SPONSOR: The University of Texas MD Anderson Cancer Center

TITLE: Atorvastatin in triple-negative breast cancer (TNBC) patients who did not achieve a pathologic complete response (pCR) after receiving neoadjuvant chemotherapy; a multicenter pilot study

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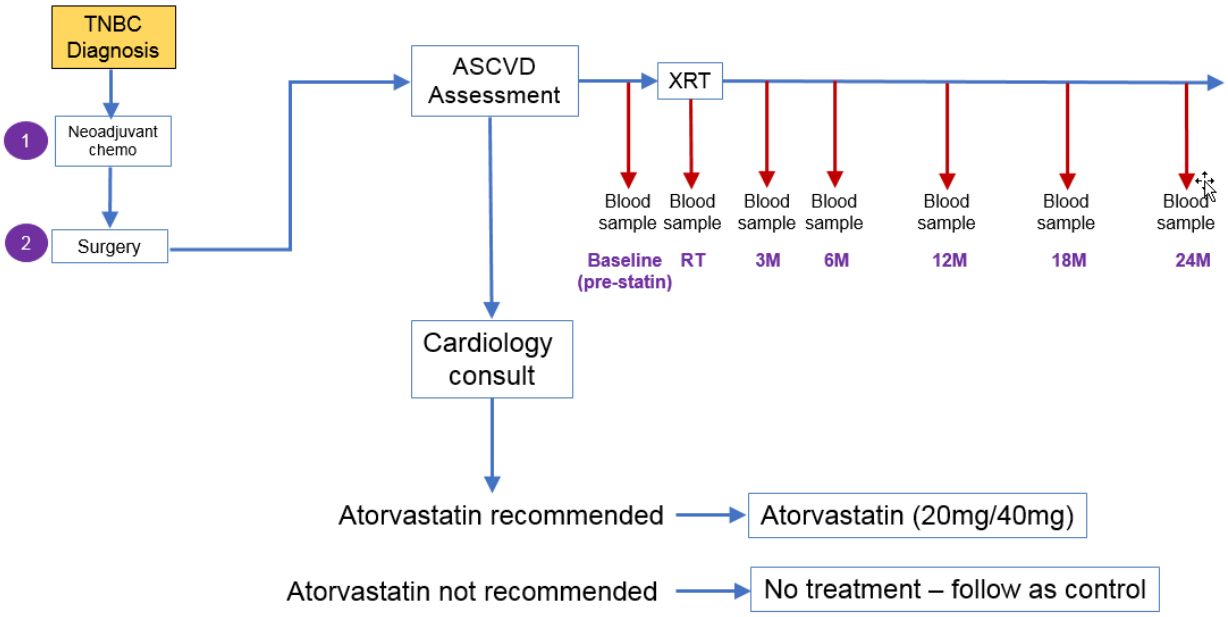
1.0 TRIAL SUMMARY

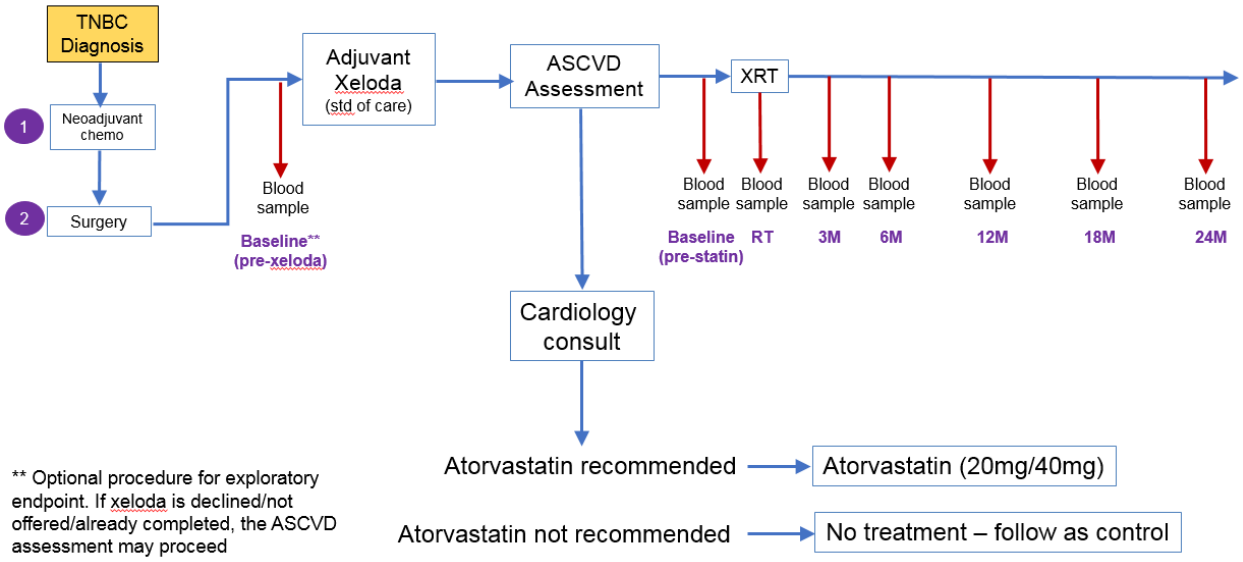
Abbreviated Title	Atorvastatin as adjuvant therapy in TNBC patients which did not achieve pCR
Trial Phase	Pilot Study
Clinical Indication	Role of Atorvastatin in TNBC in the adjuvant setting
Trial Type	Open labeled, non- randomized, multicenter
Type of control	TNBC patients who were not recommended to receive Atorvastatin by ASCVD
Route of administration	Oral
Trial Blinding	No
Treatment Groups	Two
Number of trial subjects	80
Estimated duration of trial	3 years
Duration of Participation	2 years

