Detection and Prevention of Anthracycline-Related Cardiac Toxicity with Concurrent Simvastatin

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AC	Doxorubicin/Cyclophosphamide
ACE	Angiotensin Converting Enzyme
ADL	Activities of Daily Living
AE	Adverse Event
AJCC	American Joint Committee on Cancer
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ARB	Angiotensin II Receptor Blocker
AST	Aspartate Aminotransferase
BCIRG	Breast Cancer International Research Group
CK	Creatine Kinase
cTnI	Cardiac Troponin I
CRF	Case Report Form
CRO	Clinical Research Office
CRRMC	Clinical Research Review and Monitoring Committee
CTCAE	Common Toxicity Criteria for Adverse Events
CYP3A4	Cytochrome P450 3A4
DNA	Deoxyribonucleic Acid
DSMP	Data and Safety Monitoring Program
ECOG	Eastern Cooperative Oncology Group
EF	Ejection Fraction
EKG	Electrocardiogram
ER	Estrogen Receptor
FDA	Food and Drug Administration
GLS	Global Longitudinal Strain
HER2	Human Epidermal Growth Factor Receptor 2
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
IND	Investigational New Drug
IRB	Institutional Review Board
JHM	Johns Hopkins Medicine
LV	Left Ventricular
MANTICORE	Multidisciplinary Approach to Novel Therapies in Cardiology Oncology Research
MUGA	Multigated Acquisition
NCI	National Cancer Institute
NSABP	National Surgical Adjuvant Breast and Bowel Project
NYHA	New York Heart Association
PRADA	Prevention of Cardiac Dysfunction During Adjuvant Breast Cancer Therapy
PR	Progesterone Receptor
QA	Quality Assurance
RFS	Recurrence Free Survival
ROC	Receiver Operating Curve
SAE	Serious Adverse Event

SCUSF	Sun Coast University of South Florida
SKCCC	Sidney Kimmel Comprehensive Cancer Center
TOP2B	Topoisomerase II Beta
TOP2A	Topoisomerase II Alpha
ULN	Upper Limit of Normal
WMA	Wall Motion Abnormality

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1. Summary

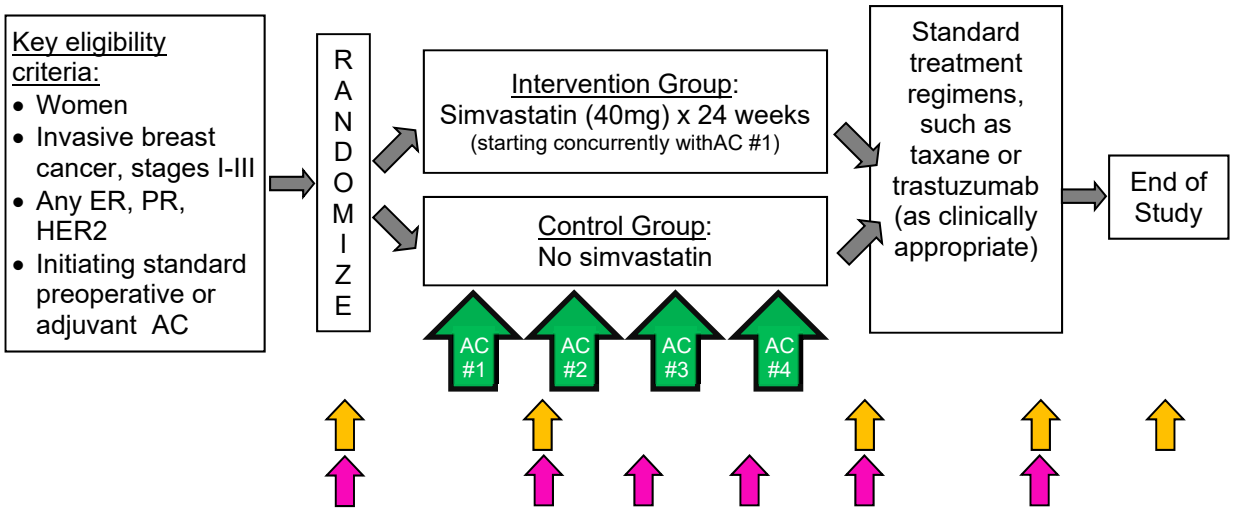
Although rare, cardiac toxicity can be a late consequence of adjuvant anthracycline-based chemotherapy for early stage breast cancer. Standard clinical practice dictates confirmation of a normal ejection fraction (EF) by echocardiogram or multigated acquisition (MUGA) scan prior to initiation of adjuvant anthracycline therapy. However, in the United States, cardiac monitoring beyond baseline confirmation of a normal EF is not routinely performed for adjuvant breast cancer patients unless additional potentially cardiotoxic agents, such as trastuzumab, are prescribed or possible signs or symptoms of heart failure are observed.

A decline in EF is known to be a late stage in the development of cardiac toxicity and identification of cardiac damage at an earlier stage could offer the opportunity for an intervention to prevent potentially irreversible anthracycline-induced cardiac toxicity. Myocardial strain is a new echocardiographic technique which can detect subclinical heart disease prior to a decline in EF. Data suggests that statins may attenuate the cardiac toxicity of anthracyclines by minimizing anthracycline-induced mitochondrial damage.

This is a prospective, randomized study to assess statin therapy for cardiac protection during anthracycline-based chemotherapy and myocardial strain for early detection of anthracycline-induced cardiac toxicity. Eligible patients include women with early stage invasive breast cancer (stage I-III) with normal organ function for whom adjuvant or neo-adjuvant anthracycline-based chemotherapy is planned. Women with known cardiac disease and/or who are on therapy with a statin at baseline are not eligible. Patients will undergo serial assessment of echocardiographic parameters including myocardial strain before, during, and after anthracycline therapy. Patients will be randomized in a 1:1 ratio to the intervention group treated with simvastatin therapy (40 mg daily) concurrently with doxorubicin/cyclophosphamide (AC) chemotherapy or to the control group treated with AC chemotherapy alone. Echocardiographic parameters will be compared between the two groups. Patients will be monitored for hepatic and muscular toxicity and for signs or symptoms of heart failure.

The overarching objective is to assess the detectable change in myocardial strain over time in the breast cancer patients receiving anthracycline-based adjuvant chemotherapy who do and do not receive simvastatin treatment. The hypothesis is that statin therapy will minimize reduction in myocardial strain associated with anthracycline therapy. Results gained from this study will be used in designing future definitive trials assessing the clinically important difference in strain between patients who do and do not receive statin therapy as well as the protective effect of statin on AC-induced cardiotoxicity with long-term follow-up.

2. Schema



Standard AC chemotherapy (doxorubicin and cyclophosphamide) in the preoperative/neoadjuvant or adjuvant setting, every 2-3 weeks, for up to 4 cycles

Echocardiogram with strain analysis: baseline, immediately before AC #2, 1-3weeks after AC #4, 24 weeks after AC #1, and 52 weeks after AC #1

Blood sampling: baseline and prior to cycles 2-4 of AC, 2-3 weeks after AC #4, and 24 weeks after AC #1 (liver function tests to be drawn at each time point; creatine kinase, CBC and chemistry to be drawn at baseline only)

3. Background and Rationale

3.1 Cardiac Toxicity of Adjuvant Anthracycline Therapy for Early Stage Breast Cancer

3.1.1 Clinical Presentation of Anthracycline-Induced Cardiac Toxicity

The development of anthracycline-based chemotherapy regimens in the adjuvant treatment of early stage breast cancer resulted in reduced breast cancer recurrence and mortality.[1 2] While non-anthracycline-based regimens are currently an option for some patients, anthracycline-based chemotherapy still remains a cornerstone of curative therapy for many women with early stage breast cancer.[3] For women with human epidermal growth factor receptor-2 (HER2) -positive breast cancer, the addition of trastuzumab to adjuvant anthracycline-based chemotherapy further reduces breast cancer recurrence and mortality.[4-6]

Unfortunately, cancer survivors are known to face increased risk of cardiovascular disease.[7-9] While cancer therapy may cause a variety of types of cardiovascular toxicity, the most frequent is left ventricular (LV) dysfunction leading to heart failure.[10] Indeed, the American College of Cardiology and American Heart Association Guidelines have recently classified cancer patients receiving chemotherapy as having stage A heart failure, defined as being at increased risk for developing cardiac dysfunction but without structural heart disease or symptoms.[11]

Cardiac toxicity is a rare, yet serious, complication of both anthracycline and trastuzumab therapies. Anthracycline-associated cardiac toxicity has typically been described as left ventricular dysfunction and heart failure which can be asymptomatic or symptomatic and which presents long after chemotherapy.[12-14] In contrast, trastuzumab-associated cardiac toxicity typically presents during trastuzumab therapy and is most frequently characterized by asymptomatic decline in EF which is reversible.[15-21]

Anthracycline-associated cardiac toxicity has traditionally been considered irreversible, although recent data has challenged this notion. A small case-control study demonstrated improvements in cardiac function in patients with anthracycline-induced heart failure with beta-blocker treatment comparable to that observed in patients with idiopathic heart failure.[22] In addition, a small retrospective review and a small prospective study evaluating cardiac function in patients with anthracycline-associated heart failure who were treated with standard heart failure medications such as beta-blockers and angiotensin converting enzyme (ACE) inhibitors revealed improvements in EF over time. [23, 24] Despite the new findings that anthracycline-induced heart failure may indeed be reversible, it has historically been associated with inferior prognosis in comparison to heart failure of many other etiologies.[25]

Cardiac toxicity associated with anthracyclines is dose-dependent with reported rates as high as 18-48% in patients treated with a cumulative dose of 700 mg/m^2 of doxorubicin.[12,26] However, abnormalities in left ventricular performance after exposure to low-moderate doses of anthracyclines have recently been reported.[27] Besides higher dose, risk factors for anthracycline-associated cardiac toxicity include coronary artery disease, diabetes, hypertension, concurrent treatment with trastuzumab, older age, cardiac exposure to radiation and black race.[12,25,26,28] Duration of anthracycline infusion may also be linked to the risk for cardiac toxicity.[29] In addition, animal data suggests that genetic alterations in genes involved in

intracellular doxorubicin transport, such as MRP1, and in free radical metabolism, such as NAD(P)H oxidase, may predispose to anthracycline-induced cardiac toxicity.[30]

The clinical significance of asymptomatic reduction in EF after anthracycline therapy is unknown. However, data indicates that asymptomatic reduction in EF due to causes other than anthracycline exposure is associated with substantial risk of future cardiovascular events and mortality.[31,32] Thus, it would be expected that asymptomatic reduction in EF in patients treated with anthracyclines is associated with poor cardiovascular clinical outcomes also.

3.1.2 Frequency of Anthracycline-Induced Cardiac Toxicity

True rates of cardiac toxicity from anthracycline chemotherapy are difficult to discern as it may occur years after anthracycline exposure and may not be clinically detected because it often involves asymptomatic reduction in EF without symptomatic heart failure. In addition, long-term follow-up to assess for cardiac toxicity has not been performed for most recipients of anthracycline therapy and the definitions used for cardiac toxicity have varied in the studies which have been performed, making precise determination of the prevalence of cardiac toxicity from anthracyclines difficult to discern.[33] According to a recent meta-analysis, rates of cardiac toxicity are more than 5-fold higher with anthracycline than non-anthracycline containing chemotherapy regimens.[34] Data from long-term follow-up of children treated with anthracyclines reveals reported rates of subclinical heart failure ranging from 0-57% in comparison to rates of clinical heart failure generally reported as less than 5%.[35-40] For example, the Childhood Cancer Survivor Study cohort revealed rates of heart failure of 1.7% among survivors compared to 0.2% among unaffected siblings, with heart failure rates continuing to rise over a 30-year follow-up period.[38]

In the breast cancer setting, reported rates of clinical and subclinical anthracycline-induced cardiac toxicity have also varied. The Early Breast Cancer Trialists' Collaborative Group reported a 33% higher risk of death from heart disease among breast cancer patients treated with adjuvant anthracycline chemotherapy compared to those treated with non-anthracycline chemotherapy, but rates of heart failure were not reported.[41] Estimates of short-term cardiac toxicity from modern anthracycline-based adjuvant chemotherapy for breast cancer can be determined from the adjuvant trastuzumab trials in which assessment of EF was performed immediately after completion of doxorubicin (cumulative dose 240 mg/m2) and cyclophosphamide. In the N9831 study, 5% of patients were unable to continue to the trastuzumab-containing portion of therapy due to asymptomatic decline in EF after AC. Similarly, in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B31 study, 6.6% of patients were unable to continue on to receive trastuzumab due to asymptomatic decline in EF or heart failure symptoms after AC. A slightly lower rate of 2.1% was reported for patients in the Breast Cancer International Research Group (BCIRG) 006 trial who were unable to begin trastuzumab therapy after completion of AC due to reduced EF.[4,15,16]

With longer follow-up, reported rates of cardiac toxicity after anthracycline therapy for early stage breast cancer patients have varied. For example, Ganz et al evaluated EF in 180 patients who had previously participated in a trial comparing an anthracycline-based chemotherapy regimen to a non-anthracycline based regimen and found no difference in the proportion of patients with EF less than 50% 5-8 years after treatment (5% in the non-anthracycline arm versus

7% in the anthracycline arm, p-value 0.68) or 10-13 years after treatment (3% in the non-anthracycline versus 0%,in the anthracycline arm, p-value 0.16) between the two groups. Exploratory analysis, however, revealed that EF measured 5-8 years after treatment was lower in the women who received anthracycline therapy compared to those who did not (mean EF 61.4% versus 64.8%, p-value 0.01).[42] These results, however, may underestimate long-term cardiac toxicity as recruitment for this follow-up study was likely biased towards healthy survivors.[43] Zambetti et al identified similarly low rates of cardiac toxicity in their report of cardiac outcomes in 1000 women treated on three adjuvant breast cancer trials with 14 years median follow-up. In this report, 1% of patients treated with doxorubicin experienced heart failure and 0.6% experienced cardiac death. Reduced EF was noted in 8% of patients who received doxorubicin, with the majority being asymptomatic.[44]

The results of Ganz and Zambetti differ substantially from the retrospective population-based analysis performed by Pinder et al which showed that as many as 38% of older women treated with anthracyclines for breast cancer had a subsequent diagnosis of heart failure. In comparison to these rates of heart failure in older women who received anthracyclines, Pinder et al reported that 32.5% of older women treated with non-anthracycline chemotherapy for breast cancer and 29% of older women with breast cancer not treated with chemotherapy had subsequent diagnoses of heart failure.[28] Notably, these estimates are higher than the prevalence of heart failure in the general population of older women and raise concern regarding limitations of the population-based data on which they are based, but they do suggest that cardiac toxicity from anthracyclines may be more common than previously suspected, especially among older women.[45] In further support of this data are the findings of Du et al who also performed a population-based study of older women with breast cancer. They concluded that the cumulative incidence of heart failure after 10 years of follow-up was 31.9% among anthracycline-treated patients compared to 26.4% among those who didn't receive chemotherapy (4.7% excess risk).[46]

