Response

All participants will be assessed for compliance once they are randomized to treatment and receive at least one dose of treatment. The primary analysis will consist of all randomized patients who receive at least one dose of study drug. Secondary analyses will consist of patients who either complete the trial or who drop out for adverse events that are related to the use of statins. Patients who drop out for non-related adverse events or die for reasons not related to statin use will be removed from the secondary analysis. Adverse events described by the Data Safety Monitoring Committee as related to statin use will be differentiated so as to render decisions regarding study drug compliance.

12.10. Interim Analysis

Interim reviews will be performed by the Data Safety Monitoring Committee every six months to assess accrual, retention, compliance, the incidence of AEs, SAEs, and diabetes and, LV function measures.

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