1.1 Study Schemas:

Cohort 1 – If patient will receive XRT and xeloda is not planned

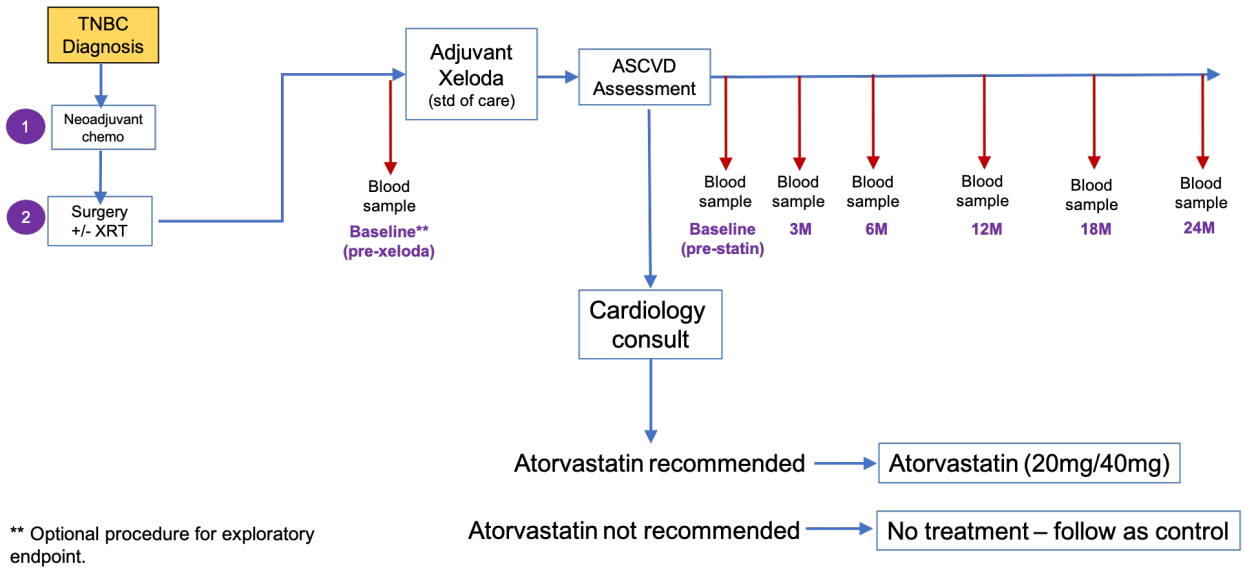


Cohort 2 – If patient will receive xeloda prior to XRT



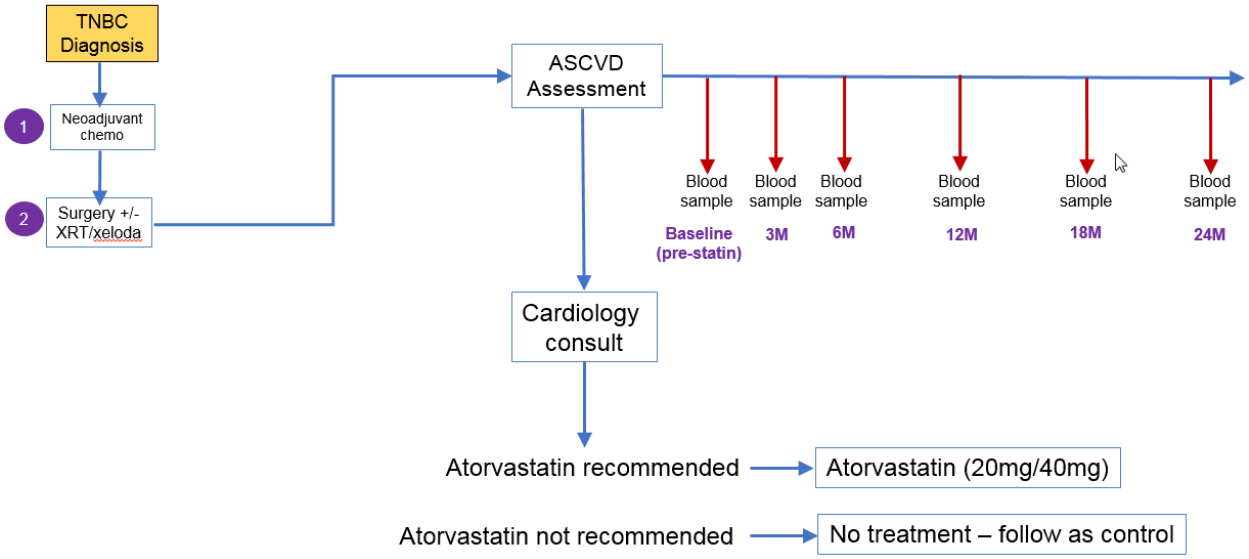
** Optional procedure for exploratory endpoint. If xeloda is declined/not offered/already completed, the ASCVD assessment may proceed

Cohort 3 – If patient will receive xeloda after XRT or XRT is not planned



** Optional procedure for exploratory endpoint.

Cohort 4 – If patient will not receive XRT and/or xeloda (or has already completed these prior)



2.0 OBJECTIVE(S) & HYPOTHESIS (ES)

Central Hypothesis:

Adjuvant statin therapy is effective in reducing the risk of recurrence in TNBC patients who did not achieve a pCR to neoadjuvant chemotherapy.

Primary Objective:

- To determine the proportion of patients with undetectable circulating tumor cells (CTCs) at 6 months in patients with stage IIB/III TNBC who did not achieve a pCR or Residual Cancer Burden-I (RCB-I) after receiving neoadjuvant chemotherapy (NAC) with and without atorvastatin therapy.

Secondary Objectives:

- To determine if baseline fasting lipid profile level (LDL-C) and/or change in serum lipid levels are a predictive biomarker of change in the proportion of patients with CTCs.

- To assess effect of biomarkers on atorvastatin treatment response, defined as CTCs, circulating tumor DNA (ctDNA), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum Interleukin-6 (IL-6) and other inflammatory cytokines, for the purpose of identifying the optimal patient population for future larger scale adjuvant studies.

- To determine if baseline fasting lipid profile level (LDL-C) and/or change in serum lipid levels are associated with 2-year relapse free survival (RFS) rate.

- To determine if baseline CRP and/or change in serum lipid levels are a predictive biomarker of change in the proportion of patients with CTCs.

- To determine if baseline C-reactive protein (CRP) and/or change in CRP are associated with 2-year RFS rate.

- To determine if baseline absolute number of CTCs and/or CTC change are associated with 2-year RFS rate.

- To estimate the 2-year RFS rate of patients with TNBC who did not achieve pCR with and without atorvastatin therapy.

- To describe the toxicity and adverse events profile of atorvastatin treatment when given concurrently with standard doses of radiotherapy to the chest wall and regional nodes.

Exploratory Objectives

- To evaluate the correlation between multiplexed imaging biomarkers in the normal or tumor tissue taken at the time of surgery, and response to atorvastatin-induced CTC changes or with measured outcomes.

- To evaluate whether adjuvant xeloda treatment changes CTC counts and other inflammatory biomarkers.

3.0 BACKGROUND & RATIONALE

3.1 Need for Adjuvant Therapies for Patients with TNBC

TNBC is a subtype of breast cancer that affects approximately 15% of breast cancer patients and was, until recently, defined as having estrogen receptor (ER) and progesterone receptor (PR) <10% positivity by IHC, and Her2 normal, which is 0 or 1+ by IHC, or HER2 2+ by IHC and negative by FISH or HER2 negative by FISH if IHC is not performed. More recently, the hormone receptor expression cutoff was changed to <1% to qualify as TNBC, although the clinical relevance of this has been questioned and patients with 1-9% ER and PR positivity rates are still often managed similar to patients with ER and PR <1%. While we have adjuvant treatments for hormone receptor positive breast cancer with endocrine therapy that have resulted in significant survival improvements, there are currently no approved adjuvant treatments for patients with TNBC. TNBC is the most aggressive subtype of breast cancer, and these worse outcomes are most pronounced among patients who did not achieve a pCR after neoadjuvant chemotherapy, as shown in Figure 1 [1, 2]. Efficacious adjuvant treatments with acceptable toxicity profiles are therefore needed for these breast cancer patients who are at highest risk of relapse. One of those efforts recently reported was treatment with adjuvant capecitabine [3]. This study reported that disease-free survival was longer in the group treated with capecitabine than the group without treatment (HR 0.7; 95%CI 0.53-0.92). Among the subgroup of patients with TNBC, disease-free survival remained significantly better in capecitabine group (HR 0.58 p5%CI 0.39-0.87).

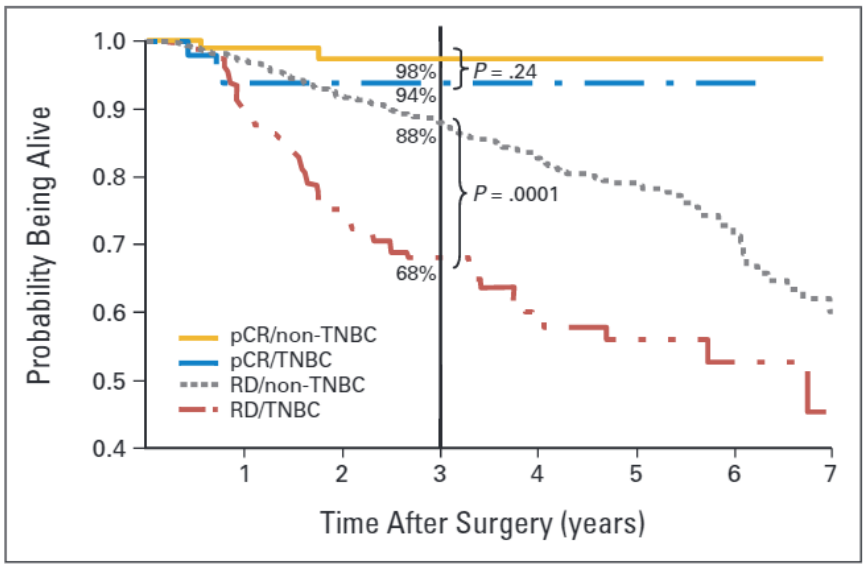


Figure 1 [1]

3.1.1 Statins as Possible Adjuvant Therapy for Breast Cancer based on Retrospective Datasets of Statins in Breast Cancer

Statins, 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are cholesterol-lowering agents that have been long established as agents that significantly reduce

cardiovascular events and overall mortality. They are more recently being recognized to have tumor protective effects, which in combination with their favorable toxicity profile makes statins a potential candidate for adjuvant therapy of patients with TNBC. Statin use after diagnosis has been shown to be associated with improved breast cancer relapse-free survival and lower breast cancer mortality in large nationwide and single-institution cohorts [4-9]. Figure 2 summarizes findings of several of these studies [10].