3.1.3 Mechanism of Anthracycline-Induced Cardiac Toxicity

Anthracycline-induced cardiac toxicity has traditionally been thought to result from reactive oxygen species generated from reduction-oxidation cycling of anthracycline quinones. These reactive oxygen species damage deoxyribonucleic acid (DNA), proteins and lipids, ultimately resulting in cardiomyocyte dysfunction and death. Recently, this theory has been challenged as research has revealed that anthracycline-induced cardiac toxicity is mediated via mitochondrial dysfunction resulting from the interaction between anthracyclines and cardiomyocyte topoisomerase II beta (TOP2B). Anthracyclines block the function of both topoisomerase II alpha (TOP2A), which is often expressed in malignant cells, and of TOP2B, which is expressed in cardiomyocytes. In the heart, anthracyclines intercalate into cardiomyocyte DNA, forming a complex with TOP2B which inhibits its action. This results in reduced transcription of genes involved in the regulation of mitochondrial biogenesis and function, leading to changes in

mitochondrial structure and function and ultimately to the generation of reactive oxygen species.[14,47,48]

3.2 Assessment of Cardiac Function in Breast Cancer Patients Treated with Anthracyclines

3.2.1 Traditional Approaches to Assessing Cardiac Function

Traditionally, normal baseline cardiac function has been confirmed via echocardiographic or MUGA scan measurement of EF prior to the initiation of anthracycline therapy. For breast cancer patients receiving current standard cumulative adjuvant doxorubicin doses of 240-300 mg/m^2, repeat assessment of cardiac function after completion of doxorubicin therapy is not typically performed unless therapy with trastuzumab is planned or signs or symptoms of heart failure are present. However, recent guidelines suggest consideration of repeat assessment of EF over time for adult patients who have been treated with potentially cardiotoxic chemotherapy agents such as anthracyclines, although evidence to support these guidelines is currently lacking.[11,49,50]

Unfortunately, standard echocardiography and MUGA imaging both have limitations and neither can reliability detect cardiac damage prior to a decline in EF.[33] Research suggests that EF drops late in the process of anthracycline-induced cardiac toxicity with data revealing that extensive cardiac remodeling precedes overt clinical heart failure after an index event such as exposure to anthracycline therapy.[43,51,52] Thus, even if performed at regular intervals after anthracycline therapy, standard echocardiography and MUGA imaging may not be able to detect early cases of anthracycline-induced cardiac toxicity. Newer techniques to try to detect anthracycline-induced cardiac toxicity at an earlier stage at which it may be reversible are needed.

3.2.2 Newer Approaches to Assessing Cardiac Function

Multiple potential biomarkers for assessing cardiac function and risk are currently being evaluated. These include advanced imaging techniques such as echocardiographic myocardial strain and cardiac magnetic resonance imaging and serum markers such as cardiac troponins, among others. The optimal technique(s) and frequency of assessment are not known. This protocol will further assess the role of echocardiographic myocardial strain.

3.2.2.1 Echocardiographic Myocardial Strain

Myocardial strain is an echocardiographic measure of myocardial tissue deformation or contractility. During contraction, the myocardium shortens in the circumferential and longitudinal directions (resulting in negative circumferential and longitudinal strain) and lengthens in the radial direction (resulting in positive radial strain). Changes in myocardial strain can identify subclinical heart disease prior to a drop in EF and predict subsequent cardiovascular outcomes.[53] Numerous studies have demonstrated that reduced global longitudinal strain (GLS) is associated with higher risk of death and hospitalization for cardiovascular problems in patients with cardiac diseases such as acute myocardial infarction, chronic ischemic cardiomyopathy and chronic heart failure.[54-59]

As is the case in cardiac disease, evidence suggests that changes in myocardial strain can detect anthracycline-induced cardiac damage in the absence of a decline in EF. In a pilot study of 16

elderly women treated with pegylated liposomal doxorubicin, the mean GLS was -22.7% at baseline and dropped to -18.8% after 6 cycles of chemotherapy. No significant change in EF was noted in this time period.[60] Ho et al performed a cross sectional study in 70 asymptomatic breast cancer survivors without known cardiovascular disease who had previously received anthracyclines with or without trastuzumab up to 6 years earlier and 50 asymptomatic controls without known cardiovascular disease. Echocardiographic strain analysis performed in the study participants revealed reduced mean GLS in the chemotherapy group in comparison to the controls (- 18.1% versus -19.6%). GLS was below the lower limit of normal in 26% of the patients previously treated with anthracyclines. No significant differences were observed in EF between the two groups.[61] Stoodley et al observed similar findings in a prospective study of 52 breast cancer patients treated with anthracycline therapy. Echocardiography with strain analysis was performed one week prior to initiating chemotherapy and one week after the completion of all cycles of chemotherapy. Mean GLS decreased from -17.7% to -16.3% with 48% of patients experiencing at least 10% decrease in GLS. Radial strain was also noted to decrease with mean values dropping from 40.5% to 34.5%. No patients experienced greater than 10% decline in EF.[62]

Stoodley et al recently reported a follow-up study in which 50 of the anthracycline-treated breast cancer patients underwent serial echocardiography over 12 months, thus providing, to the best of our knowledge, the first reported long-term prospective cardiac strain data in this population. In comparison to baseline, reductions in LV longitudinal peak systolic strain were noted immediately after completing anthracycline therapy and at the 6-month time-point, but resolved by the 12-month time-point in 84% of patients, demonstrating that they tend to be transient. Among the eight patients with persistent reductions in LV longitudinal peak systolic strain exceeding one standard deviation at the 12-month time-point, no significant change in EF was noted. A LV longitudinal peak systolic strain value < -17.2% at the 6 month time-point predicted a low LV longitudinal peak systolic strain value at the 12 month time-point with 100% sensitivity and 80% specificity.[63]

Long-term prospective data associating a reduction in myocardial strain with subsequent drop in EF and inferior cardiac outcomes is not available for breast cancer patients treated with standard adjuvant anthracycline therapy alone, but several studies in breast cancer patients treated with trastuzumab, many of whom also received anthracyclines, demonstrate the prognostic capacity of echocardiographic myocardial strain measurements in the breast cancer population. Hare et al performed echocardiograms measuring EF, strain and strain rate at baseline and every 3 months in 35 breast cancer patients receiving trastuzumab. In this small study, 51% of patients experienced a drop in longitudinal strain rate and 37% experienced a drop in radial strain rate. Of those with decreased longitudinal strain rate, EF was noted to decrease more than 10% concurrently in three patients and subsequently in two additional patients.[64]

Fallah-Rad et al performed a similar study of 42 breast cancer patients treated with adjuvant trastuzumab, most of whom also received anthracyclines. Echocardiography with strain analysis was performed at baseline, prior to trastuzumab and every 3 months afterwards in these patients. Drug-induced cardiac toxicity, defined as symptomatic heart failure and a drop in EF of at least 10% to below 55% necessitating discontinuation of trastuzumab was observed in 24% of the study population. When compared to the patients who did not develop cardiac toxicity, those

who did were noted to have lower longitudinal and radial strain measurements after 3 months of trastuzumab (mean peak GLS in cardiac toxicity patients compared to patients without cardiac toxicity: -16.4% versus -19.9%; mean peak global radial strain in cardiac toxicity patients compared to patients without cardiac toxicity: 32.5% versus 42.4%). Receiver operating curve (ROC) analysis of the ability of a 2% difference between baseline and 3 month peak GLS to identify subsequent development of cardiac toxicity revealed sensitivity, specificity, positive predictive value and negative predictive value of 79%, 82%, 60% and 92% respectively. Similarly, ROC analysis of the ability of a 0.8% difference between baseline and 3 month peak global radial strain to identify subsequent development of cardiac toxicity revealed sensitivity, specificity, positive predictive value and negative predictive value of 86%, 81%, 60% and 95% respectively.[65]

Sawaya et al also prospectively followed echocardiographic measures in 81 breast cancer patients, all of whom received anthracyclines, taxanes and trastuzumab and underwent echocardiography with strain analysis every 3 months. During the study period, 32% developed cardiac toxicity as defined by a drop in EF of at least 5% to less than 55% with symptoms of heart failure or by an asymptomatic drop in EF by at least 10% to less than 55%. In this study, peak systolic longitudinal myocardial strain measured at the completion of anthracycline therapy was predictive of subsequent development of cardiac toxicity. ROC analysis of a value of longitudinal strain < 19% after the completion of anthracycline chemotherapy revealed sensitivity 74%, specificity 73%, positive predictive value 53% and negative predictive value 87%. On multivariate analysis, GLS < 19% after anthracycline therapy was the only independent predictor of subsequent cardiac toxicity. This study also revealed that a drop in GLS greater than 10% between baseline and the end of anthracycline therapy predicted subsequent development of cardiac toxicity. Of note, radial and circumferential strain measurements were not predictive of cardiac toxicity in this study.[66]

More recently, Negishi et al performed a similar study of echocardiographic assessment every 6 months in 81 women treated with trastuzumab, 37 of whom also received anthracyclines. In this study, 24% of women experienced cardiac toxicity, defined as a reduction in EF exceeding 10%. The strongest predictor of a subsequent decline in EF was the difference between GLS at baseline and after 6 months of therapy, with the optimal cut point of an 11% reduction (sensitivity 65%, specificity 94%). Changes in global longitudinal peak systolic strain rate and global longitudinal early diastolic strain rate between baseline and 6 months of follow-up were also predictive of subsequent declines in EF. Notably, only longitudinal strain indices were significant predictors of subsequent cardiac toxicity in this study.[67]

To date, most investigations into the role of echocardiographic myocardial strain in detecting subclinical anthracycline-induced cardiac toxicity have focused on systolic measures. However, Stoodley et al recently observed changes in diastolic function in breast cancer patients immediately after completing 4-6 cycles of anthracycline-based chemotherapy. Furthermore, they identified reduced baseline systolic strain as a predictor of reduced diastolic strain rate after chemotherapy.[68]

Together, these findings suggest significant clinical utility for echocardiographic myocardial strain analysis in the prediction of subsequent cardiac outcomes in breast cancer patients treated

with cardiotoxic therapy. Ultimately, risk stratification based on strain could offer the potential to identify individuals for whom enhanced cardiac monitoring or prevention interventions could be directed. Indeed, early data suggests that interventions can improve strain in both patients with cardiovascular disease and in breast cancer patients. For example, Blondheim et al performed echocardiography 2 hours prior to and 2 hours after the administration of heart failure medications to a group of 21 patients with ischemic cardiomyopathy. They identified an improvement in segmental strain in initially dysfunctional segments, but no change in global strain measurements.[69] In a study with longer follow-up, Leong et al performed echocardiography at baseline and after 4-7 months of treatment in newly diagnosed patients with idiopathic dilated cardiomyopathy. GLS improved between baseline and follow-up assessments (baseline mean -12% compared to follow-up mean -16%).[70]

To our knowledge, only three studies to date have reported on whether interventions can improve strain in cancer patients at risk for chemotherapy-induced cardiac toxicity. Negishi et al presented non-randomized, retrospective data regarding GLS at baseline and follow-up in 159 women receiving anthracyclines, trastuzumab or anthracyclines followed by trastuzumab. Of the 159 patients, 52 experienced a decline in GLS > 11%. Beta-blocker therapy was administered to 24 of the women with a decline in GLS > 11% while 28 women who experienced similar declines in GLS did not receive beta-blocker therapy. Subsequent assessment of GLS revealed greater improvement in the women treated with beta-blocker therapy.[71] El-Shitany et al reported a prospective randomized trial in 50 leukemia patients, half of whom were pre-treated with carvedilol prior to anthracycline-based chemotherapy and half of whom received anthracycline-based chemotherapy alone. Post-treatment peak GLS was noted to be improved compared to baseline in the group which received carvedilol.[72] Cadeddu et al performed a study in 49 patients receiving epirubicin for a variety of solid tumors who were randomized to telmisartan or placebo. Epirubicin was noted to impair strain rate peak, but it subsequently normalized in only the telmisartan arm, with the effect noted to persist until follow-up assessment after 18 months.[73,74]

Although these data are promising, it should be noted that prospective randomized data indicating that strain can be improved in breast cancer patients with chemotherapy-related cardiac toxicity are currently limited. More importantly, it is not yet known whether interventions to improve strain can attenuate the expected subsequent decline in EF predicted by an abnormal strain and whether this would ultimately lead to superior clinical cardiac outcomes.

In addition, it should be noted that the use of myocardial strain as a clinical measure is still in development and several issues remain to be clarified before widespread clinical use. For example, the optimal measure of strain (longitudinal strain, radial strain, circumferential strain or strain rate) is not yet known, although the largest body of data to date supports the use of GLS. In addition, the cut-off values to define normal and abnormal strain require further clarification as different investigators have used different definitions, although a recent meta-analysis suggested a normal range of -15.9% to -22.1% for GLS.[75] Potential differences in normal values for strain between men and women have also not yet been clarified. Furthermore, the potential impact of the echocardiographic technique used to obtain the images required to calculate strain, the frame rate of the machine used and the impact of the type of software used for the calculation of strain are also not well defined. Reproducibility of strain assessments and measurement error

also remain a potential concern. Finally, at this time, availability of software to calculate strain and echocardiography technicians trained to obtain the images required for strain analysis are limited.[53,67,75,76]

Despite these limitations, the data described above suggest that echocardiographic myocardial strain is a promising new tool which can predict subsequent decline in EF and, potentially predict inferior cardiac outcomes in cancer patients undergoing therapies which may be cardiotoxic. Thus, it may be able to detect anthracycline-induced cardiac toxicity at an earlier stage at which it may be reversible. Johns Hopkins has extensive experience to date in measuring echocardiographic myocardial strain, making it an ideal site for further study of this new echocardiographic tool. In our lab, the intra-observer reproducibility coefficient of variation for GLS measurements is 5% and the inter-observer reproducibility coefficient of variation for GLS measurements is 9%.

3.3 Cardioprotective Interventions to Prevent Anthracycline-Induced Cardiac Toxicity

3.3.1 Agents Previously Evaluated for Cardioprotection

To date, multiple agents have been studied as potential interventions to prevent anthracycline-induced cardiac toxicity, although only dexrazaxone has been approved by the United States Food and Drug Administration (FDA) for this purpose. Evidence suggests that earlier intervention with standard heart failure medications in patients affected with anthracycline-induced heart failure is associated with greater cardiac recovery, thus the notion of intervention prior to the development of cardiac toxicity is appealing.[23]

In 2011, a Cochrane review evaluated randomized controlled trials of N-acetylcysteine, coenzyme Q10, L-carnitine, carvedilol, phenethylamines, amifostine, dexrazaxone and the combination of Vitamin E, Vitamin C and N-acetylcysteine for the prevention of anthracycline-induced cardiotoxicity. Unfortunately, this review found no benefit for any of these agents other than dexrazaxone in the prevention of cardiac toxicity. As part of the Cochrane review, a meta-analysis of 8 randomized controlled trials evaluating dexrazaxone in 1,561 patients receiving anthracycline therapy was performed. This revealed an 82% reduction in the risk of heart failure with dexrazaxone. Of note, while there exists some concern about the possibility of reduced efficacy of chemotherapy with concurrent administration of dexrazaxone, no reduction in response rate was noted in this meta-analysis.[77] Consideration of dexrazaxone use is currently recommended for patients who have received at least 300 mg/m2 of doxorubicin and who plan to continue to be treated with anthracycline therapy.[78]

Recently, attention has turned to evaluation of drugs used for the treatment of heart disease, such as beta-blockers, ACE inhibitors or angiotensin II receptor blockers (ARBs), as potential preventive agents for anthracycline-induced cardiac toxicity. For example, Kalay et al performed a small randomized trial evaluating carvedilol in patients planning to receive anthracycline chemotherapy in which they found that carvedilol prevented a decline in EF.[79] A similar study utilizing nebivolol for cardiac protection in anthracycline-treated patients also revealed higher follow-up EF in the patients who received the beta-blocker compared to controls.[80] Seicean et al reported a retrospective study revealing that breast cancer patients treated with anthracyclines and trastuzumab who were on beta-blockers continuously throughout

their cancer treatments had lower risk of subsequent diagnoses of new heart failure than those who did not take continuous beta-blockers.[81] Using a different approach, Cardinale et al selected patients expected to be at high risk for chemotherapy-induced cardiac toxicity based on elevated cardiac troponin-I levels immediately after high-dose chemotherapy and randomized these patients to treatment with an ACE inhibitor or none. This study revealed a lower rate of decline in EF among the patients treated with the ACE inhibitor.[82] Similarly, Bosch et al demonstrated less reduction in EF and lower risk of death and heart failure with the administration of enalapril and carvedilol to patients with acute leukemia and other malignant hematologic diseases being treated with stem cell transplant.[83] In another study, Nakamae et al demonstrated that valsartan, prevented increases in left ventricular end diastolic diameter, QTc interval on electrocardiogram (EKG) and QTc dispersion on EKG in lymphoma patients treated concurrently with an anthracycline-based chemotherapy regimen.[84] Ongoing studies such as the Multidisciplinary Approach to Novel Therapies in Cardiology Oncology Research (MANTICORE), Prevention of Cardiac Dysfunction During Adjuvant Breast Cancer Therapy (PRADA) and Sun Coast University of South Florida (SCUSF) 0806 (clinicaltrials.gov identifier NCT01009918) trials aim to further assess the role of ACE inhibitors, ARBs and beta-blockers in the prevention of cardiac toxicity induced by oncologic therapies.[85,86]

3.3.2 Statins as Cardioprotective Agents

While typically used for the management of hyperlipidemia and coronary artery disease, several lines of evidence suggest that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors ("statins") may be effective for prevention of anthracycline-induced cardiac toxicity. Simvastatin has been shown to attenuate mitochondrial dysfunction in rat cardiomyocyte cell cultures exposed to oxidant stress.[87] This potential mechanism of action is particularly promising given that recent evidence suggests that doxorubicin-induced cardiac toxicity is mediated by mitochondrial dysfunction. Mice treated with both statins and doxorubicin demonstrate less short term cardiac toxicity as measured by troponin release and hemodynamic measurements compared to mice treated with doxorubicin alone.[88,89] Furthermore, concurrent treatment of rats with rosuvastatin and doxorubicin compared to doxorubicin alone and to doxorubicin plus carvedilol resulted in less cardiac damage, suggesting statin therapy may be more beneficial than beta-blocker therapy in the prevention of anthracycline-induced cardiac toxicity.[90]

To date, there is only minimal clinical data evaluating the ability of statin therapy to prevent cardiac toxicity in patients receiving anthracyclines. A retrospective study of 67 breast cancer patients on uninterrupted statin therapy during anthracycline chemotherapy for breast cancer and during a follow-up period of 2.55 +/- 1.68 years compared to 134 propensity-matched controls not on uninterrupted statin therapy during chemotherapy and the follow-up period revealed a 70% reduction in incident heart failure requiring hospitalization after initiation of anthracycline treatment in the statin group (6% versus 17.2%). Notably, the presence of cardiovascular risk factors was associated with higher risk for heart failure in this study.[91,92] Acar et al performed a small study randomizing 40 patients receiving anthracycline chemotherapy to atorvastatin 40 mg oral daily starting prior to chemotherapy and continuing for 6 months or to no intervention. Echocardiography was performed at baseline and 6 months later. Mean EF was noted to drop in the control group but not in the atorvastatin group.[93] A randomized pilot trial evaluating 3 months of rosuvastatin for cardiac protection in breast cancer patients receiving anthracycline,

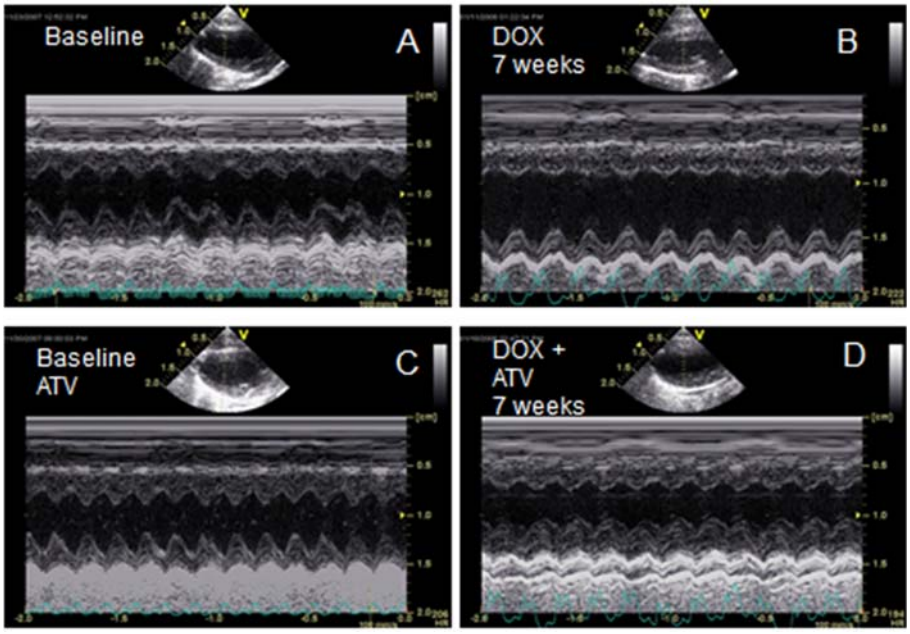
cyclophosphamide, paclitaxel and trastuzumab therapy is currently ongoing with a primary endpoint of adherence to rosuvastatin therapy (clinicaltrials.gov identifier NCT01051401).

While published data regarding statins as cardioprotective agents in chemotherapy patients is limited at this time, there is no data to suggest safety concerns for the combination of anthracyclines and statins. Indeed, many patients take both drugs concurrently in the clinical setting. Furthermore, cell line data has suggested that statins may potentiate the anti-tumor effects of anthracyclines.[88] However, a small phase II study evaluating pravastatin therapy plus anthracycline-based chemotherapy (epirubicin, cisplatin, capecitabine) in gastric cancer revealed no significant anti-cancer benefit from the addition of the statin. Notably, there was no additional toxicity observed from co-administration of the statin and the anthracycline-based chemotherapy in this trial.[94]

Dr. Theodore Abraham's Translational Cardiovascular Ultrasound Research Laboratory at Johns Hopkins has further explored the potential benefit of statins for the prevention of anthracycline-induced cardiac toxicity. Baseline echocardiography in rats treated with doxorubicin alone and in rats treated with doxorubicin plus atorvastatin was performed. Follow-up echocardiography 7 weeks later provided *in vivo* morphologic and functional evidence of the benefit of atorvastatin. The rats treated with doxorubicin alone displayed ventricular dilatation and impaired function compared to baseline. In contrast, the rats treated concurrently with atorvastatin did not

demonstrate significant ventricular dilatation and the decrease in ventricular function was blunted (Figure A) (unpublished data).

Figure A:

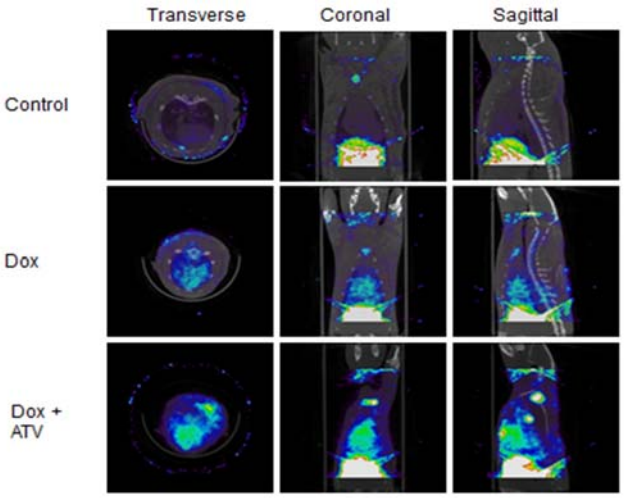


Echocardiography: Rats treated with doxorubicin for 7 weeks develop substantial ventricular dilatation (B) compared to baseline (A) and reduction in function. In contrast, rats treated with doxorubicin but also pre-treated with statin do not develop significant heart dilatation and the decrease in function is blunted (C and D).

Further work in Dr. Abraham's lab using transverse, coronal and sagittal views on annexin imaging to perform *in vi*vo quantification of apoptosis in the heart demonstrated that atorvastatin therapy can diminish apoptosis induced by doxorubicin therapy. The extent of apoptosis was compared in control rats, rats treated with doxorubicin and rats treated with doxorubicin plus atorvastatin (Figure B). No apoptosis was observed in the control rats (upper panel). Less

apoptosis was observed in the rats who received doxorubicin and atorvastatin (lower panel) than the rats who received doxorubicin alone (middle panel) (unpublished data).

Figure B:



Gross pathologic analysis was also performed on hearts from rats treated with doxorubicin alone and those who received concurrent atorvastatin therapy in Dr. Abraham's lab (Figure C). This confirmed the imaging findings with greater dilatation observed in the hearts from the rats who received doxorubicin alone (lower panel) than those who received doxorubicin plus statin therapy (upper panel) (unpublished data).

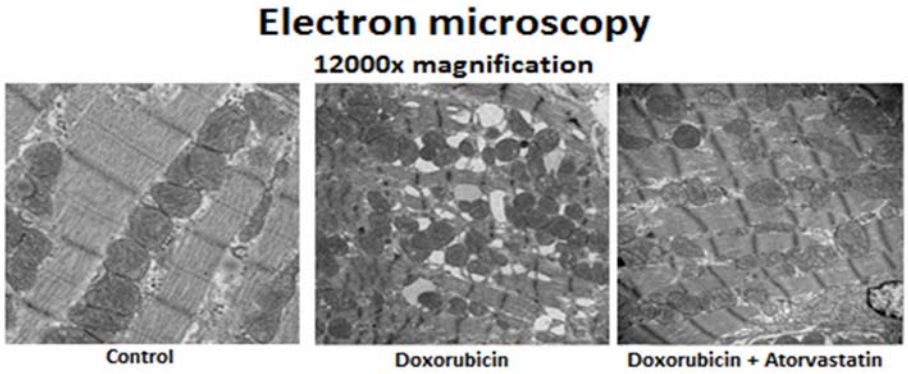
Figure C:

Gross pathology: Hearts from doxorubicin treated animals who also received statin therapy (upper panel) were less dilated than those not treated with statin (lower panel). Thus these data corroborated the morphologic data from echocardiography.

Finally, ultrastructural evidence supporting the use of statins to attenuate cardiac toxicity induced by anthracyclines was observed in Dr. Abraham's lab by the use of electron microscopy. Mitochondrial density and structure in cardiomyocytes from control rats, rats treated with

doxorubicin alone and rats treated with doxorubicin plus atorvastatin were analyzed by electron microscopy (Figure D). Control rats showed normal mitochondrial density and structure (left panel). In contrast, substantial mitochondrial damage was observed in rats treated with doxorubicin alone (middle panel). Less evidence of mitochondrial damage was observed in the rats treated with both doxorubicin and atorvastatin (right panel) consistent with the hypothesis that doxorubicin-induced cardiotoxicity is mediated by mitochondrial dysfunction and that statin therapy can block this effect (unpublished data).

Figure D:



At this time, much is unknown regarding the potential use of statins for the prevention of anthracycline-induced cardiac toxicity. For example, since most investigators have waited a few months to perform cardiac evaluation after anthracycline exposure (either with or without statins), it is not known if the potential cardioprotective effect of statins is mediated by prevention of cardiac damage or by repair of cardiac damage induced by anthracyclines. In addition, it is not known whether one statin may be more a more effective cardioprotective agent against anthracycline-induced cardiac toxicity than another. Indeed, this is a potentially relevant question as evaluation of statins for other potential uses in cancer patients has revealed benefits may be limited to the lipophilic class.[95]

In addition, the optimal dose, duration and timing of administration of statin therapy for cardioprotection in patients receiving anthracyclines is currently not unknown. However, key characteristics of an optimal statin for use as a cardioprotective agent during anthracycline-based chemotherapy include ease of dosing and minimal interactions with chemotherapy.

For this study, we have selected simvastatin which is dosed once daily. For the purpose of this trial, we have selected a moderate dose of 40 mg daily. Like doxorubicin and paclitaxel, simvastatin is a substrate of the cytochrome P450 3A4 (CYP3A4) enzyme, but it does not significantly inhibit or induce CYP3A4, making clinically significant interactions between simvastatin and the commonly used chemotherapy regimen of AC followed by paclitaxel for early stage breast cancer unlikely.[97] We have selected a 6 month course of simvastatin starting concurrently with anthracycline therapy based on the previous small trial of Acar et al which evaluated a similar duration of statin therapy for cardioprotection.[93] After discussion with Dr. Abraham we have decided that administering the first dose of simvastatin the day of cycle 1 AC

closely approximates the previous small trial of Acar et al while permitting patients to enroll with fewer delays from time of consent to time of first AC therapy.