The initial report of the protective effect of statin use came from a cohort study of 1945 early stage breast cancer survivors participating in the Life After Cancer Epidemiology (LACE) study [6]. Pharmacy records showing post-diagnosis statin use in the Kaiser Permanente health plan that these patients were enrolled in showed a trend towards decreased risk of recurrence HR 0.67 (0.39-1.13) if > 100 days statin use was recorded, and HR 0.38 (0.12-1.19) if > 2 years of statin use was recorded, with average exposure to statin use of 1.96 years and average follow-up of 7 years from diagnosis. Lovastatin was the primary statin used in 84.4% of patients in this study, followed by simvastatin in 10.9% of patients. Another United States (US) study by Chae et al retrospectively assessed statin use in 703 patients with stage II or III breast cancer diagnosed between 1999 and 2005 [5]. Use of any statin for greater than 6 months was associated with lower recurrence rate (adjusted HR 0.48-0.82), with prescriptions for atorvastatin and simvastatin accounting for 60% and of 17%, respectively. The largest US study of 4,216 patients with stage I or II breast cancer enrolled in the Group Health health-care delivery found that use of lipophilic statins (atorvastatin, simvastatin, lovastatin, fluvastatin, and cerivastatin) was associated with a trend towards decreased recurrence, HR 0.76 (0.54-1.05) [7]. The largest and strongest evidence of benefit of statin use comes from a nationwide Danish prospective cohort study that included 18,769 patients diagnosed with stage I-III breast cancer between 1996 and 2003 [4]; in this study, simvastatin use was associated with decreased breast cancer relapse at 10 years after diagnosis (adjusted HR 0.70, 95% CI 0.57-0.86, and absolute relapse reduction of 10%).

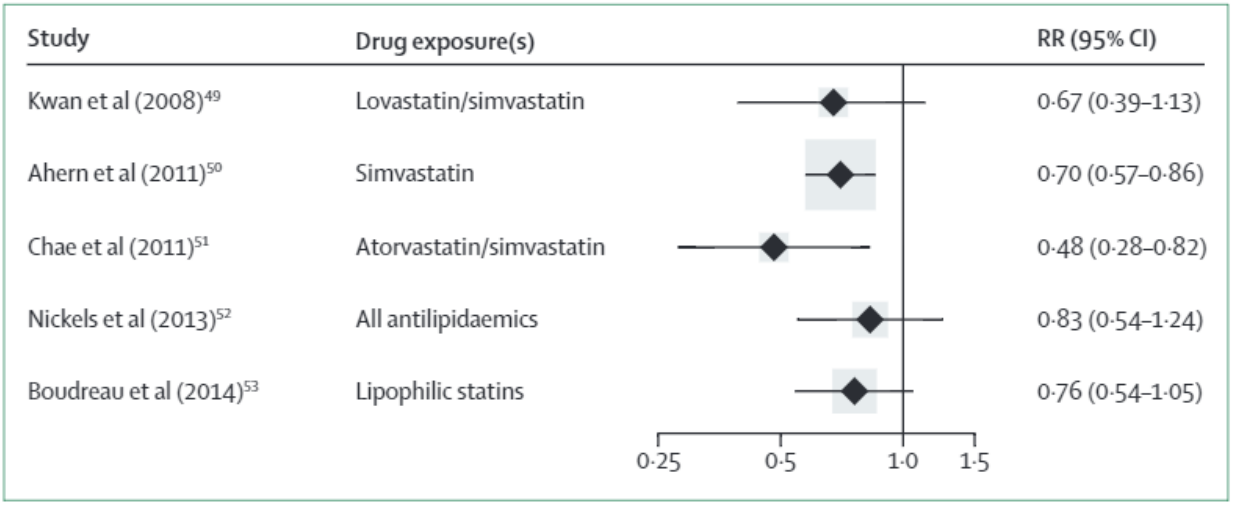


Figure 2. Association between statin use and the risk of breast cancer recurrence [10]

Our group provided additional support for this protective effect in 723 primary inflammatory breast cancer (IBC) patients, in whom we showed that use of atorvastatin at the time of initial evaluation was significantly associated with prolonged relapse-free survival (HR 0.48, 95% CI 0.28-0.84) [8]. Of note, simvastatin use was not found to be significantly associated with improved outcomes in this study but the limited patient number (total of 73 patients were on statin therapy) may have limited the power of this analysis. In addition to its association with overall decreased relapse rates, statin use and simvastatin use in particularly has been studied as a radiosensitizing agent. Our group retrospectively studied this in 519 stage III inflammatory breast cancer patients who had adjuvant radiation treatment and found that statin use was associated with decreased locoregional relapse (adjusted HR 0.40, 95% CI 0.16-1.00) [11], with 2 year and 5 year local control rates of 76% and 69%, respectively in the no-statin group compared to 92% and 85%, respectively, in the statin group. We further showed that in this cohort locoregional control was strongly related to HDL plasma level (discussed in more detail below, Wolfe, IJROBP 2015) and that the expression of the HDL cholesterol transporter ABCA1 dictates radio-responsiveness to HDL in vitro (Wolfe et al, IJROBP, in press). These retrospective findings justify our central hypothesis (Section 2.0) and support our proposal of this clinical trial. These studies additionally provide strong hypothesis based leads for the biomarkers studies proposed below.

3.1.2 Preclinical and Clinical Trial Data of statins in breast cancer

3.1.2.1 Preclinical Data

The scientific rationale for studying statins, inhibitors of HMG CoA-reductase, in patients with TNBC is supported by preclinical in vitro and in vivo data demonstrating the efficacy of these agents in preventing breast cancer growth and progression, respectively. Our group has shown in in vitro viability assays that therapeutic levels of simvastatin decrease proliferation of TNBC (SUM 159, MDA-231, SUM 149) cell lines [12] via cell cycle arrest in G1 phase, a process that is reversed by adding mevalonate, a cholesterol pathway component downstream to the effect of statin. These effects on proliferation are seen after just 24 hours of pre-treating with simvastatin and appear between 48 hours and 8 days after treatment. Simvastatin caused impaired migration of these TNBC cell lines, as tested by the Boyden chamber migration assay, and the migratory ability of simvastatin treated cells was rescued again by addition of mevalonate. Effect of simvastatin on TNBC cancer stem cells was also assessed, since these cells have been hypothesized to be metastasis-initiating cells. First, the mammosphere formation assay, which correlates to cancer stem cell self-renewal capacity was used and showed that primary sphere formation was reduced 75-78% in the TNBC cell lines tested after simvastatin pre-treatment. Additionally the spheres that were formed were smaller compared to control DMSO treated cells. Self-renewal properties were restored with mevalonate addition. Mouse models using orthotopic injection of green fluorescent protein–labeled SUM 149 cells showed a decreased rate in primary tumor formation 20 weeks after injection of cells, from 95% with cells treated with DMSO solution to 67% with cells treated with therapeutic concentration of simvastatin (p=0.04). There was also a decreased rate of metastasis, from 86% with DMSO solution treated cells to 22% with simvastatin treatment (p=0.04). To more closely simulate a clinically relevant environment, mice were injected with TNBC cell lines in the tail and then after 1 week, half the mice were given drinking water containing simvastatin while the other half received water alone. Corroborating the other in vivo

experiments, metastasis rate decreased from 70% in control to 20% in simvastatin treated mice (p=0.06). Of note, we also showed that simvastatin enhances sensitivity of TNBC and inflammatory breast cancer cell lines to radiation treatment.

Several mechanisms have been shown to mediate statin induced decrease in proliferation and metastatic potential of breast cancer cells. Several groups have shown that simvastatin causes increased expression of the tumor suppressor PTEN and subsequent downregulation of PI3K pathway components such as phosphorylated Akt [12-15]. In support of this the Akt inhibitor LY294002 has been shown to augment the effect of simvastatin in TNBC cell lines [13]. Our group performed proteomic analyses of signaling pathways in the study detailed above and showed significant decreases in levels of FOXO3a (pS318) and Akt (pS473) with simvastatin treatment. FOXO3a is a tumor suppressor protein that is targeted for degradation by phosphorylation by Akt, and was found to be a downstream mediator of the effects of simvastatin. Simvastatin was found to decrease phosphorylation of FOX03a and thereby prevent degradation and allow for tumor suppressive ability of FOXO3a [12]. Further investigation with knockdown and overexpression studies confirmed that FOXO3a, a tumor suppressor transcription factor, mediates simvastatin's antitumor effects and that the decreased proliferation and migratory ability induced by simvastatin is negated by knocking out FOXO3a expression. The relevance of FOXO3a expression to breast cancer metastases and importance as a possible target with statin treatment was evaluated with analysis of 1479 breast cancer patients in 8 publicly available datasets, and showed that decreased FOXO3a correlates to decreased metastasis free survival in patients with TNBC.