3.3.3 Other Potential Benefits of Statins in Breast Cancer Patients

In addition to potentially preventing cardiac toxicity associated with anthracycline therapy, statins have been shown to have *in vitro* anti-tumor effects.[98] To this end, the ability of statins to help prevent breast cancer incidence and to help reduce the risk of breast cancer recurrence has been evaluated. With regard to chemoprevention, data to support statins has not been consistently promising, although there may be a class effect, with the potential chemoprevention benefit limited to the lipophilic statins, such as simvastatin.[96] On the other hand, a reduction in the risk of breast cancer recurrence with statin use has been more consistently observed, again with the benefit primarily linked to the lipophilic statins. Kwan et al first evaluated post-diagnosis use of statins in 1,945 breast cancer survivors enrolled in the prospective Life After Cancer Epidemiology cohort. They identified a 33% lower risk of recurrence among women who started statin therapy after breast cancer diagnosis compared to those who did not start statin therapy, although this finding was not statistically significant. Most patients who received statins in this study were treated with lipophilic statins.[99] This finding was confirmed in a nationwide, population-based prospective cohort study of 18,769 Danish women with early stage breast cancer. Risk of recurrence was reduced among the women who had been prescribed statins, with the benefit limited to prescription of lipophilic statins (10 year adjusted hazard ratio 0.73, 95% confidence interval 0.60-0.89).[95] More recently, population-based data from the entire Danish population has demonstrated a reduction in cancer-related mortality among patients who used statins prior to diagnosis (hazard ratio 0.85, 95% confidence interval 0.83-0.87). When these results were examined separately by tumor type, the findings were essentially unchanged (hazard ratio for death from breast cancer among breast cancer patients who used statins compared to those who did not use statins 0.88, 95% confidence interval 0.80-0.99).[100] Finally, data from the German MARIEplus population-based prospective cohort of breast cancer patients demonstrates a trend towards reduced risk of breast cancer recurrence and breast cancer-specific mortality among statin users with early stage breast cancer compared to non-users (hazard ratio for recurrence 0.83, 95% confidence interval 0.54-1.24; hazard ratio for breast cancer-specific mortality 0.89, 95% confidence interval 0.52-1.49), although these findings were not statistically signficant.[101] At this time, there have been no reported randomized trials evaluating the use of statins for the reduction of breast cancer recurrence and the available data remains observational in nature.

3.4 Study Rationale and Design

This is a prospective, randomized study evaluating the effects of AC chemotherapy concurrent with statin therapy versus AC chemotherapy alone on change in strain parameters over time in women with early stage breast cancer. We plan to randomize women undergoing neo-adjuvant or adjuvant anthracycline-based chemotherapy for breast cancer in a 1:1 ratio to receive chemotherapy plus simvastatin or chemotherapy alone. We will evaluate patients who do and do not receive simvastatin for evidence of cardiac toxicity by using the echocardiographic myocardial strain, echocardiographic EF and by assessment for symptoms of heart failure. Our hypothesis is that simvastatin will minimize reduction in myocardial strain. This will serve as a surrogate endpoint for symptomatic cardiac toxicity. Since the bulk of the data regarding

myocardial strain as a tool for evaluating cardiac toxicity from cancer therapy suggests that GLS is the strongest measure, we will use that as our primary measure. However, we will also measure velocity-based GLS rate and we will evaluate other measures of systolic and diastolic strain in an exploratory manner in addition to routine measurements of left ventricular diastolic function and filling pressures. We will also explore the capacity of strain and changes in strain to predict subsequent cardiac endpoints including reduction in EF and development of symptomatic heart failure if these endpoints occur in our study population.

To our knowledge, this will be the first prospective study to provide information about echocardiographic strain in breast cancer patients treated with anthracyclines who receive statin therapy. In addition, since prior studies evaluating strain in breast cancer patients have included small sample sizes, our study population, although also small, will add significantly to the available data describing serial changes in strain in breast cancer patients treated with anthracycline therapy. Furthermore, unlike prior studies evaluating strain in breast cancer patients which typically utilized echocardiography with strain analysis at baseline and after anthracycline treatment, we will also obtain an echocardiogram with strain analysis early during the course of anthracycline chemotherapy to detect any early impact of chemotherapy and simvastatin on myocardial strain. Comparison of this echocardiogram to subsequent echocardiograms in the patients who do and do not receive simvastatin could potentially shed light on whether simvastatin can prevent cardiac toxicity or whether it can repair cardiac toxicity after anthracycline exposure.

This study is not intended or powered to detect a specific difference in strain between those who do and do not receive statin therapy, but it will be able to find a minimal detectable difference with sufficient power (Section 13.3). The data collected will help plan future studies to establish a clinically important difference in strain between breast cancer patients who do and do not receive statin therapy and to assess the protective effect of statin on AC-induced cardiac toxicity with long-term follow-up.

4. Hypothesis

We hypothesize that simvastatin will provide cardiac protection, as evidenced by minimizing reduction in myocardial strain, during (neo)adjuvant anthracycline-based chemotherapy for early stage breast cancer.

5. Objectives

5.1 Primary Objective

To compare the absolute change in echocardiographic GLS from baseline to 2-3 weeks after completion of 4 cycles of (neo)adjuvant anthracycline-based chemotherapy in early stage breast cancer patients who do and do not receive concurrent simvastatin therapy

5.2 Secondary Objectives

5.2.1 To compare the relative change in echocardiographic GLS from baseline to 2-3 weeks after completion of 4 cycles of (neo)adjuvant anthracycline-based chemotherapy in early stage breast cancer patients who do and do not receive concurrent simvastatin therapy

5.2.2 To compare the absolute and relative changes in echocardiographic GLS rate from baseline to 1-3 weeks after completion of 4 cycles of (neo)adjuvant anthracycline-based chemotherapy in early stage breast cancer patients who do and do not receive concurrent simvastatin therapy.

5.2.3 To describe echocardiographic GLS and GLS strain rate and change in these parameters at multiple time points from baseline to 52 weeks in breast cancer patients treated with (neo)adjuvant anthracycline-based chemotherapy who do and do not receive 24 weeks of concurrent simvastatin therapy.

5.2.4 To evaluate the feasibility of concurrent administration of simvastatin therapy with (neo)adjuvant anthracycline-based chemotherapy in early stage breast cancer patients.

5.2.5 To assess the feasibility of performing serial echocardiographic myocardial strain assessments in early stage breast cancer patients undergoing (neo)adjuvant anthracycline-based chemotherapy.

5.2.6 To evaluate the safety and tolerability of concurrent administration of simvastatin with (neo)adjuvant anthracycline-based chemotherapy in early stage breast cancer patients.

5.2.7 To describe the recurrence free survival (RFS) in early stage breast cancer patients treated with anthracycline-based chemotherapy with and without concurrent simvastatin.

5.3 Exploratory Objectives

5.3.1 To describe the relationships between early measurements of echocardiographic GLS and GLS rate and of change in these parameters with subsequent measurements of EF and subsequent development of symptomatic heart failure in early stage breast cancer patients

treated with (neo)adjuvant anthracycline-based chemotherapy who do and do not receive 24 weeks of concurrent simvastatin therapy.

5.3.2 To explore risk factors that may predict early change in GLS in breast cancer patients treated with neo(adjuvant) anthracycline-based chemotherapy who do and do not receive 24 weeks of concurrent simvastatin therapy

5.3.3 To describe additional echocardiographic strain parameters besides GLS and GLS rate, including right ventricular strain, in addition to routine assessment of left ventricular diastolic function and filling pressures, at multiple time points from baseline to 52 weeks in breast cancer patients treated with (neo)adjuvant anthracycline based chemotherapy who do and do not receive 24 weeks of concurrent simvastatin therapy.

6. Patient Population

6.1 Inclusion Criteria

The following individuals are eligible for participation in this study:

6.1.1 Female Sex

Note: Patients may be pre-menopausal or post-menopausal

6.1.2 Age 18 years or older

6.1.3 Histologically confirmed invasive breast carcinoma, stage I-III (see Appendix A for staging)

Note: Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2 status must be known. In newly diagnosed patients planning neoadjuvant treatment, a formal assessment of axillary lymph nodes is not required.

6.1.4 Planning to initiate adjuvant or neoadjuvant AC chemotherapy (doxorubicin 60 mg/m2 and cyclophosphamide 600 mg/m2 every 2-3 weeks x 4 cycles).

Note:
- Participants may be planning to receive additional adjuvant therapy after the completion of AC chemotherapy.
- Receipt of all standard chemotherapy and/or targeted therapy regimens after AC that deemed clinically appropriate by the treating physician are permitted. For example, patients may receive taxanes or carboplatin/paclitaxel. Her2 positive patients may receive trastuzumab with or without pertuzumab.
- HER2 positive patients must be planning to initiate trastuzumab therapy after AC chemotherapy.

6.1.5 Eastern Cooperative Oncology Group (ECOG) performance status 0-1 (see Appendix B)

6.1.6 Normal organ function and marrow function as defined below:

- Absolute neutrophil count (ANC) $\geq 1000/mm^3$
- Platelet count $\geq 100{,}000/mm^3$
- Total bilirubin less than or equal to the upper limit of normal
- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq$1.5 times the upper limit of normal
- Creatinine $\leq$1.5 times the upper limit of normal
- Creatine kinase (CK) $\leq$2.5 times the upper limit of normal

6.1.7 Left ventricular ejection fraction (LVEF) as assessed by baseline echocardiogram at or above the lower limit of normal

6.1.8 Women of childbearing potential must agree to use adequate contraception (non-hormonal or barrier method of birth control or abstinence) prior to study entry and for the

duration of participation. Should a woman become pregnant or suspect she is pregnant while participating in the study, she should inform her treating physician immediately.

6.1.9 Ability to understand the study regimen and the willingness to sign a written informed consent document

6.1.10 Negative pregnancy test (women of childbearing potential only)

6.2 Exclusion Criteria

The following individuals are not eligible for participation in this study:

6.2.1 Prior anthracycline therapy

6.2.2 Currently pregnant or lactating

6.2.3 Currently receiving investigational agents

6.2.4 Known active liver disease (cirrhosis, chronic viral hepatitis, autoimmune liver disease or other known clinically significant active liver disease)

6.2.5 Known myopathy or history of rhabdomyolysis

6.2.6 Uncontrolled hypothyroidism

6.2.7 History of allergic reaction or intolerance to statin treatment

6.2.8 Currently receiving statin therapy or have received any statin therapy within the last 3 months

6.2.9 Known history of ischemic cardiac disease (including angina requiring anti-anginal medications, myocardial infarction, coronary artery disease documented on cardiac catheterization or ischemia documented on stress test), congestive heart failure, clinically

significant arrhythmia or conduction system abnormalities, clinically significant valvular disease, clinically significant pericardial effusion or EF below the lower limit of normal

6.2.10 Uncontrolled inter-current illness including, but not limited to, ongoing or active serious infection, other active cardiac disease or psychiatric illness/social situations which would limit compliance with study requirements

6.2.11 Inability to swallow tablets or use of a feeding tube

6.2.12 Gastrointestinal disease, surgery or malabsorption that could potentially impact the absorption of the study drug

6.2.13 Daily consumption of alcohol exceeding 3 standard drinks a day (defined as 10 grams of alcohol, which is equivalent to 285 mL of beer, 530 mL of light beer, 100 mL of wine or 30 mL of liquor)

6.2.14 Women currently taking drugs which are strong inhibitors or inducers of CYP3A4 are not eligible. These may be found at the Indiana University Clinical Pharmacology website at http://medicine.iupui.edu/clinpharm/ddis/main-table/.

6.2.15 Women taking drugs associated with a substantial risk of myopathy when co-administered with simvastatin are not eligible. These drugs are listed in the simvastatin package insert (available at: http://www.merck.com/product/usa/pi_circulars/z/zocor/zocor_pi.pdf).

6.2.16 Women taking medications for which interaction with simvastatin may result in increased levels of simvastatin are not eligible. Such drugs are listed in the simvastatin package insert (available at: http://www.merck.com/product/usa/pi_circulars/z/zocor/zocor_pi.pdf).

6.2.17 Any medical condition which, in the opinion of the investigator, puts the patient at risk of potentially serious complications while on study treatment

6.3 Inclusion of Women and Minorities

This study is open to individuals of all races and ethnic groups. There is no bias towards age or race in the clinical trial outlined. This trial is open to accrual of women only because breast cancer is rare in men and because normal values for myocardial strain may differ between men and women.[53]

7. Study Design and Treatment Plan

7.1 Recruitment

Patients will be recruited through the breast cancer medical oncology clinics that are part of the Johns Hopkins Medical Institutions. Only patients willing to come to the East Baltimore Campus of Johns Hopkins Hospital for echocardiograms will be eligible to participate.

7.2 Determination of Eligibility

Subjects will be registered with the study coordinator once an informed consent form is signed. The study coordinator will assign a *study number* – the study number is a sequential number beginning with "001". Subjects will not begin protocol-specified treatment until eligibility is confirmed and randomization takes place.

Subjects who sign a consent form, but do not initiate protocol treatment for any reason [i.e., subjects who are screen failures, those who do not initiate the planned course of AC chemotherapy or those who do not initiate simvastatin (if randomized to receive it)] will be replaced.

7.3 Study Drug and Management of Toxicities

7.3.1 Treatment Assignment and Randomization

This is a non-blinded, non-placebo controlled, randomized, prospective study. Eligible patients will be randomized in a 1:1 ratio to receive either simvastatin with AC chemotherapy or AC chemotherapy alone. Randomization will occur after registration and prior to the planned first cycle of chemotherapy. All participants and providers will know the arm to which they are randomized. A master list of randomization assignments will be made by the protocol biostatistician and will be delivered to the Research Pharmacy at Johns Hopkins and to other key personnel, as identified. Upon successful registration of a subject, randomization will occur by the appropriate study staff using the participant's study number and initials to the correct group.

7.3.2 Control Group: AC Chemotherapy alone

Patients not randomized to simvastatin will participate in all aspects of the study with the exception of simvastatin administration and completion of the study drug diary (Appendix C). All other assessments (e.g., study visits, toxicity assessments, questionnaires, laboratory studies and echocardiograms) will be performed according to the same schedule as that which is used for the intervention group.

7.3.3 Intervention Group: AC chemotherapy with concurrent Simvastatin

7.3.3.1 Simvastatin Administration

For those participants randomized to receive study drug, simvastatin will be administered on an outpatient basis orally at a dose of 40 mg once daily. Treatment will start on the evening of the first dose of AC and will continue for a total of 24 weeks. No pre-medications are required.

Patients will be instructed to take simvastatin at approximately the same time each evening. Missed doses will not be made up.

Patients will be asked to record administration of each dose of the study drug in the study drug diary (Appendix C). Study staff will fill in the calendar in the diary to reflect the appropriate dates for which the patient should be taking simvastatin prior to giving the calendar to the patient to complete. This calendar will be compared with the drug accountability records (number of returned pills, if any) in order to assess compliance.

No other investigational agents should be administered concurrently. Any questions about concurrent enrollment in other clinical trials should be discussed with the Protocol Chair in advance.

7.3.3.2 Safety Assessments and Dose Modifications

If possible, symptoms should be managed symptomatically. In case of toxicity, appropriate medical treatment should be used (including anti-emetics for nausea/vomiting, anti-diarrheals for diarrhea, etc.).

If statin treatment is interrupted due to an adverse event, treatment can be resumed as soon as possible **if the adverse event is deemed unrelated** to the study drug. If the drug is stopped, due to the hospitalization of the patient, the principal investigator should be contacted to confirm if it is safe to resume the drug. The maximum allowable interruption is 4 weeks for the duration of time that the patient is on study.

7.3.3.2.1 Hepatotoxicity

Simvastatin can cause rare hepatotoxicity characterized by elevations in transaminases (ALT and AST). In clinical trials of simvastatin, persistent increases in transaminases were observed in approximately 1% of patients. Literature indicates that this is likely an idiosyncratic event which is often not clinically significant and there is controversy about the need for routine monitoring of transaminases when simvastatin is used in the clinical setting. However, guidelines suggest assessment of transaminases at baseline, 12 weeks after initiation of statin therapy, whenever the dose is changed and yearly thereafter.[102] Given that chemotherapy can also cause hepatotoxicity, transaminases will be monitored carefully in this study. The schedule for monitoring transaminases in this study is more frequent than that recommended for statin therapy alone due to the potential impact of chemotherapy on the liver.

Liver function panels will be assessed at baseline. In addition, liver function panels are recommended immediately prior to the second, third and fourth cycles of AC. Liver function panels will also be performed 1-3 weeks after the fourth cycle of AC and monthly during any additional treatment period after AC. The liver assessment performed 1-3 weeks after the fourth

cycle of AC will coincide approximately with the recommended standard of care assessment for hepatotoxicity 12 weeks after initiation of statin therapy.

In case of abnormalities in ALT or AST, dose adjustments of simvastatin (for patients in the intervention group only) and repeat assessments of transaminases (for patients in both the intervention and control groups) will be made as follows:

AST and/or ALT level	Guidelines for Dose Adjustment (for patients randomized to receive simvastatin) and for Repeat Assessment of AST and ALT (for all patients)
≤ 1.5 times ULN	Continue current dose of simvastatin. Re-assess AST and ALT according to study calendar
> 1.5 times ULN and < 3 times ULN	Continue current dose of simvastatin. Re-assess AST and ALT in 4 weeks (+/- 3 days) or according to study calendar (whichever occurs first).
≥3 times ULN	Hold simvastatin and re-assess AST and ALT in 4 weeks (+/- 3 days) or according to study calendar (whichever occurs first). Evaluation for other causes of abnormal AST and ALT such as viral hepatitis, autoimmune hepatitis alcohol, other medications or biliary disorders may be performed at the discretion of the investigator. • If AST and/or ALT remain ≥3 times ULN when re-assessed, simvastatin will be permanently discontinued. If possible, patient will stay on study for clinical assessments and echocardiography but will no longer receive study drug. AST and ALT will be monitored every 4 weeks (+/- 3 days) or according to the study calendar (whichever occurs first) until AST and ALT are < the ULN or until the investigator determines no further improvement can be expected. • If AST and ALT become < 3 times ULN when re-assessed, the investigator thinks it is safe to re-challenge with simvastatin, and < 24 weeks have passed since simvastatin was initiated, the study drug may be restarted at 20 mg oral daily with repeat assessment of AST and ALT in 4 weeks (+/- 3 days) or according to the study calendar (whichever occurs first). If repeat AST and/or ALT are again > 3 times ULN after simvastatin is re-started at the lower dose, the drug will be permanently discontinued. If possible, the patient will stay on study for clinical assessments and echocardiography but will no longer receive study. If AST and ALT are < 3 times ULN on the lower dose, simvastatin will be continued at 20 mg oral daily and AST and ALT will be monitored every 4 weeks (+/- 3 days) or according to the study calendar (whichever occurs first) until AST and ALT are < ULN or until the investigator determines no further improvement can be expected.

7.3.3.2.2 Skeletal Muscle Toxicity

Simvastatin can cause myopathy which manifests as muscle pain, tenderness or weakness. In some cases CK is elevated and, rarely, rhabdomyolysis can occur. The risk of skeletal muscle toxicity is dose related. [103] There are no guidelines regarding screening for skeletal muscle toxicity or managing skeletal muscle toxicity for patients receiving simvastatin therapy.

Predisposing factors for skeletal muscle toxicity in patients taking simvastatin include older age, female gender, renal impairment and uncontrolled hypothyroidism.

In this trial, CK will be assessed at baseline and only patients with CK less than or equal to 2.5 times the ULN will be able to participate. Repeat CK will be assessed within 72 hours of patient report of grade 2 or higher myalgia or investigator suspicion of skeletal muscle toxicity.

If skeletal muscle toxicity occurs, dose adjustments of simvastatin (for patients in the intervention group only), repeat assessments of skeletal muscle symptoms and repeat

assessments of CK (for patients in both the intervention group and the control group) will be made as follows:

Skeletal Muscle Toxicity	Guidelines for Dose Adjustment (for patients randomized to receive simvastatin) and for Repeat Assessment of Skeletal Muscle Toxicity (for all patients)
Grade 1 myalgia and CK ≤10 X ULN (if CK checked) or CK ≤10 X ULN (if checked) and no myalgia or Grade 2 myalgia and CK ≤10 X ULN	Continue current dose of simvastatin. Pain control at the discretion of the investigator. Repeat assessment for muscle toxicity symptoms as per study calendar. Repeat CK (if has been checked) in 4 weeks (+/- 7 days) with management as per this table.
Grade 3 myalgia with any level of CK or Grade 2 myalgia with CK > 10 X ULN or Grade 1 myalgia with CK > 10 X ULN (if CK checked) or CK > 10 X ULN (if checked) and no myalgia	Hold Simvastatin. Pain control at the discretion of the investigator. Re-assess skeletal muscle toxicity symptoms and CK in 2 weeks (+/- 3 days) or per study calendar (whichever occurs first) • If repeat assessment for skeletal muscle toxicity reveals grade 3 myalgia and/or CK remains > ULN, permanently discontinue simvastatin. If possible, patient will stay on study for clinical assessments and echocardiography but will no longer receive study drug. • If repeat assessment for skeletal muscle toxicity reveals ≤ grade 2 myalgia and CK becomes normal, may restart simvastatin at 20 mg oral daily if the investigator thinks it is safe and if < 24 weeks have passed since simvastatin was initiated. If simvastatin is restarted at the lower dose, the patient will be assessed for muscle toxicity symptoms and CK at the next study visit and managed according to this table. If grade 3 myalgia and/or CK > 10 X ULN occurs again, permanently discontinue simvastatin. If possible, patient will stay on study for clinical assessments and echocardiography but will no longer receive study drug.
Clinical evidence of rhabdomyolysis	Permanently discontinue simvastatin. Clinical management of rhabdomyolysis at the discretion of the investigator. If possible, patient will stay on study for clinical assessments and echocardiography but will no longer receive study drug.

Rhabdomyolysis with renal failure occurs more commonly with administration of statin therapy in certain high-risk situations. If the following clinical scenarios occur for patients randomized

to simvastatin, the study drug will be discontinued until resolution of the problem at the discretion of the investigator:

- Severe infection
- Hypotension
- Major surgery (not including planned breast surgeries or axillary lymph node evaluations)
- Trauma
- Uncontrolled seizures
- Severe metabolic abnormalities
- Severe endocrine abnormalities
- Severe electrolyte abnormalities

If any of the above clinical scenarios resolve, simvastatin may be re-started at the same dose as prior at the discretion of the investigator. Such patients would continue to participate in clinical assessments and echocardiography for the study.

The risk for skeletal muscle toxicity associated with simvastatin is higher with co-administration with certain other medications. Drugs which must be avoided during participation in this study are described below (Section 7.3.4.2).

7.3.3.2.3 Cardiac Toxicity

Simvastatin is not expected to cause cardiac toxicity. To the contrary, this study aims to use simvastatin to protect against chemotherapy-induced cardiac toxicity with myocardial strain as a surrogate endpoint for cardiac toxicity. However, since the primary endpoint of this study is GLS instead of clinically apparent cardiac toxicity, guidelines for assessment and management of clinically apparent cardiac toxicity, should it occur, are provided here.

Participants will be assessed for clinical signs of cardiac toxicity by investigator evaluation at the time of study visits and by echocardiographic evaluation of EF and wall motion abnormalities. During study visits, investigators will assess for symptoms of heart failure including exertional dyspnea, lower extremity swelling, paroxysmal nocturnal dyspnea, and orthopnea. If present, investigators will attribute whether these symptoms are due to heart failure or to other causes. Physical examination to detect signs of heart failure such as a S3 heart sound, jugular venous distention, peripheral edema, hepatomegaly and pulmonary crackles will be performed at the investigators' discretion. Investigators will record New York Heart Association (NYHA) functional classification (Appendix D) for participants thought to have cardiac disease at each visit. In addition echocardiographic myocardial strain will be assessed according to the study schedule, but results of strain analysis will not be available to investigators or participants during study participation.

Clinical management of cardiac toxicities will be at the discretion of the investigator, although cardiology consultation is encouraged. Decisions regarding cessation of chemotherapy, delay in chemotherapy and/or reduction in dose of chemotherapy in the face of cardiac toxicities will be at the discretion of the investigator. Patients may continue to participate in this study in the face of cardiac toxicity if it is thought to be safe by the investigator. The decision of whether to

discontinue simvastatin will be at the discretion of the investigator. In cases in which simvastatin is discontinued, patients may stay on study for clinical assessments and echocardiography but will no longer receive study drug.

7.3.3.3 Other Toxicity

Management of toxicities other than hepatic, skeletal muscle and cardiac toxicity that the investigator attributes to simvastatin (for the intervention group only) is described in the table below:

Toxicity Grade	Guidelines for dose adjustment (for patients randomized to receive simvastatin only)
Grade 1 or 2	Continue simvastatin if investigator thinks it is safe. Management of toxicity at discretion of investigator. Re-assess toxicity at frequency at the discretion of the investigator
Grade 3 or 4	Hold simvastatin if investigator thinks toxicity is attributable to simvastatin. Re-assess toxicity at frequency determined by investigator. If toxicity resolves to $\leqslant$ grade 2 and investigator deems it to be safe, may restart simvastatin at 20 mg oral daily. If any grade 3 or 4 toxicity occurs again, discontinue simvastatin permanently. If possible, patient will stay on study for clinical assessments and echocardiography but will no longer receive study drug.

7.3.3.4 Special Considerations

- For toxicities which are considered by the treating investigator unlikely to develop into serious or life–threatening events (e.g. alopecia, altered taste etc.), simvastatin treatment may be continued at the same dose without reduction or interruption.
- The treating investigator may reduce a subject's simvastatin dose for a toxicity of any grade/duration where s/he believes it to be in the best interests of the subject.
- Any consideration to modify the above dose modification guidelines should be discussed with the Principal Investigator for approval or disapproval in advance.

7.3.4 Concomitant Therapy

7.3.4.1 Cancer-Directed Therapies and Supportive Care

All patients will receive chemotherapy (with or without trastuzumab) as per usual clinical care. AC may be administered every 2 weeks or every 3 weeks. If administered, taxane therapy may be given weekly, every 2 weeks or every 3 weeks. Either paclitaxel or docetaxel may be used. If administered, trastuzumab may be given weekly or every 3 weeks or a combination of these dosing schedules. Any other chemotherapy regimens will be administered as directed at the discretion of the treating physician. Administration of anti-emetics, growth factors, chemotherapy pre-medications and any other supportive medications will be at the discretion of the investigator. Any necessary dose modifications and delays in chemotherapy or trastuzumab will also be at the discretion of the investigator. Since study assessments are timed in relation to chemotherapy administration, the study assessment schedule during the period of AC administration will be adjusted appropriately if chemotherapy is delayed. Details regarding adjustment of the study assessment schedule in the event that AC chemotherapy is discontinued before the planned 4 cycles are completed are provided in the study calendar (section 8.0). For patients receiving neoadjuvant AC chemotherapy, surgery will be performed when deemed

appropriate by the clinical team. Every attempt will be made to perform study assessments on time in patients undergoing surgery during the course of the study, but if unable to do so, the missed assessments will be performed as soon as possible post-operatively. After completion of AC, patients will continue to receive appropriate therapy such as additional chemotherapy, HER2 targeted therapy, and/or radiation therapy as clinically indicated at the discretion of the investigator.

7.3.4.2 Prohibited Concomitant Medications

Drugs which are strong inhibitors or inducers of CYP3A4 should be avoided during study participation. A list of CYP3A4 inhibitors and inducers can be found at the Indiana University Clinical Pharmacology website at http://medicine.iupui.edu/clinpharm/ddis/main-table/. All medications study participants are taking should be cross-referenced with the medications listed in this online table.

In addition, certain drugs are associated with a higher risk of myopathy if co-administered with statins and simvastatin can increase levels of certain other drugs. Such drugs are listed in the simvastatin package insert (available at: http://www.merck.com/product/usa/pi_circulars/z/zocor/zocor_pi.pdf). If possible, use of these drugs should be avoided during study participation.

In addition, study participants should not consume > 8 ounces of grapefruit juice or carbonated grapefruit beverages daily.

Co-administration of ACE inhibitors, ARBs and beta-blockers is allowed. Administration of these medications will be tracked as part of review of concomitant medications.

All concomitant medications will be recorded by study staff at each study visit.

7.4 Study Assessments

7.4.1 Medical Records

Charts will be reviewed and information regarding tumor characteristics (e.g., stage, hormone receptor status, HER2 status, grade, surgery performed or planned, radiation plans, endocrine therapy plans) and relevant cardiovascular medical history will be entered into a case report form at baseline. Medical records related to cardiovascular medical history will be reviewed if appropriate. Medical records will be reviewed every 6 months from randomization for 5 years to assess for recurrence (defined as in-breast recurrence, distant metastases, new contralateral primary breast cancer, and death from breast cancer).

7.4.2 Echocardiogram

Two-dimensional echocardiography will be at Johns Hopkins Hospital. The standard clinical echocardiography protocol will be followed. Assessment of EF and myocardial strain will be performed. Myocardial strain analysis will be performed using FDA approved software. Results of clinical assessment of EF will be provided to the investigators to share with patients. Results of the clinical assessment of EF from the study echocardiograms will be available in the electronic medical record. For baseline study echocardiograms, every effort will be made to

have results of EF available in the electronic medical record within 3 business days. For subsequent study echocardiograms, every effort will be made to have results of EF available in the electronic medical record within 10 business days. Myocardial strain results will not be available in the electronic medical record and will not be shared with the treating investigators or patients.

For patients who continue to receive trastuzumab after completion of AC, every effort will be made to time the study echocardiogram due after the fourth dose of AC such that it can serve as the pre-trastuzumab assessment of EF.

If a clinically concerning abnormality is detected on echocardiogram performed for the study, the treating physician will be notified. Management of such abnormalities will be at the discretion of the treating physician and may include termination of therapy.

7.4.3 Participant Questionnaire

Participants will complete a baseline questionnaire describing risk factors for heart disease (Appendix E). This questionnaire has been modified from a questionnaire used for multiple other trials in the Johns Hopkins Breast Cancer Program.

7.5 Duration of Study Participation

All patients who initiate AC therapy while on this study and who complete at least one echocardiogram after initiating AC will be included in the analysis in an intent-to-treat fashion. The total duration of study participation is approximately 52 weeks (from first cycle of AC and initiation of study drug until end of study assessments 52 weeks after first dose of AC). While the duration of study participation is only 52 weeks, all patients will be followed by chart review for 5 years after randomization to identify breast cancer recurrence.