Other suggested intracellular mechanisms include inhibition of NF-kB transcription factor [15-18] and promotion of apoptosis. NF-kB proteins are a family of transcription factors that are involved in control of many normal cellular processes, such as immune and inflammatory responses, development, cellular growth, and apoptosis. The NK-kB system is dysregulated in a number of disease states such as cancer. Simvastatin was shown to inhibit the DNA binding and transcriptional activities of NF-kB [15] with resultant downstream effect on PTEN (increased transcription) as well as anti-apoptosis protein BCL-XL (decreased transcription) [15, 17-19]. In addition to suppression of anti-apoptotic protein BCL-XL, simvastatin has been shown to upregulate pro-apoptotic c-Jun N-terminal kinase (JNK), death receptor-5, and CCAAT/enhancer binding protein homologous protein pathway, leading to simvastatin induced apoptosis. Both NF-kB and apoptotic processes were shown to be dependent on HMG-CoA reductase activity as the effects of simvastatin were reversed with addition of mevalonate [16, 19].

The mevalonate and cholesterol pathways appears to mediate many of the anti-tumor effects of simvastatin as many of the above processes are negated with addition of mevalonate. Statins are inhibitors of HMG CoA reductase and this enzyme is the rate limiting step of cholesterol precursors such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) [20]. These 2 proteins are responsible for translocation of Ras and Rho, respectively, to the cell membrane. Ras is a GTPase that is an important component of intracellular signaling and is frequently dysregulated in human cancers. Delocalization of RhoA from the cell membranes results in disorganization of actin fibers and disappearance of focal adhesion sites, which is reversed after statin treatment with adding GGPP back to cell media. The mevalonate pathway also provides metabolites for post-translational modifications that have been found to stimulate

histone deacetylases and DNA methyltransferase that affect cancer signaling pathways through epigenetic changes [21]. Genome wide RNAi analysis showed that inhibition of multiple components of the mevalonate pathway potentiates statin induced apoptosis [22].

Systemic cholesterol reduction has also been suggested to be an important antitumor-mediating mechanism [23] of statin treatment. Our group showed that VLDL increases and HDL decreases mammosphere formation in IBC cell lines SUM 149 (TNBC) and KPL4. Retrospective analysis of IBC cohorts supported this effect by showing that higher VLDL values >30 mg/dL and low HDL values < 60 mg/dL predicted for lower 5-year overall survival. For hormone receptor positive breast cancer, it was shown that 27-hydroxycholesterol is a primary metabolite of cholesterol as well as an ER and liver X receptor ligand and was found to cause ER-dependent growth and LXR-dependent metastasis in mouse models of breast cancer.

Statins have been well established to have anti-inflammatory properties and decrease high sensitivity CRP (shCRP) and ESR in cardiac patients. Statin immunomodulatory roles are only beginning to be understood, however, particularly in the cancer setting where the importance of immune surveillance and immunotherapeutics are now fully being realized. Suppression of TNF-a induced IL-6 secretion has been demonstrated in MCF-7 breast cancer cell lines after treatment with pitavastatin [16]. Our group has also shown that simvastatin suppresses recruitment of macrophages to tumor via IL-6 inhibition (data not published). Statin effect on tumor associated macrophages (TAM) was also seen in a melanoma mouse model in which a long acting liposomal encapsulated formulation of simvastatin mediated potent antitumor activity through a reduction in TAM induced oxidative stress and production of hypoxia-inducible factor 1 α (HIF-1 α) in tumors (Alupei MC). Additionally, in healthy individuals as well as acute coronary syndrome patients, statins have been shown to increase frequency of CD4+FOXP3+ regulatory T cells [24, 25].

Based on these findings, we hypothesize that atorvastatin has antitumor effects and improve recurrence free survival in patients with TNBC who did not achieve a pCR after preoperative chemotherapy.

3.1.2.2 Clinical Data

Several pre-surgical window of opportunity trials have been completed or are underway evaluating the efficacy of neoadjuvant statin treatment in breast cancer patients. Bjarnadottir et al showed in 50 patients with primary invasive breast cancer that treatment with 2 weeks of atorvastatin 80 mg before surgery led to a statistically significant decrease in Ki-67 by 7.6% and increase in HMG-CoA reductase [26]. This group then performed another phase II trial to explore mechanisms of reduced Ki-67 expression in 42 patients with primary invasive breast cancer and again did a 2 week window of opportunity study with atorvastatin 80 mg for 2 prior to surgery [27]. They found that there was significant decrease in cyclin D1 and increase in tumor suppressive protein p27. A similarly designed phase 2 trial with fluvastatin in stage 0/1 breast cancer showed a decrease in Ki-67 and increase in markers of apoptosis with cleaved caspase-3 in high grade patients only [28]. Additional pre-surgical studies are underway to assess effect of simvastatin on proliferation and apoptosis in basal subtype of primary breast cancer (NCT00807950), effect of rosuvastatin on proliferation in hormone receptor positive breast cancer (NCT02483871), assessment of

mechanisms of action of atorvastatin in reducing Ki-67 (NCT02416427), and efficacy of combination of statin treatment with metformin (NCT01980823) again assessed by change in Ki-67. No unexpected toxicities aside from what is known from treatment of cardiovascular patients have been reported in these studies.

In addition to studies in primary breast cancer, additional studies have evaluated statin therapy in patients at high risk of developing breast cancer. Patients with BRCA 1, BRCA 2, CDH1, or TP53, personal history of breast cancer, or lifetime risk >20% by Claus model were treated with 40 mg lovastatin for 6 months and had mammographic density and breast duct cytology assessed [29]. The study showed technical feasibility and that the drug was well tolerated but showed no significant change in endpoints. Another trial underway is a single arm phase 2 of simvastatin for 24-48 weeks in patients with DCIS or stage I-III breast cancer to prevent second new breast cancer, assessing changes in biomarkers such as hsCRP, AKT and p-AKT, cyclin D2, Twist, and contralateral tumor density (NCT00334542). Chemoprevention of atorvastatin is being evaluated in a randomized phase II trial of 100 patients at high risk of breast cancer treating, treating patients with 1 year atorvastatin and measuring change in breast density (NCT00914017). Another atorvastatin chemoprevention trial instead is administering 3 months of drug in patients with DCIS or LCIS and measuring Ki-67 on breast biopsies as a biomarker trial (NCT00637481). Simvastatin is also being tested in this setting in a phase II trial of patients at high risk for non-hormone receptor responsive breast cancer, assessing markers such as Ki-67 in ductal lavage as endpoint [30].

The improvement in proliferation markers seen in the above completed studies highlight that statin therapy has biological activity in breast cancer patients. Notably, the studies listed above are trials with either laboratory biomarker or imaging endpoints without clinical evaluation of efficacy. Additionally, due to easier feasibility, they have evaluated patients in pre-surgical settings but not in the adjuvant setting of high risk patients where there is currently a critical need for effective and well tolerated treatments. The trial detailed in this protocol, therefore, is an important first step to understanding clinical activity of statin treatment in breast cancer management, identifying biomarkers of this clinical response, studying efficacy in the adjuvant setting where there is currently a lack of data on statin treatment, and addressing a high patient subpopulation for whom adjuvant treatments are critically needed.

Given support of biologic effect seen in above clinical studies, we hypothesize that adjuvant statin use can be effective in reducing the risk of recurrence in TNBC patients who did not achieve a pCR to neoadjuvant chemotherapy, a breast cancer subpopulation for which we do not have standard of care adjuvant treatment options and is at high risk for relapse. We propose to administer atorvastatin to this patient cohort in a phase 2 trial to assess clinical and biologic efficacy as well as identify biomarkers of response.

3.1.3 Rationale for Dose Selection/Regimen/Modification

The American College of Cardiology and American Heart Association released new lipid guidelines in 2018 with recommendations on statin use for lipid management as shown in Figure 3 below [31]. According to these guidelines, patients are eligible for either moderate intensity statin therapy or high intensity statin therapy based on age, presence of diabetes, clinical

atherosclerotic cardiovascular disease risk (ASCVD), elevated 10 year ASCVD risk score based on an online risk calculator (http://tools.acc.org/ASCVD-Risk-Estimator/), and baseline lipid profile. Clinical ASCVD consists of ACS, those with history of MI, stable or unstable angina or coronary other arterial revascularization, stroke, transient ischemic attack (TIA), or peripheral artery disease (PAD) including aortic aneurysm, all of atherosclerotic origin. Figure 3 shows the classification of available statin drugs by level of intensity. For our study we will be employing 2 dose levels so that we appropriately treat patients based on lipid guidelines. We will use atorvastatin 20 mg as our moderate intensity statin and atorvastatin 40 mg as our high intensity statin. The use of atorvastatin for choice of statin treatment in this study is supported by evidence of biologic activity demonstrated in pre-surgical window of opportunity breast cancer trials as described in 3.1.2.2.