Duration of individual subject treatment will depend on individual tolerance. Should a patient decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. If a patient discontinues AC prior to completing 4 cycles, assessments due after AC #4 will be performed after the last cycle administered whenever possible. Further assessments will then be performed according to the study calendar. If a patient discontinues simvastatin, all efforts will be made to keep the patient on the study for clinical assessments and

echocardiography but study drug will no longer be administered. Reason(s) for discontinuation should be recorded in the medical record. Reasons for premature withdrawal may include:

- Disease progression
- Unacceptable adverse events
- Inter-current illness that prevents further administration of treatment or would affect assessment of clinical status to a significant degree
- Non-compliance with protocol or treatment
- Subject becomes pregnant
- Subject refuses to continue treatment
- Subject is lost to follow-up

7.6 Additional Information

Participants will be offered parking stickers in appreciation of time at each study-specific echocardiogram with strain analysis procedure. Participants will also be asked to agree to optional future contact for possible participation in other clinical trials.

Follow-up of participants will continue for 5 years after randomization for ongoing collection of information pertaining to breast cancer treatment and outcomes, and changes in medical history, such as development of cardiovascular disease.

8. Study Calendar

Parameter	Baseline[1]	AC	During AC[2] (within 3 days prior to each cycle)			Post-AC Period[3]			End of Treatment[4]
		Day1	Pre-AC #2	Pre-AC #3	Pre-AC #4	1-3 weeks post last AC (±3 days)[3a]	Additional treatment period[3b]	24 weeks after AC #1 (±14 days)[3c]	
CLINICAL EVALUATIONS:									
History and Physical[5]	X		X	X	X	X	X	X	X
ECOG PS[6]	X		X	X	X	X	X	X	X
Height	X								
Vitals signs[7]	X		X	X	X	X	X	X	X
Waist and hip circumference	X								
NYHA Functional Classification[8]	X		X	X	X	X	X	X	X
LABORATORY/IMAGING EVALUATIONS:									
Echocardiogram, strain analysis[9]	X		X			X		X	X
Hematology and Chemistry panel[10]	X								
Creatine Kinase	X								
Hepatic/Liver Function Panel (LFTs)[11]			X	X	X	X	X		
Pregnancy Test [12]	X								
STUDY ASSESSMENTS/TREATMENT ADMINISTRATION:									
Participant questionnaire[13]	X								
Concomitant medications	X	X X							
Symptoms/ Adverse Events[14]	X	X X							
Randomization[15]	X								
Simvastatin[16]		X X							
Follow-Up[17]									X

Note: Additional tests may be performed at the discretion of the treating investigator as clinically indicated.

8.0 Study Calendar (continued)

1. Baseline assessments should be performed ≤21 days prior to randomization, as noted.
2. Pre-cycle assessments to be performed ≤3 days prior to each planned cycle. If a cycle of AC is delayed, LFTs should be repeated if delay is >7 days; repeat of other assessments is not required.
3. This period refers to the time after the last cycle of AC and until the End of Treatment assessments:
   a. Post-last dose of AC: assessments due after the last dose of AC administered and prior to start of any additional treatment; preferred window for study echocardiogram is 1-3 weeks after last dose (±3 days), no window applies to other assessments.
   b. This refers to patients who receive chemotherapy with or without trastuzumab. These patients should have assessments about every 4 weeks during the period of post-AC chemo.
   c. 24 weeks after AC #1: assessments are required in all patients regardless of dose holds/delays.
4. End of study assessments to be performed 52 weeks after AC #1 (+/- 14 days), regardless of delays.
5. History will include documentation of specific symptoms of heart failure and muscle disease, including exertional dyspnea, lower extremity swelling and/or edema, paroxysmal nocturnal dyspnea, orthopnea and muscle pain - if cardiac symptoms are present, investigators will attribute whether due to heart failure or another cause. If clinically indicated, physical examination will include evaluation for signs of heart failure such as presence of a S3 heart sound, jugular venous distention, peripheral edema, hepatomegaly and pulmonary crackles.
6. ECOG Performance Status (Appendix B).
7. Vital signs include weight, heart rate, respiratory rate, and blood pressure.
8. NYHA Classification to be determined only if cardiac disease is thought to be present (Appendix D).
9. Echocardiogram will include assessment of EF and myocardial strain. Note: Baseline test should be done after majority of eligibility testing is complete and prior to randomization.
10. Complete blood count (CBC) with differential and comprehensive chemistry panel, including measurement of sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total bilirubin, calcium, total protein, albumin, AST, ALT, and alkaline phosphatase. In follow-up, these assessments should be done as per standard of care/provider discretion.
11. LFTs include AST, ALT, total bilirubin, alkaline phosphatase, and albumin. (Note: Assessments done as part of a comprehensive chemistry panel may be used.) In cases of sample hemolysis, at least the total bilirubin and either the AST or ALT must be reported – if results ≤ ULN, no tests need to be repeated. If the total bilirubin is > ULN and either the AST or ALT are > 1.5 X ULN, LFTs should be repeated to monitor simvastatin toxicity, but treatment may proceed without waiting for results; any actions or dose modifications for simvastatin should be addressed when repeat results are received. LFTs are required at baseline and are included in the chemistry panel at that time LFTs are recommended but not required prior to each AC cycle and at least monthly in the additional treatment period. For patients with normal LFTs, no additional LFTs are required after the completion of chemotherapy. For patients who had abnormalities in LFTs during chemotherapy, LFTs must be followed until they resolve to a grade 1 or less.
12. Pregnancy test (blood or urine), for women of childbearing potential only.
13. Participant questionnaire at baseline only (Appendix E).
14. Symptoms and adverse events that will be tracked and reported include those related to simvastatin (treatment arm only, last assessment due 30 days after last dose of simvastatin), and to cardiac disease, muscle disease, liver disease (both arms). The collection of toxicities related to chemotherapy and other

breast cancer therapy administration is not required. Symptoms of uncertain etiology will be collected and adjudicated by the Principal Investigator.

15. Randomization to occur after eligibility confirmation; allow time for simvastatin supply to be prepared and provided to the participant for dosing to start on day of AC #1..
16. For patients randomized to receive it: Simvastatin 40 mg oral daily starts on day of AC #1 and continues daily x 24 weeks unless held due to toxicity or at the discretion of the investigator. Participants will complete a pill diary for simvastatin administration (Appendix C).
17. Follow-up for changes in disease status, recurrence, and cardiovascular health will continue for up to 5 years from randomization.

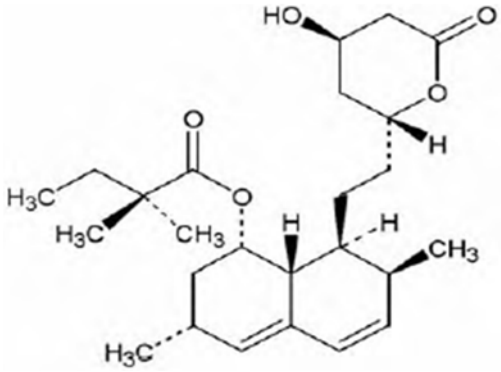
NOTE: The schedule should be followed as closely as is realistically possible; however, the schedule may be modified due to problems such as scheduling delays or conflicts (e.g., clinic closure, poor weather conditions, vacations, etc.) with the guidance of the Protocol Chair/designee, as appropriate, and will not be reportable as a deviation unless the endpoints of the study are affected.

9. Pharmaceutical Information

Pharmaceutical information described below is derived from the simvastatin package insert, available at http://www.merck.com/product/usa/pi_circulars/z/zocor/zocor_pi.pdf.

9.1 Simvastatin Product Identification

- Mode of action: Simvastatin is a pro-drug which is hydrolyzed to its β-hydroxyacid form, which is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme is responsible for catalyzing the conversion of HMG-CoA to mevalonate, a precursor of sterols, including cholesterol. This is a rate-limiting step in cholesterol biosynthesis.
- Other names in the United States: Zocor
- Classification: Statin
- Molecular formula: butanoic acid, 2,2-dimethyl-,1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetarhydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenylester, [1S-1α,3 α,7β,8 β (2S*, 4S*)-8a β]]
- Empirical formula: $C_{25}H_{38}O_5$
- Molecular weight: 418.57
- Structural formula:



9.2 Simvastatin Pharmacokinetics

Simvastatin is orally absorbed. It undergoes extensive hepatic first-pass metabolism. Both simvastatin and its β-hydroxyacid metabolite are highly bound to plasma proteins. Metabolism

is via the cytochrome P450 enzyme system (CYP 3A4 substrate). Plasma half-life is 3 hours. Simvastatin is primarily excreted in feces.

9.3 Contraindications to Simvastatin Therapy

Simvastatin is contraindicated in women who are pregnant, women who may become pregnant or nursing women. Simvastatin is contraindicated in patients with acute liver disease and in patients taking strong CYP 3A4 inhibitors, gemfibrozil, cyclosporine or danazol.

9.4 Drug Interactions with Simvastatin

Concomitant administration of simvastatin and strong CYP3A4 inhibitors such as itraconazole, ketoconazole, posaconazole, voriconazole, erythromycin, clarithromycin, telithromycin, human immunodeficiency virus (HIV) protease inhibitors, boceprevir, telaprevir, nefazodone, gemfibrozil, cyclosporine and danazol is associated with increased risk of skeletal muscle toxicity. In addition, simvastatin doses must be limited in patients also taking amiodarone, amlodipine, verapamil, diltiazem, ranolazine and dronedarone due to increased risk of skeletal muscle toxicity with higher doses of simvastatin. Grapefruit juice should be avoided in patients taking simvastatin due to increased risk of skeletal muscle toxicity. Caution should be used with co-prescription of fibrates or niacin and simvastatin due to increased risk of skeletal muscle toxicity. Simvastatin can enhance anti-coagulant effects of coumadin, thus frequent monitoring is recommended for patients on coumadin and simvastatin. Co-administration of colchicine and simvastatin has also been associated with increased risk of rhabdomyolysis. Simvastatin may increase plasma concentrations of digoxin.

9.5 Reported Simvastatin Toxicities

The primary side effects of concern for the statins include elevated transaminases and myopathy. Elevated transaminases occur in up to 0.7% of patients, are dose-dependent and typically occur within the first 3 months of therapy. Monitoring of hepatic transaminases is recommended. Statin-associated myopathy ranges from mild aches to more severe myopathy causing pain and immobility associated with elevations in creatine kinase (CK) and, rarely, in rhabdomyolysis. The reported incidence of statin-associated myopathy is 0.1-0.2%.[97]

Increases in fasting serum glucose levels and in hemoglobin A1c have also been observed in patients taking simvastatin. Other common side effects of simvastatin reported in the pre-marketing clinical trials experience included gastrointestinal disorders, myalgias, arthralgias, headache, upper respiratory infection, rash and asthenia. The post-marketing experience for simvastatin also identified several other possible toxicities including cognitive impairment, hypersensitivity syndrome, skin changes, dizziness, paresthesias, peripheral neuropathy,

depression, interstitial lung disease, pancreatitis, pruritis, alopecia, vomiting, anemia and erectile dysfunction in addition to the known risks of hepatotoxicity and skeletal muscle toxicity.

9.6 Simvastatin Monitoring Parameters

Simvastatin levels are not monitored.

9.7 Storage and Stability of Simvastatin

Simvastatin is stored at room temperature.

9.8 Simvastatin Preparation and Administration

Simvastatin is available in 5 mg, 10 mg, 20 mg, 40 mg and 80 mg tablets. It is administered orally. Simvastatin 20 mg tablets will be used for this study.

9.9 Simvastatin Availability

Simvastatin is an FDA approved drug. Simvastatin will be provided to study participants randomized to the simvastatin arm. The drug will be purchased for use in this study.

9.10 Accountability for Study Drug

The Investigational Drug Service of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins will keep records of study drug receipts and dispensation as per standard practice. Documentation of study drug destruction will be maintained in the research study binder.

10. Adverse Events

10.1 General

This study will use the descriptions and grading scales found in the revised NCI CTCAE Version 4 for adverse event reporting that can be found at http://ctep.cancer.gov/reporting/ctc.html.

Information about adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate. Adverse events will be collected from the time of consent, throughout the study and until the completion of all end-of-study assessments (52 weeks +/- 14 days after the first cycle of AC).

Specific symptoms of cardiac, liver, and muscle disease, such as exertional dyspnea, lower extremity swelling and/or edema, paroxysmal nocturnal dyspnea, orthopnea and muscle pain will be captured; collection of toxicity related to chemotherapy administration is not required. Elevations of AST, ALT and CK will be closely monitored and tabulated in all participants; these will be reported as adverse events if felt to be possibly related to simvastatin. Changes in EF or the development of other concerning findings on echocardiograms will also be closely monitored and reported in the study data in the presence or absence of symptoms; these will also be reported as adverse events.

Subjects who have an ongoing adverse event related to the study procedures and/or simvastatin may continue to be periodically contacted by a member of the study staff until the event is resolved or determined to be irreversible by the investigator.

10.2 Definitions

10.2.1 Adverse event (AE)

Any undesirable sign, symptom or medical condition occurring after starting study participation even if the event is not considered to be related to the study. An undesirable medical condition

can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram).

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment. Adverse events occurring before starting study treatment but after signing the informed consent form will be recorded.

10.2.2 Serious adverse event or reaction

A serious adverse event (SAE) is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening;
- requires or prolongs hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or a birth defect;
- is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Events **not** considered to be SAEs are hospitalizations for the:

- routine treatment or monitoring of a pre-existing condition, not associated with any deterioration in condition, or for elective procedures (for example, venous access placement);
- treatment, which was elective or pre-planned, and/or for a pre-existing condition that did not worsen – including hospitalizations for pre-planned breast and/or axillary surgeries for patients who receive chemotherapy in the neoadjuvant setting;
- treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious given above and **not** resulting in hospital admission.

Specific exclusions for events **not** considered to be reportable SAEs as part of a routine/expedited report on this study include the following:

- events for participants on the intervention/simvastatin arm believed to be definitely unrelated to statin treatment
- expected toxicities related to chemotherapy administration (eg, febrile neutropenia of a participant receiving AC or subsequent chemotherapy);
- any event meeting SAE criteria for any participant on the non-intervention/control arm of the study.

NOTE: The exception to this is the death of a participant within the study intervention/observation period; deaths should be reported per IRB requirements regardless of attribution and regardless of whether the participant is in the control or statin arm.

The definition of serious adverse event (experience) also includes *important medical event*. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life

threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

10.2.3 Expectedness

- Unexpected adverse event: An adverse event, which varies in nature, intensity or frequency from information on the package insert or safety reports. Any adverse event that is not included in the informed consent is considered "unexpected".

- Expected (known) adverse event: An adverse event, which has been reported. An adverse event is considered "expected", only if it is included in the informed consent document as a risk.

10.3 Relationship

The relationship of all adverse events and serious adverse events to study medication will be assessed by an investigator and assigned as follows:

- Definitely**:** An adverse event which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, and for which no alternative cause is present.

- Probably**:** An adverse event which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, but for which a potential alternative cause may be present.