All TNBC patients who are not receiving statin therapy and do not have a known contraindication to statin therapy will be eligible to participate in this study. Baseline lipids and risk factors will be assessed at time of initiation of study. For patients who are recommended to receive statins based on the most recent 2018 lipid guidelines, the algorithm illustrated in Figure 4 (Primary prevention) and Figure 5 (Secondary prevention) will be applied and patient will be started on moderate intensity atorvastatin 20 mg or high intensity atorvastatin 80 mg by mouth daily as described. Of note, though the majority of preclinical work done in our lab is based on simvastatin, our retrospective study of IBC patients and pre-surgical window of opportunity breast cancer trials as described in 3.1.2.2 support that the anti-breast cancer effect is seen with atorvastatin in addition to other statins. Importantly, unlike simvastatin which is only a moderate intensity statin, atorvastatin can be dosed both as a moderate intensity as well as high intensity statin as needed to comply with standard of care cardiovascular guidelines (http://tools.acc.org/ASCVD-Risk-Estimator/).

	High Intensity	Moderate Intensity	Low Intensity
LDL-C Lowering	>=50%	30-49%	<30%
Statins	Atorvastatin 40mg/80mg Rosuvastatin 20mg/40mg	Atorvastatin 10mg/20mg Rosuvastatin 5mg/10mg Simvastatin 20-40mg	Simvastatin 10mg
		Pravastatin 40mg/80mg Lovastatin 40mg/80mg Fluvastatin XL 80mg Fluvastatin 40mg BID Pitavastatin 1-4mg	Pravastatin 10-20mg Lovastatin 20mg Fluvastatin 20-40mg

Figure 3 Dose recommendation for each risk category

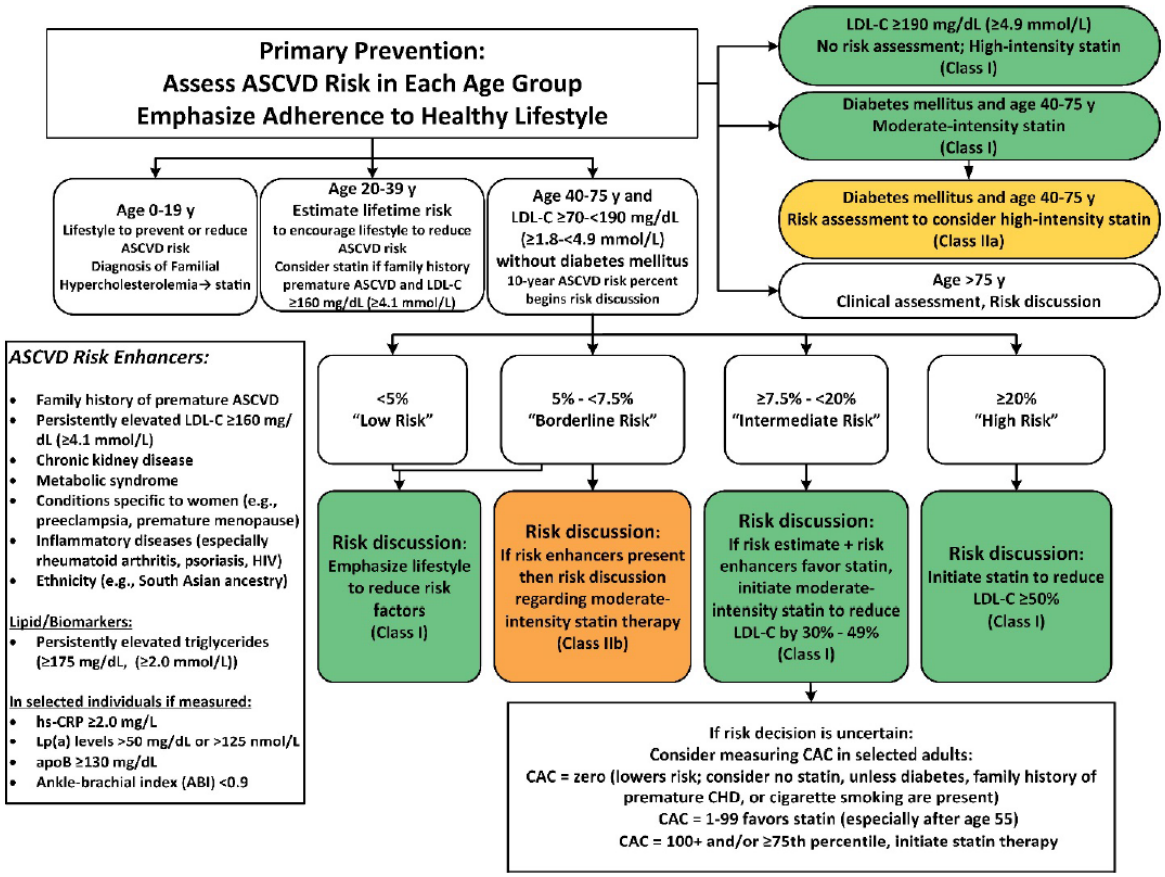
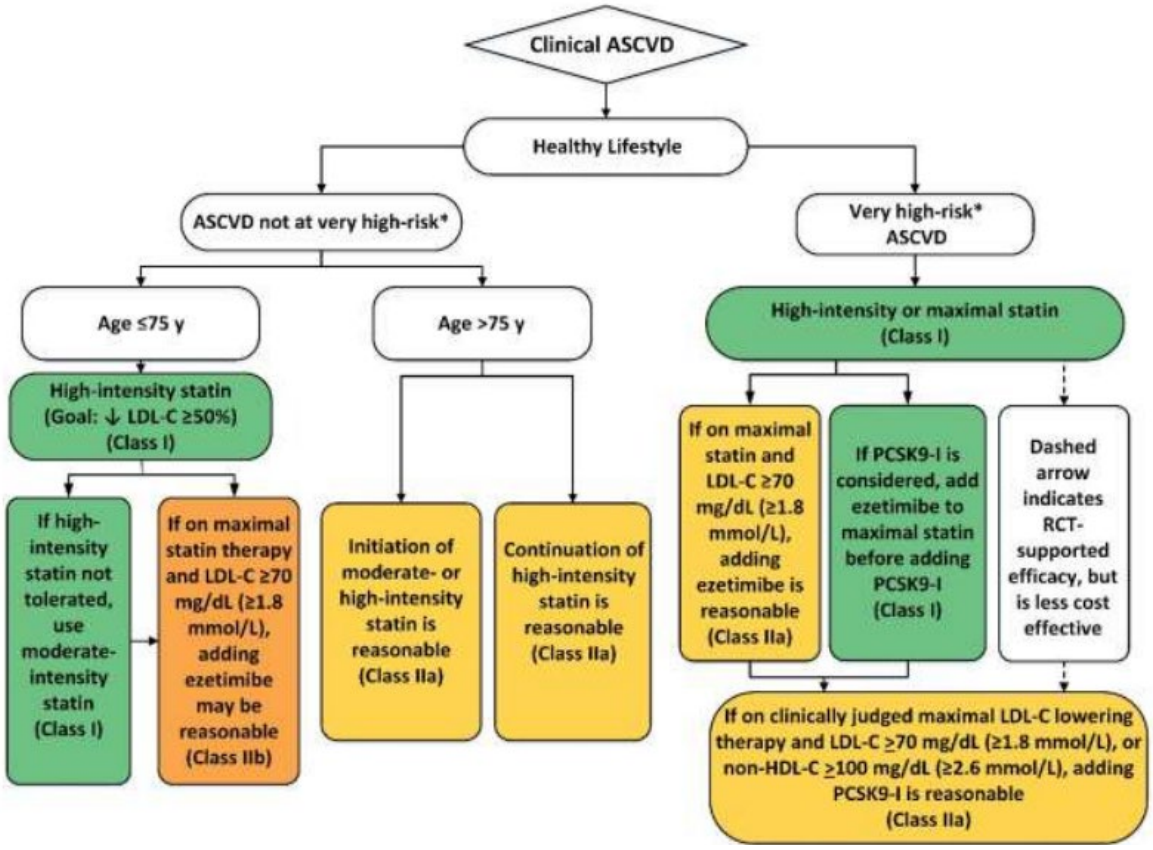


Figure 4 ASCVD risk stratification algorism (Secondary prevention) [31]



Major ASCVD Events
Recent ACS (within the past 12 mo)
History of MI (other than recent ACS event listed above)
History of ischemic stroke
Symptomatic peripheral arterial disease (history of claudication with ABI <0.85, or previous revascularization or amputation (S4.1-39))
High-Risk Conditions
Age ≥65 y
Heterozygous familial hypercholesterolemia
History of prior coronary artery bypass surgery or percutaneous coronary intervention outside of the major ASCVD event(s)
Diabetes mellitus
Hypertension
CKD (eGFR 15-59 mL/min/1.73 m^2) (S4.1-15, S4.1-17)
Current smoking
Persistently elevated LDL-C (LDL-C ≥100 mg/dL [≥2.6 mmol/L]) despite maximally tolerated statin therapy and ezetimibe
History of congestive HF

*Very high risk includes a history of multiple major ASCVD events or 1 major ASCVD event and multiple high-risk conditions.

ABI indicates ankle-brachial index; ACS, acute coronary syndrome; ASCVD, atherosclerotic cardiovascular disease; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HF, heart failure; LDL, low-density lipoprotein cholesterol; and MI, myocardial infarction.

Figure 5 ASCVD risk stratification algorism (Secondary prevention) [31]

3.1.4 Rationale for Correlatives

1) We will measure CTCs and ctDNA as a burden of metastatic cells and will assess their changes during statin treatment. CTCs can be found in human blood when cancers undergo metastatic dissemination and spread to distant organs [32]. Our previous work demonstrated that circulating tumor cells at the end of neoadjuvant chemotherapy in patients with TNBC are predictive of worse outcomes, including 2 year recurrence rate [33]. CTCs have been reported as a surrogate marker for tumor response and linked to shorter survival in metastatic breast, prostate and colorectal lung cancer patients [32, 34-38]. We will therefore also calculate changes in CTCs and ctDNA during treatment and correlate to 2 year RFI. These correlatives will therefore both assist in evaluating efficacy of statin treatment as well as developing and testing these platforms to monitor adjuvant treatment in breast cancer patients.

2) We will test serial serum lipid profiles, markers of inflammation: ESR, high sensitivity CRP, IL-6, and immune profiling, and evaluate if these behave as biomarkers of response to atorvastatin treatment. Please see section 3.1.2 for preclinical work supporting the rationale for testing of these studies.

3) Up to 5 unstained slides of tumor and normal tissue from the mastectomy specimen will be used for multiplexed imaging of hypothesis based biomarkers and staining correlated to clinical outcomes as well as other correlatives. We will include imaging such as, but not limited to IL6R, gp130, cholera toxin for lipid rafts, multi-marker labelling of macrophages and stem cells.

4) Additional blood samples and mastectomy tumor formalin fixed paraffin embedded slides will be banked for future testing of additional studies pending the above studies. Additional tests that may be run in the future include components of the cancer signaling pathways shown to mediate statin effects as detailed in section 3.1.2 and components of the mevalonate/HMG Co-A reductase pathway.

3.1.5 Study Endpoint

This is a multicenter pilot study including MD Anderson Cancer Network sites. The primary endpoint is the proportions of patients with undetectable CTC at 6 months in patients with stage IIB/III TNBC who did not achieve a pCR nor had a residual cancer burden (RCB)-I after neoadjuvant chemotherapy with and without atorvastatin; we will enroll patients who had an RCB-II or RCB-III (if TNBC), or non-pCR (if IBC). The positive outcome is defined either CTC's remaining non-detected when baseline CTC is not detected or reduction of the absolute number in CTCs when baseline CTC is detected. If positive outcome is observed with atorvastatin in more than 6 patients among the first 30 patients enrolled, the next step in this clinical study is to enroll 50 additional patients to assess CTC changes in patients who were treated either with atorvastatin (N=18) or without atorvastatin (N=32). CTC change is defined either a CTC measurement becoming negative in patients who have baseline positive CTCs, or a CTC measurement becoming positive in patients who have a baseline negative CTC.

The secondary endpoints will align with the secondary objectives to understand the underlying biology of atorvastatin-induced changes and potential associations with the clinical outcome of interest, 2-year RFS. For this reason, study treatment will continue for 2 years in order to explore the kinetics and magnitude of pharmacodynamic changes in biomarkers including inflammation markers (C-reactive protein, ESR, serum IL-6 and other cytokines), CTCs and lipid profiles over the course of the study. The patients on the study who will not receive atorvastatin will be followed and provide longitudinal data on the natural behavior of inflammation and lipid pathways in patients with TNBC including triple negative IBC.

The exploratory objective that relates to patients in cohort 4 who will not receive xeloda or have already completed prior to the determination of need for atorvastatin is added to learn about the changes in CTCs and inflammatory biomarkers as a result of xeloda. Xeloda is currently a standard of care based on the CREATE-X study, yet no CTC work has been published showing if CTCs are eliminated by this type of chemotherapy.

4.0 METHODOLOGY

4.1 Entry Criteria

Informed consent from each subject must be obtained prior to enrolling at the clinic visit or remotely (electronic informed consent). The method of obtaining and documenting the informed consent and the contents of the consent must comply with ICH-GCP and all applicable regulatory requirements. Informed consent for this study may only be obtained by the Principal Investigator or the assigned designee. This delegation will be included on a protocol delegation log that will be signed by the site's PI. Informed Consent Forms for enrolled patients and for patients who are enrolled but not eligible to receive study treatment (screening failures) will be maintained at the study site. The site investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

All participants must be registered in the Clinical Oncology Research (CORe) system at MD Anderson with the correct corresponding institution and investigator. This will result in a unique study number being assigned to each patient. Patients must not start protocol intervention prior to the registration in CORe.

To register a patient, participating Cancer Network Site should upload the following documents into the institutionally approved cloud-based storage folder:

- Signed informed consent and documentation of the informed consent process
- HIPAA authorization form (if separate from the informed consent document)
- Completed eligibility checklist with supporting source documents for each criterion

Patients will be registered in CORe by 2 steps by a member of the Cancer Network Research Team and an email receipt will be sent to the participating Cancer Network Site including the registration details and assigned protocol patient identification number (PPID#).

4.1.1 Inclusion Criteria

1. Is willing and able to provide written informed consent for the trial.

2. Is a female or male and ≥ 18 years of age at diagnosis of TNBC (including patients with a clinical diagnosis of triple negative inflammatory breast cancer).

3. Has histological confirmation of breast carcinoma.

4. Have stage IIB or III disease as defined by the American Joint Committee on Cancer version 7 or 8.

5. Has confirmed TNBC, defined as having estrogen and progesterone receptor <10% positivity by IHC and HER2 normal, which is 0 or 1+ by IHC and negative by FISH if performed or HER2 2+ by IHC and negative by FISH or HER2 negative by FISH if IHC is not performed.

6. Received neoadjuvant chemotherapy and did not achieve pCR nor had an RCB-I (we will enroll patients with an RCB-II or RCB-III) following neoadjuvant chemotherapy. Since the RCB index has not been validated in IBC, any amount of residual disease will be allowed. pCR is defined as:

   a) the absence of residual invasive cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0/Tis ypN0 in the current AJCC staging system).

   Or

   b) the absence of residual invasive and in situ cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0 ypN0 in the current AJCC staging system)

7. Has a performance status of 0-1 on the ECOG Performance Scale.

8. Has adequate organ function as determined by the following laboratory values:

   ANC ≥ 1,500 /mcL, Platelets ≥ 100,000 /mcL, Hgb ≥ 8 g/dL, creatinine levels < 2.0 x ULN, Total bilirubin ≤ 1.5 x ULN, ALT and AST ≤ 3.0 x ULN

9. Subjects of childbearing potential should be willing to use effective methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through at least 4 months after the last dose of study drug. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year. Effective methods of birth control include 1). Use of hormonal birth control methods: pills, shots/injections, implants (placed under the skin by a health care provider), or patches (placed on the skin); 2). Intrauterine devices (IUDs); 3). Using 2 barrier methods (each partner must use 1 barrier method) with a spermicide. Males must use the male condom (latex or other synthetic material) with spermicide. Females must choose either a Diaphragm with spermicide, or Cervical cap with spermicide, or a sponge (spermicide is already in the contraceptive sponge).

10. Within 12 months from completion of definitive surgery after neoadjuvant chemotherapy at the time of consent.

11. Willing to take statin for minimum of two years.

4.1.2 Exclusion Criteria

1. Has not recovered from adverse events due to prior therapies, i.e. monoclonal antibody, chemotherapy, targeted small molecule therapy, radiation therapy, or surgery.

    - Note: Subjects with ≤ Grade 2 neuropathy, alopecia and general disorders and administration site conditions (per CTCAE version 4.0) are an exception to this criterion and may qualify for the study.

2. Has a known malignancy (other than breast cancer) except basal cell carcinoma or squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

3. Has known psychiatric or substance abuse disorders and assessed by attending physician that would interfere with cooperation with the requirements of the trial.

4. Has received prior therapy with a statin within past 6 months or is currently receiving statin therapy. Patients who previously received a statin more than 6 months prior to beginning study therapy and who discontinued treatment for reasons other than severe toxicity or allergic reaction are eligible.