- Possibly**:** An adverse event which has a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, but a potential alternative cause does not exist.

- Unlikely**:** An adverse event which does not have a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, does not reappear or worsen after re-administration of the investigational drug/agent (if applicable), and for which there is evidence that it is related to a cause other than the investigational drug/agent.

- Unrelated**:** An adverse event for which there is evidence that it is definitely related to a cause other than the investigational drug/agent. In general, there is no timely relationship

to the administration of the investigational drug/agent, or if there is a timely relationship, the event does not follow a known pattern of response, and there is an alternative cause.

10.4 Reporting Procedures

10.4.1 General

All reportable adverse events as described above will be captured on the appropriate study-specific case report forms (CRFs). In addition, all events meeting the definition of serious adverse events above, regardless of causality to study drug, will be reported promptly to the Principal Investigator and/or the Study Coordinator.

10.4.2 Institutional Review Board

All adverse events and serious adverse events will be reported to the institutional review board (IRB) per current institutional standards. If an adverse event requires modification of the study protocol and informed consent, these modifications will be provided to the IRB with the report of the adverse event or as soon as possible thereafter.

10.4.3 Food and Drug Administration (FDA)

The clinical trial outlined uses an FDA-approved medication and has been determined to be IND-exempt. Any unexpected adverse events believed to be definitely, probably, or possibly related to the medication (s) will be reported to the Food and Drug Administration via MedWatch (using the online form available at https://www.accessdata.fda.gov/scripts/medwatch/; by telephone 1-800-FDA-1088; or by fax 1-800-FDA-0178 using form available at http://www.fda.gov/medwatch/report/hcp.htm).

11. Data and Safety Monitoring

11.1 Data Management

All information will be collected on study-specific case report forms (CRFs) by study staff. These data will be reviewed for completeness and accuracy by the Principal Investigator as well as the Sidney Kimmel Comprehensive Cancer Center Clinical Research Office.

11.2 Meetings

Scheduled meetings will take place as needed and will include the protocol principal investigators, study coordinator(s), data manager(s), sub-investigators (as appropriate), collaborators (as appropriate), and biostatisticians (as appropriate) involved with the conduct of the protocol. During these meetings, matters related to the following will be discussed: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for objectives.

11.3 Monitoring

This is a DSMP Level I study under the SKCCC Data Safety Monitoring Plan (12/6/2012). The Clinical Research Office QA Group will perform an audit at the end of the first year and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee.

12. Administrative Procedures

12.1 Protocol Amendments

Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB before implementation. Any modifications made to the protocol or informed consent document according to local requirements or any other reason may also require approval from sponsoring agencies.

12.2 Informed Consent

An investigator will explain to each subject the nature of the study, its purpose, procedures involved, expected duration, potential risks and benefits. Each subject will be informed that participation in the study is voluntary and that she may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment. This informed consent will be given by means of a standard written statement and will be submitted for IRB approval prior to use. No patient will enter the study before her informed consent has been obtained. In accordance with the Health Insurance Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

12.3 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

12.4 Regulatory Authorities

12.4.1 Institutional Review Board

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards..

12.4.2 Food and Drug Administration (FDA)

This trial does not involve an Investigational New Drug (IND) application and no reporting is required with regards to the clinical trial outlined at this time.

13. Statistical Considerations

13.1 Study Design

This is a prospective, randomized, open-label, non-placebo controlled study evaluating change in myocardial strain with anthracycline (AC) chemotherapy plus simvastatin (investigational arm) or chemotherapy alone (control arm) in women with early stage breast cancer. Patients will be randomized 1:1 to the investigational arm or the control arm.

A number of recent studies suggest anthracyclines may reduce myocardial strain, a sign of cardiac toxicity which can be detected prior to decline in EF. Data suggests that simvastatin may attenuate the risk of anthracycline-related cardiac toxicity. The primary goal of this study is to assess change in global longitudinal strain (GLS) from baseline to 1-3weeks after completion of 4 cycles of chemotherapy in early stage breast cancer patients receiving AC with and without concurrent simvastatin. We hypothesize that simvastatin may have a cardio-protective effect by minimizing reduction in myocardial strain during treatment with anthracycline-based chemotherapy.

The study will accrue 90 patients, 45 in each arm. Safety of concurrent administration of statin and AC chemotherapy will be monitored continuously. The design includes interim monitoring for futility, i.e., we will suspend enrollment for re-evaluation if there is high probability that more than 30% of patients receiving statin therapy experience >5% absolute decline in GLS for the first two consecutive visits post therapy.

This study is not intended or powered to detect a specific difference in GLS declines between arms, but it will be able to find a minimal detectable difference with sufficient power (Section 13.3). Results will be used in designing future definitive studies assessing the clinically

important difference in strain between patients who do and do not receive statin therapy as well as the protective effect of statin on AC-induced cardiotoxicity with long-term follow-up.

13.2 Endpoints

13.2.1 Primary Endpoint

The primary endpoint is the difference in mean absolute change in GLS from baseline until 1-3 weeks after the completion of AC chemotherapy between patients who do and do not receive concurrent simvastatin therapy.

13.2.2 Secondary Endpoints

Secondary endpoints include the difference in the relative change, measured as percent change in GLS from baseline until 1-3weeks after completion of AC chemotherapy between patients who do and do not receive concurrent simvastatin therapy.

Additionally, we will measure the differences in the absolute and relative change in velocity-based GLS rate from baseline until 1-3weeks after the completion of AC chemotherapy between arms.

Changes in GLS and GLS strain rate will also be evaluated over multiple time points from baseline to 52 weeks in both arms.

Feasibility of administering simvastatin during AC chemotherapy will be assessed by the proportion of doses which are documented as missed by patients randomized to receive simvastatin based on pill diaries.

Feasibility of using myocardial strain as a measure of cardiac toxicity in breast cancer patients receiving AC will be assessed by the proportion of patients who complete all five study-mandated echocardiograms and by the overall proportion of missing echocardiograms.

Safety and tolerability of concurrent administration of simvastatin with AC chemotherapy will be evaluated according to the NCI CTCAE version 4.

Finally, RFS will be assessed by review of medical records every six months for 5 years after randomization.

13.2.3 Exploratory Endpoints

Exploratory endpoints will include correlations between early measurements (defined as measurements taken at baseline and prior to AC#2) of GLS and GLS rate with subsequent measurements of EF and subsequent development of symptomatic heart failure, risk factors that may predict early change in GLS in addition to changes in other echocardiographic parameters (e.g.,right ventricular strain, left ventricular diastolic function and filling pressures) over time.

13.3 Sample Size and Accrual Rate

Following the intent-to-treat approach, we will include all randomized patients in the primary efficacy analysis. We plan to have 80 patients, 40 in each arm, for the primary analysis.

Previous studies showed that breast cancer patients receiving chemotherapy alone have an average of 1-5% absolute decline in strain post-therapy, which is associated with subsequent development of cardiotoxicity. Other published studies collectively suggest that the standard deviation of baseline GLS ranges from 1.5% to 3%. Based on these results, we estimated that the standard deviation of changes across patients from baseline to follow-up approximately ranged from 2% to 4%. The changes in GLS in the statin and control arms will be compared using a two-sample t-test. The table below (table 1) shows the minimal detectable difference for a range of assumed standard deviations with 80% power and a one-sided type I error of 0.05. If the actual difference between arms is larger than we expect, we will be able to detect it with greater statistical power.

To account for approximately 10% attrition, a total of 90 patients will be enrolled and randomized, 45 in each arm of the study. With an accrual goal of approximately 3 patients per month and total duration of study participation of approximately 52 weeks, the data collection for the primary endpoint should be complete in about 3.5 years; follow-up for disease recurrence will continue for at least 5 years after enrollment of each subject.

Table 1: Detectable difference assuming equal variances in a two-sample t test with 80% Power and one-sided alpha of 0.05 (n=80)

Mean Absolute Change (Pre-Post) in GLS		Standard Deviation of Change	Detectable Difference between Arms, Δ GLS (%)
Chemo Alone	Statin + Chemo		
1%	-0.11%	2.0%	1.11%
	-0.39%	2.5%	1.39%
	-0.66%	3.0%	1.66%
	-0.96%	3.5%	1.96%
	-1.21%	4.0%	2.21%
3%	1.89%	2.0%	1.11%
	1.61%	2.5%	1.39%
	1.34%	3.0%	1.66%
	1.06%	3.5%	1.96%
	0.76%	4.0%	2.24%
5%	3.89%	2.0%	1.11%
	3.61%	2.5%	1.39%
	3.34%	3.0%	1.66%
	3.04%	3.5%	1.96%
	2.79%	4.0%	2.21%

13.4 Stratification factors

None.

13.5 Analysis Plan

13.5.1 Analysis of the Primary Endpoint

For the echocardiography, digital data will be direct image output. GLS will be obtained automatically and expressed as a percentage. Absolute change in GLS from baseline to 1-3weeks after completion of AC chemotherapy will be calculated in each arm. The descriptive statistics (mean, standard deviation, median, and range) along with the 95% confidence intervals will be estimated. GLS may be transformed if necessary to achieve normality. If so, estimates of means and confidence intervals of the transformed outcome will be back-transformed so that parameter estimates and confidence intervals are interpretable. A two-sample t-test will be used to determine if the detected changes from baseline are significantly different between arms. Missing data at post-baseline, if at all, will be handled using a mixed-effects regression model that includes all randomized subjects, assuming missing completely at random. Additionally, we will perform a sensitivity analysis following the as-treated approach (also referred to as a

'complete cases' approach) including only patients that have a baseline GLS value and at least one post-AC GLS value, particularly at 1-3weeks after completion of 4 cycles of AC.

13.5.2 Analyses of the Secondary Endpoints

Percent changes in GLS from baseline to 2-3 weeks after completion of AC chemotherapy will be calculated as (baseline – follow-up)*100% / baseline. Analyses of the percent change will be performed in a similar fashion as described above.

Changes in velocity-based GLS rate from baseline to 1-3 weeks after completion of AC chemotherapy will be summarized in each arm and compared between arms as described above. Echocardiography will be performed prior to the first dose of AC (baseline), prior to the second dose of AC, 1-3 weeks after 4 cycles of AC, 24 weeks after the first dose of AC and 52 weeks after the first dose of AC. A mixed-effects model will be used to assess changes in GLS and GLS strain rate over time, which accounts for correlation among measures from the same subject and meanwhile provides a flexible structure at the presence of missing data (assuming missing at random). Within patient changes over time will be described via such model. Difference in strain parameters between arms will be evaluated with the adjustment for whether or not trastuzumab is received as a time-dependent covariate and other potential confounders.

Feasibility of administering simvastatin during AC chemotherapy will be assessed by the proportion of doses which are documented as missed by patients randomized to receive simvastatin based on pill diaries. These analyses will be descriptive only.

Feasibility of using myocardial strain as a measure of cardiac toxicity in breast cancer patients receiving AC will be assessed by the proportion of patients who complete all five study-mandated echocardiograms and by the overall proportion of missing echocardiograms. These analyses will be descriptive only.

Safety and tolerability of concurrent administration of simvastatin with AC chemotherapy will be described using summary statistics with frequencies and percentages. All subjects receiving at least one dose of the study drug(s) will be included in the safety analysis.

RFS will be calculated as the time from randomization until the time of the first documentation of breast cancer recurrence at any site (in-breast recurrence, new contralateral primary recurrence or distant metastases) or death due to breast cancer, whichever occurs first. Subjects who do not recur but die of other unrelated cause will be censored at the time of death. Subjects who remain alive with no disease recurrence will be censored at the date of their last chart review. RFS probabilities will be estimated in each arm (chemotherapy + simvastatin versus chemotherapy alone) separately using the Kaplan-Meier method. Effect of statin treatment on RFS will be explored through the use of the Cox proportional hazards model.

13.5.3 Analyses of the Exploratory Endpoints

Changes in EF over time will be described. All time points will be combined in a mixed-effects model that includes change in EF as the outcome and change in GLS (and GLS rate), time point and their interaction as covariates, while accounting for the correlation of measurements from the same patient. Mean strain parameters along with EF measurements over time will be visually co-

displayed using a scatterplot. We will also consider change in EF as a binary outcome by classifying patients into those who have a clinically significant drop in EF at 1-year follow-up, i.e., an absolute decrease of ≥ 10% from baseline to < 50%, and those who do not. We will utilize a logistic regression with generalized estimating equations (GEE) method to assess the independent effect of early change in strain in predicting EF decline and simultaneously account for important sources of variation including baseline measurement, age, stage, cardiac risk factors, receipt of hormonal therapy, receipt of radiation, type of surgery, schedule of AC (q 2 week vs. q 3 week), receipt of other medications which may impact cardiac function (such as ACE inhibitors, ARBs or beta blockers), receipt of taxanes, receipt of HER2 targeted therapy, laterality of cancer and other potentially relevant breast cancer factors. We will also examine whether treatment arm modifies the association between change in strain parameters and EF decline (i.e., interaction). Association of early change in strain with subsequent heart failure within 1-year follow-up will be explored in a similar fashion. Heart failure will be classified as symptomatic or asymptomatic, where symptomatic heart failure will be considered to be grade 2 or higher according to the NCI CTCAE v. 4.0 and/or NYHA functional classification class II or higher. Considering only less than a handful of patients will experience a clinically significant drop in EF or symptomatic heart failure within the study follow-up, these analysis will be largely descriptive and hypothesis generating in nature; however, they will provide valuable data for future studies.

We will use a linear regression model to explore risk factors collected through questionnaires (including but not limited to hypertension, diabetes, obesity, and smoking history) that may predict early change in GLS.

We will describe additional echocardiographic strain parameters besides GLS and GLS rate, including right ventricular strain, in addition to routine assessment of left ventricular diastolic function and filling pressures, at multiple time points from baseline to 52 weeks in this patient population treated with (neo)adjuvant anthracycline based chemotherapy with and without concurrent simvastatin therapy.

13.6 Early Stopping

13.6.1 Early Stopping Guidelines for Safety

The most likely toxicity associated with simvastatin that we expect to encounter in this study is hepatotoxicity, although only approximately 1% of patients on statin drugs experience persistent transaminitis. We will monitor transaminases regularly while patients are on chemotherapy and statin therapy in this trial. In order to avoid potentially concerning hepatotoxicity related to our intervention, we plan to suspend enrollment for a safety assessment if we are 65% certain that the risk of ≥ grade 3 elevation in AST and/or ALT exceeds 5%. The prior for the stopping rule is beta (0.1, 9.9), representing our prior guess at the risk of liver function toxicities is 1% on average and there is 90% certainty that this proportion is between 0.006% and 3.8%. The following tables (table 2 and table 3) show the stopping rules and associated operating characteristics based on 5000 simulations.