5. Is currently receiving another anti-lipidemic agent other than statin: fibric acid derivatives (i.e. fenofibrate, gemfibrozil), bile acid sequestrants (i.e. cholestyramine, colestipol), Ezetimibe, niacin, Lovaza (omega-3-acid ethyl esters), red yeast rice, Orlistat, phytosterol, and lomitapide.

6. Known hypersensitivity to statin or any component of the formulation.

7. Active liver disease or unexplained persistent elevations of serum transaminases, defined as elevated transaminases > 3x ULN on at least 2 separate occasions 1 week apart.

8. Pregnancy or women who may become pregnant and not on acceptable form of contraception. Lactating women.
9. Has evidence of distant metastasis.
10. Record of myocardial infarction within 6 months before starting therapy, symptomatic congestive heart failure (New York Heart Association > class II), unstable angina, or unstable cardiac arrhythmia requiring medication.
11. Chronic steroid use as this may prevent any immunomodulatory roles of statin treatment, defined as anticipating need of supraphysiologic dose of steroids for at least 12 weeks while on study.

4.2 Treatment Plan

- After surgery, the attending physician will determine the need for adjuvant xeloda and radiation, and decide the sequence of these. Based on this information, the patient will be enrolled into cohorts 1-4 as applicable.
- Patient will have a consultation with study cardiologist who will determine the need for statin treatment based on ASCVD criteria and clinical judgement. If the patient is planned for adjuvant xeloda, the cardiology consult (and baseline labs) will be done at the end of xeloda, prior to when atorvastatin would be considered.
- All patients who are recommended for high- or moderate-intensity statin assessed by cardiologist will start atorvastatin with the dose recommended in the guideline (Figures 3, 4, and 5.)
- All patients who do not meet the criteria for statin treatment based on ASCVD risk stratification and/or clinical judgement will be followed and have blood collected at the same time points without statin treatment.
- All patients enrolled in Cohort I and Cohort II whoever are eligible for atorvastatin treatment will start atorvastatin treatment with radiation therapy; and all patients enrolled in Cohort III whoever are eligible for atorvastatin treatment will start atorvastatin treatment after the completion of xeloda treatment; and all patients enrolled in Cohort IV whoever eligible for atorvastatin treatment will start atorvastatin treatment after cardiology consultation
- After 2 years treatment or follow up, patients will be off study, but may continue statin treatment based on physician discretion.

4.2.1 Dose Selection:

Atorvastatin 20 mg or 40 mg will be administered by mouth daily depending on whether the patient requires high intensity statin (atorvastatin 40 mg) based on cardiovascular risk factors (3.1.3) or moderate-intensity (atorvastatin 20 mg).

4.2.2 Toxicities and Dose Modification: Atorvastatin

- Adverse events (both non-serious and serious) associated with statin exposure may occur shortly after the first dose or several months after the last dose of treatment. Statin therapy must be withheld for drug-related toxicities and severe or life-threatening adverse events as per Table 2 below.

- The most commonly observed significant adverse reactions and associated frequencies are diarrhea (7-14%), arthralgias (9-12%), nasopharyngitis (13%), hemorrhagic stroke (2%), insomnia (5%), diabetes mellitus (6%), nausea (7%), dyspepsia (6), urinary tract infection (7-8%), elevated serum transaminase (</- 2%), limb pain (9%), myalgias (4-8%), musculoskeletal pain (5%), muscle spasms (4-5%), and pharyngolaryngeal pain (3-4%).

- Other rare and significant toxicities seen in < 2% of patients include abdominal pain, abnormal hepatic function tests, alopecia, anaphylaxis, anemia, angioedema, anorexia, cholestasis, cholestatic jaundice, cognitive dysfunction (reversible), confusion (reversible), depression, elevated glycosylated hemoglobin (HbA_{1c}), epistaxis, eructation, erythema multiforme, gynecomastia, hematuria, hepatic failure, hepatitis, hyperglycemia, hypoesthesia, increased creatinine phosphokinase, increased serum alkaline phosphatase, increased serum glucose, jaundice, joint swelling, muscle fatigue, myasthenia, myopathy, myositis, neck stiffness, nightmares, pancreatitis, paresthesia, peripheral edema, peripheral neuropathy, rhabdomyolysis, rupture of tendon, Stevens-Johnson syndrome, thrombocytopenia, and toxic epidermal necrolysis

<u>Hepatotoxicity</u>

If serious hepatotoxicity with clinical symptoms and/or hyperbilirubinemia/jaundice occur during treatment, atorvastatin therapy should be discontinued. If alternate etiology is not identified, atorvastatin should be permanently discontinued. If alternate etiology is identified and corrected, atorvastatin can be restarted once liver function tests return to normal values. Excessive ethanol consumption should be avoided as this can potentiate adverse hepatic effects of statin therapy.

<u>Myopathy/Rhabdomyolysis</u>

Patients should be instructed to report unexplained muscle pain, tenderness, weakness, or brown urine, particularly if accompanied by malaise or fever. Manage according to Table 2.

Baseline hypothyroidism, Vitamin D, etc. should be tested and corrected if found to be abnormal to minimize risk of statin induced myopathy. Concurrent use of medications (see section 6.3.2)

that potentiate myopathy such as CYP3A4 inhibitors of fibrates should be avoided. Uncomplicated myalgia immune-mediated necrotizing myopathy (IMNM) associated with HMG-CoA reductase inhibitors use has also been reported.

Renal Impairment

No dose changes are required for renal impairment but this increases risk of myopathy so patient should be closely monitored.

Pregnancy and Lactation

Studies in animals and pregnant women have shown evidence of fetal abnormalities and use is contraindicated in women who are or may become pregnant. There are reports of congenital anomalies following maternal use of HMG-CoA reductase inhibitors in pregnancy. Cholesterol biosynthesis may be important in fetal development; serum cholesterol and triglycerides increase normally during pregnancy. HMG-CoA reductase inhibitors should be discontinued prior to pregnancy (ADA 2013). The manufacturer recommends administration to women of childbearing potential only when conception is highly unlikely and patients have been informed of potential hazards.

It is not known if atorvastatin is excreted in breast milk. Due to the potential for serious adverse reactions in a nursing infant, use while breast-feeding is contraindicated by the manufacturer.

Table 2 Dose Modification Guidelines for Statin Related Adverse Events:

Toxicity	**Severity**	**Management including Treatment Discontinuation**
Myopathy	Mild symptoms	Continue without change in statin treatment. . Evaluate patient for other conditions that may increase the risk for muscle symptoms (i.e. hypothyroidism, reduced renal or hepatic function, rheumatologic disorders such as polymyalgia rheumatica, steroid myopathy, vitamin D deficiency, or primary muscle diseases). Measure CK and follow algorithm as detailed in CK section below.
	Moderate symptoms	Evaluate for other conditions that may increase risk for muscle symptoms. Discontinue use until symptoms can be evaluated. Hold treatment until symptom resolution. Upon resolution, resume the original dose of atorvastatin. If muscle symptoms recur, dose reduce or change to alternate statin (pravastatin). If symptoms re-occur at lower dose, change to alternate statin. Measure CK and assess for rhabdomyolysis.
	Severe symptoms	Discontinue use until symptom resolution. Re-challenge at lower dose or alternate statin (pravastatin). If re-occurs at lower dose, change to alternate statin. Measure CK and assess for rhabdomyolysis.
CK elevation	< 10x ULN	Continue statin at same dose with close monitoring of symptoms. Workup for secondary causes and treat as indicated.

Toxicity	Severity	Management including Treatment Discontinuation
	>10x normal	Evaluate for rhabdomyolysis (creatinine, myoglobinuria, and treat if patient has). If no rhabdomyolysis, repeat 1 week later and discontinue if persistently > 10x ULN. Workup for secondary causes and treat as indicated.
Rhabdomyolysis	n/a	Permanently discontinue. Treat with hydration, etc. per guidelines.
Hepatic injury	Bilirubin > 1.5 ULN or clinical evidence of hepatotoxicity	Discontinue treatment. If alternate etiology is not identified, atorvastatin should be permanently discontinued. If alternate etiology is identified and corrected, atorvastatin can be restarted once liver function tests return to normal values.
	AST or ALT 1-3x ULN	Continue same dose and type of statin and monitor closely.
	AST or ALT > 3x ULN 1 week apart	Repeat 1 week later. If still elevated at 3x ULN, dose reduce or discontinue treatment. Workup for other causes of liver injury and restart statin if LFTs improve or alternate etiology identified.
Type 2 diabetes mellitus (new diagnosis) or hyperglycemia	T2DM or 3-4	Recommend standard of care management of patients if this is discovered. Refer to primary care physician. No change in statin dose or type indicated.
Kidney injury	3-4	Evaluate for rhabdomyolysis and permanently discontinue if workup supports this. Otherwise, continue at present dose. Monitor closely for statin associated adverse events.
Cognitive Impairment		If felt to be related to statin treatment, hold and see if symptoms improve in 3 weeks. Can then restart treatment if improvement. Workup for alternate etiologies.
All Other Drug-Related Toxicity[1]	Grade 1 or 2	No change in statin dose or type indicated.
	3	Hold statin if deemed by investigator to be related to statin therapy only. Defer to PI regarding re-introduction of statin upon improvement to grades 1/2 toxicity.
	4	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.
[1]Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption. The reason for interruption should be documented in the patient's study record.