Table 2: Early Stopping Rules for Safety

Suspend enrollment if	2	3	4
Out of total number of patients	2-17	18-34	35-45

Table 3: Operating Characteristics

True risk of AE	Probability declare treatment unsafe	Average sample size
0.01	1.5%	44.6
0.05	34.4%	35.9
0.10	78.2%	23.4
0.15	95.6%	14.8

13.6.2 Early Stopping Guidelines for Futility

Preliminary data are not yet available to estimate the effect that adding statin therapy to AC chemotherapy will have on change in myocardial strain over time in this patient population, neither to what extent the protective effect, if any, will be associated with a clinical benefit. As a result, we do not plan to stop early based on lacking statistically significant difference in GLS decline between arms, but rather will consider halting enrollment for re-evaluation if we are 90% certain that more than 30% of patients in the statin arm experience >5% absolute decline in GLS for the first two consecutive visits. This interim look will not occur until 15 patients have entered the statin arm and received treatment, and thereafter every 10 patients. The prior distribution for the decision rule is a beta (0.1, 9.9), representing our prior guess that it is almost unlikely we will see patients receiving statin therapy having experienced >5% absolute decline in GLS for two consecutive visits. Enrollment will be suspended if this occurs in 11 out of 15, 15 out of 25, 18 out of 35, and 21 out of 45 patients. Given this stopping rule, we will have 45% chance to stop if the corresponding true risk is 50% and 87% chance to stop the study if the true risk reaches 60%.

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APPENDICES

A American Joint Committee on Cancer Staging
B Performance Status Criteria
C Study Drug Diary
D NYHA Functional Classification
E Participant Questionnaire

APPENDIX A: American Joint Committee on Cancer Staging

T – Primary Tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	Ductal carcinoma in situ
Tis (LCIS)	Lobular carcinoma in situ
Tis (Paget)	Paget's disease of the nipple with no tumor Note: Paget's disease associated with a tumor is classified according to the size of the tumor.
T1	Tumor ≤ 2 cm in greatest dimension
T1mic	Microinvasion ≤ 0.1 cm in greatest dimension
T1a	Tumor > 0.1 cm but not > 0.5 cm in greatest dimension
T1b	Tumor > 0.5 cm but not > 1 cm in greatest dimension
T1c	Tumor > 1 cm but not > 2 cm in greatest dimension
T2	Tumor > 2 cm but not > 5 cm in greatest dimension
T3	Tumor > 5 cm in greatest dimension
T4	Tumor of any size with direct extension to (a) chest wall or (b) skin, only as described below
T4a	Extension to chest wall, not including pectoralis muscle
T4b	Edema (including peau d'orange" or ulceration of the skin of the breast, or satellite skin nodules confined to the same breast
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma

N – Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed (e.g., previously removed)
N0	No regional lymph node metastasis
N1	Metastasis in movable ipsilateral axillary lymph node(s)
N2	Metastases in ipsilateral axillary lymph nodes fixed or matted, or in clinically apparent ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastasis
N2a	Metastasis in ipsilateral axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastasis only in clinically apparent ipsilateral internal mammary nodes and in the absence of clinically evident axillary lymph node metastasis
N3	Metastasis in ipsilateral infraclavicular lymph node(s), or in clinically apparent ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
N3a	Metastasis in ipsilateral infraclavicular lymph node(s) and axillary lymph node(s)
N3b	Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastasis in ipsilateral supraclavicular lymph node(s)

APPENDIX A (CONTINUED): American Joint Committee on Cancer Staging

PN – Regional lymph nodes	
pNX	Regional lymph nodes cannot be assessed (e.g., previously removed or not removed for pathologic study)
pN0	No regional lymph node metastasis histologically, no additional examination for isolated tumor cells
pN0(i-)	No regional lymph node metastasis histologically, negative IHC
pN0(i+)	No regional lymph node metastasis histologically, positive IHC, no IHC cluster > 0.2 mm
pN0(mol-)	No regional lymph node metastasis histologically, negative molecular findings (RT-PCR)
pN0(mol+)	No regional lymph node metastasis histologically, positive molecular findings (RT-PCR)
pN1mi	Micrometastasis (> 0.2 mm, none > 2.0 mm)
pN1	Metastasis in one to three axillary lymph nodes and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN1a	Metastasis in one to three axillary lymph nodes
pN1b	Metastasis in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN1c	Metastasis in one to three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN2	Metastasis in four to nine axillary lymph nodes, or in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis
pN2a	Metastasis in four to nine axillary lymph nodes (at least one tumor deposit > 2.0 mm)
pN2b	Metastasis in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis
pN3	Metastasis in 10 or more axillary lymph nodes, or in infraclavicular lymph nodes, or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes
pN3a	Metastasis in 10 or more axillary lymph nodes (at least one tumor deposit > 2.0 mm), or metastasis to the infraclavicular lymph nodes
pN3b	Metastasis in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN3c	Metastasis in ipsilateral supraclavicular lymph nodes

M – Distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

APPENDIX A (CONTINUED): American Joint Committee on Cancer Staging

Stage Grouping	**T**	**N**	**M**
0	Tis	N0	M0
I	T1	N0	M0
IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

APPENDIX B: Performance Status Criteria

Score	Definition	Karnofsky Equivalent
0	Asymptomatic	100
1	Symptomatic, fully ambulatory	80 – 90
2	Symptomatic, in bed less than 50% of day	60 – 70
3	Symptomatic, in bed more than 50% of day, but not bedridden	40 – 50
4	Bedridden	20 – 30

APPENDIX C: Study Drug Diary

Subject: _____

Study Drug Diary – Simvastatin
Please complete this diary by signing your initials for each day that you take your simvastatin. Two pills of 20 mg simvastatin should be taken by mouth. Please take simvastatin at approximately the same time each evening. Missed doses will not be made up.

Please be sure to bring this diary with you to your next doctor's visit.

Month/Year: _____ Month/Year: _____

Sun	Mon	Tues	Wed	Thurs	Fri	Sat		Sun	Mon	Tues	Wed	Thurs	Fri	Sat

Month/Year: _____ Month/Year: _____

Sun	Mon	Tues	Wed	Thurs	Fri	Sat		Sun	Mon	Tues	Wed	Thurs	Fri	Sat

Completed by: _____ Date: _____
Initials of Participant

Reviewed by: _____ Date: _____
Initials of Study Staff

APPENDIX D: NYHA Functional Classification

Class	Description
Class I	Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations, dyspnea or anginal pain.
Class II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
Class III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea or anginal pain.
Class IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.

APPENDIX E: Participant Questionnaire

Detection and Prevention of Anthracycline-Related Cardiac Toxicity with Concurrent Simvastatin

Baseline Subject Questionnaire

Subject Study #: _____

Date: _____

Menstrual History

1. How old were you when you had your first menstrual period? _____
2. Have you had a menstrual period in the past 12 months?
   - O No – What was your age or the month and year of your last menstrual period?
     Age: _____ or _____/_____ (Month / Year)
   - O Yes – What was the start date of your last 3 menstrual periods?
     1) _____/_____/_____ (Month Day Year) 2) _____/_____/_____ (Month Day Year) 3) _____/_____/_____ (Month Day Year)
3. Have you had any of the following gynecological surgeries?
   - O Removal of ovaries - O One or O Both Age: _____
   - O Hysterectomy (removal of uterus) Age: _____
   - O None

Medical History

1. Have you ever been diagnosed with any of the following medical conditions (check all that apply)?
   - O Heart failure
   - O Heart attack or angina
   - O Irregular heart beat
   - O Problems with a heart valve
   - O Other heart problems
   - O High blood pressure
   - O Kidney failure
   - O Thyroid disease
   - O Diabetes
   - O Stroke or transient ischemic attack
   - O Connective tissue disease (such as Lupus, Rheumatoid arthritis)
   - O Liver disease
   - O Blood clot(s)
   - O High cholesterol
   - O Other medical problems:_____
   - O I have never had any of the above conditions or others.

2. If you have been diagnosed with high blood pressure, have you ever been prescribed medicines to lower your blood pressure (select one answer)?

   O I have not been diagnosed with high blood pressure
   O I have been diagnosed with high blood pressure but have never been prescribed medicines to lower my blood pressure
   O I have been diagnosed with high blood pressure and have been prescribed medicines to lower my blood pressure

Family History of Cardiovascular Disease

Please describe your family history of cardiovascular disease (check all that apply).

Family member: Mother

Cardiovascular problems	Status			Age at diagnosis
Heart attack or angina	O Yes	O No	O Unknown	____
High blood pressure	O Yes	O No	O Unknown	____
Heart failure	O Yes	O No	O Unknown	____
Irregular heart beat	O Yes	O No	O Unknown	____
Problem with heart valve	O Yes	O No	O Unknown	____
Stroke	O Yes	O No	O Unknown	____
Diabetes	O Yes	O No	O Unknown	____
High cholesterol	O Yes	O No	O Unknown	____
Other cardiovascular problem	O Yes	O No	O Unknown	____

Family member: Father

Cardiovascular problems	Status			Age at diagnosis
Heart attack or angina	O Yes	O No	O Unknown	____
High blood pressure	O Yes	O No	O Unknown	____
Heart failure	O Yes	O No	O Unknown	____
Irregular heart beat	O Yes	O No	O Unknown	____
Problem with heart valve	O Yes	O No	O Unknown	____
Stroke	O Yes	O No	O Unknown	____
Diabetes	O Yes	O No	O Unknown	____
High cholesterol	O Yes	O No	O Unknown	____
Other cardiovascular problem	O Yes	O No	O Unknown	____

Family History of Cardiovascular Disease (continued)

Family member: Sister(s) O I do not have any sisters

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Family member: Brother(s) O I do not have any brothers

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Family member: Daughter(s) O I do not have any daughters

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Family History of Cardiovascular Disease (continued)

Family member: Sons(s) O I do not have any sons

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Family member: Grandmother (mother's side)

Cardiovascular problems	Status			Age at diagnosis
Heart attack or angina	O Yes	O No	O Unknown	____
High blood pressure	O Yes	O No	O Unknown	____
Heart failure	O Yes	O No	O Unknown	____
Irregular heart beat	O Yes	O No	O Unknown	____
Problem with heart valve	O Yes	O No	O Unknown	____
Stroke	O Yes	O No	O Unknown	____
Diabetes	O Yes	O No	O Unknown	____
High cholesterol	O Yes	O No	O Unknown	____
Other cardiovascular problem	O Yes	O No	O Unknown	____

Family member: Grandmother (father's side)

Cardiovascular problems	Status			Age at diagnosis
Heart attack or angina	O Yes	O No	O Unknown	____
High blood pressure	O Yes	O No	O Unknown	____
Heart failure	O Yes	O No	O Unknown	____
Irregular heart beat	O Yes	O No	O Unknown	____
Problem with heart valve	O Yes	O No	O Unknown	____
Stroke	O Yes	O No	O Unknown	____
Diabetes	O Yes	O No	O Unknown	____
High cholesterol	O Yes	O No	O Unknown	____
Other cardiovascular problem	O Yes	O No	O Unknown	____

Family History of Cardiovascular Disease (continued)

Family member: Grandfather (mother's side)

Cardiovascular problems	Status			Age at diagnosis
Heart attack or angina	O Yes	O No	O Unknown	____
High blood pressure	O Yes	O No	O Unknown	____
Heart failure	O Yes	O No	O Unknown	____
Irregular heart beat	O Yes	O No	O Unknown	____
Problem with heart valve	O Yes	O No	O Unknown	____
Stroke	O Yes	O No	O Unknown	____
Diabetes	O Yes	O No	O Unknown	____
High cholesterol	O Yes	O No	O Unknown	____
Other cardiovascular problem	O Yes	O No	O Unknown	____

Family member: Grandfather (father's side)

Cardiovascular problems	Status			Age at diagnosis
Heart attack or angina	O Yes	O No	O Unknown	____
High blood pressure	O Yes	O No	O Unknown	____
Heart failure	O Yes	O No	O Unknown	____
Irregular heart beat	O Yes	O No	O Unknown	____
Problem with heart valve	O Yes	O No	O Unknown	____
Stroke	O Yes	O No	O Unknown	____
Diabetes	O Yes	O No	O Unknown	____
High cholesterol	O Yes	O No	O Unknown	____
Other cardiovascular problem	O Yes	O No	O Unknown	____

Family member: Aunt(s) (mother's side) O I do not have any maternal aunts

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Family History of Cardiovascular Disease (continued)

Family member: Aunt(s) (father's side) O I do not have any paternal aunts

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Family member: Uncle(s) (mother's side) O I do not have any maternal uncles

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Family member: Uncle(s) (father's side) O I do not have any paternal uncles

Cardiovascular problems	Status			Age at diagnosis	*If yes, how many affected?*
Heart attack or angina	O Yes	O No	O Unknown	____	____
High blood pressure	O Yes	O No	O Unknown	____	____
Heart failure	O Yes	O No	O Unknown	____	____
Irregular heart beat	O Yes	O No	O Unknown	____	____
Problem with heart valve	O Yes	O No	O Unknown	____	____
Stroke	O Yes	O No	O Unknown	____	____
Diabetes	O Yes	O No	O Unknown	____	____
High cholesterol	O Yes	O No	O Unknown	____	____
Other cardiovascular problem	O Yes	O No	O Unknown	____	____

Smoking, Drugs and Alcohol History

1. Have you ever smoked?
   - O No - Skip to # 4.
   - O Yes - Age when you first smoked: _____
2. Do you currently smoke?
   - O No - Age when you last smoked: _____
   - O Yes
3. If you are a current or former smoker, indicate for each age about how many cigarettes you smoked per day?

Age	Less than 1 per day or experimented only	1 – 4	5 – 14	15 – 24	25 – 34	35 – 44	45+
Age <15	O	O	O	O	O	O	O
Age 15-19	O	O	O	O	O	O	O
Age 20-29	O	O	O	O	O	O	O
Age 30-39	O	O	O	O	O	O	O
Age 40-49	O	O	O	O	O	O	O
Age 50-59	O	O	O	O	O	O	O
Age 60-69	O	O	O	O	O	O	O
Age 70-79	O	O	O	O	O	O	O
Age 80+	O	O	O	O	O	O	O

4. Have you lived or worked in a place, or been in other places, where people around you smoked?

	No	Yes	*If yes…* for about how many years?	*If yes…* are you currently in this place?
Home	O	O	_____	No O Yes O
Work	O	O	_____	No O Yes O
Other places	O	O	_____	No O Yes O

If you answered "yes" to other places above, please specify the other place(s) below:

_____

_____

5. During the past year how many alcoholic beverages did you have?

Beverage	Never or less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4-5 per day
Beer *(1 glass, bottle, can)*	O	O	O	O	O	O	O	O
Red wine *(4 oz. glass)*	O	O	O	O	O	O	O	O
White wine *(4 oz. glass)*	O	O	O	O	O	O	O	O
Liquor *(1shot)*	O	O	O	O	O	O	O	O
Other	O	O	O	O	O	O	O	O

Physical Activity

1. How many hours **per week** do you spend doing physical activity (walking, running, lap swimming, bicycling, and other sports)?

   _____ hours

2. How many hours **per week** do you spend sitting at work or at home using a computer, watching TV/VCR/DVD, or reading?

   _____ hours