4.2.3 Toxicities and Dose Modification: Atorvastatin and Radiation Therapy (RT)

Radiation therapy will be performed as per standard of care at each institution (i.e. once or twice per day, with a total dose of about 60-66Gy over 4-6 weeks). Statin administration will commence prior to, with/after starting radiation, after collecting baseline blood sample based on whichever cohort the patient is enrolled in.

Statin therapy must be withheld during radiation for the following adverse events:

Confluent moist desquamation, defined as presence of a single region of moist desquamation that exceeds 100 cm^2 in area (this is specified because chest wall radiotherapy with bolus to 50 Gy followed by boost to total dose of 60-66 Gy commonly leads to smaller areas of desquamation even in the absence of sensitizing agents).
* Note that areas of moist desquamation smaller than 100cm^2 in area will be scored as Grade 2 radiation dermatitis for the purposes of this study, but bleeding induced by minor trauma or abrasion will be considered Grade 3 and scored as a DLT

- Any toxicity requiring a radiotherapy delay exceeding 1 week

If statin therapy is withheld during RT, it may be started again 2 weeks after completion of RT provided that no criteria described above in section 4.2.2 has been met.

Each patient receiving RT will be followed for evaluation of toxicity according to each site's institutional policies. The recommendation is to follow for twelve weeks with weekly assessments throughout the approximate six-week course of radiotherapy and at a six-week post completion visit. Cancer Network Sites patients are required to undergo all treatments, follow-up appointments, and adverse events management at their consenting facility to collect the protocol required data.

4.3 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications specifically prohibited during the trial, discontinuation from trial therapy may be required. The final decision on any supportive therapy rests with the investigator and/or the subject's primary physician.

4.3.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. Concomitant medication will be recorded as standard of care in clinic database.

4.3.2 Prohibited Concomitant Medications

- Statins are substrates of CYP3A4 (major), P-glycoprotein, and SLCO1B1. They are also inhibitors of CYP2C8 (weak), CYP2C9 (weak), and CYP2D6 (weak). They

thereby can weakly interfere with metabolism of other drugs and vice versa. Subjects are prohibited from receiving the following therapies during the study: acipimox, azithromycin, bezafibrate, ciprofibrate, colchicine, fenofibrate, gemfibrozil, ketoconazole, voriconazole, naloxegol, niacin, niacinamide, ombitasvir, paritaprevir, ritonavir, and dasabuvir, raltegravir, red yeast rice, aprepitant, boceprevir, bile acid sequestrants, clarithromycin, cobicistat, conivaptan, cyclosporine, cyproterone , daclatasvir, danazol, dasatinib, efavirenz, dronedarone, erythromycin, diltiazem, fluconazole, fosaprepitant, itraconazole, ivacaftor, luliconazole, mifepristone, netupitant, ombitasvir, paritaprevir, ritonavir, and dasabuvir, osimertinib, posaconazole, protease inhibitors, quinine, ranolazine, sacubitril, simeprevir, stiripentol, telaprevir, telithromycin, ticagrelor, tipranavir, verapamil, fusidic acid, large quantities of grapefruit juice, amiodarone, antacids, bexarotene, bosentan, deferasirox, enzalutamide, fosphenytoin, etravirine, mitotane, osimertinib, phenytoin, rifamycin derivatives, siltuximab, St Johns Wort, tocilizumab, edoxaban, ledipasvir, prucalopride, rifaximin, silodosin, aliskiren, digoxin, midazolam, verapamil, aripiprazole, dofetilide, nimodipine, pimozide, lomitapide, flibanserin, eltrombopag, teriflunomide, lanthanum

- Atorvastatin may decrease concentration of dabigatran so caution should be used during concomitant use. Statin treatment can enhance the adverse effects of daptomycin. Consider temporarily stopping statin prior to daptomycin. If used together, regular (i.e., at least weekly) monitoring of CK concentrations is recommended. Atorvastatin can also enhance toxic effects of cimetidine and spironolactone and requires close monitoring.

- Chronic use of supraphysiologic doses of steroids as this can mitigate any immunomodulatory effects of statin treatment.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

4.4 Rescue Medications & Supportive Care

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Supportive care should therefore be provided as part of standard of care. Of note patients should be encouraged to hydrate if having symptoms of myopathy. If evidence of rhabdomyolysis, patients will likely need to be admitted for intravenous hydration.

4.5 Subject Withdrawal/Discontinuation Criteria

In this study, statin administration will continue until one of the following conditions is observed.

4.5.1. Disease progression
Disease progression will be defined as biopsy-confirmed recurrence of cancer either locally or at any distant site.

4.5.2 Noncompliance:
If the patient is not able to compliant with the treatment schedule in the absence of toxicity, the study treatment should be discontinued for the patient. Noncompliance is defined as an unscheduled dose interruption lasting ≥14 days without a documented reason agreed upon by the investigator for patient safety.

4.5.3. Sustained side effects:
Study treatment will be discontinued for patients who have sustained toxic effects that are attributed to the study drug and require a dose interruption lasting more than 12 weeks.

4.5.4. Initiation of new anticancer treatment:
In patients for whom the investigator, in his or her judgment, determines new treatment for breast cancer is warranted, the study treatment may be discontinued.

4.5.5. Patient withdraws consent.
In the event that a patient withdraws consent, the reason(s) for withdrawal must be documented. Patients must be informed that their participation in the study is voluntary and that they may choose not to take part in the study or to stop taking part at any time. If a patient chooses not to take part in the study or to stop at any time, his/her future medical care or medical benefits will not be affected.

4.5.6. Patient has completed 24 months of treatment.

4.5.7. If the patient requires or is using any prohibited medication listed on section 4.3.2 during study therapy period.

4.6 Criteria for Disease Control

The secondary purpose of this study is to estimate recurrence rate with addition of statin therapy to adjuvant treatment of patients with TNBC who did not achieve a pCR after neoadjuvant chemotherapy.

4.6.1. Response will not be considered evaluable in the following categories:

4.6.1.1. Early Deaths: Patients who die within the first 2 weeks of the initiation of drug therapy owing to concurrent disease. These cases will be considered treatment failures in the intent-to-treat analysis.

4.6.1.2. Lost to Follow-up: Patients for whom there is inadequate information to judge tumor response because of loss of contact with our institution (>2 months after a missed appointment) and with referring physician in spite of repeated attempts to locate them. These cases will be considered treatment failures in the intent-to-treat analysis.

4.6.1.3. Major Protocol Violation: Patients who significantly deviate from the treatment program by either adding or deleting another agent or another therapeutic maneuver or by modifying schedule substantially (delay treatment ≥14 days without administration reason) of the drug under evaluation. Patients who did not fulfill the requirements outlined under Patient Eligibility are also included in this category.

5.0 STUDY FLOW CHART

Trial Period:	**Screening**	**Pre-xeloda Baseline[h]**	**Baseline (pre-statin)**	**During RT[f]**	**3M**	**6M, 12 M, 18M, 24M or disease progression**
Scheduling Window (Week):			*± 2*		*± 4*	*± 6*
Informed Consent	x					
Inclusion/Exclusion Criteria	x					
Demographics and Breast Cancer History [a]	x					
Concomitant Medication Review per SOC	x		x		x	x
Physical Exam [b]	x		x		x	x
Adverse Events [b]	x		x		x	x
ECOG Performance Status [b]	x		x		x	x
Pregnancy Test – Urine or Serum β-HCG (for women with childbearing potential) [c]	x					
CBC, comprehensive metabolic profile [d]	x		x		x	x
Lipid profile [e]			x		x	x
Archived initial diagnostic breast biopsy tissue and/or surgical specimens (block or unstained slides) obtained at any time prior to study treatment	x					
Correlative Studies Blood Collection [f]		x	x	x	x	x
Cardiology consult [g]			x			
Optional collection of 5 to 20 slides if tumor sample collected as part of SOC on disease progression, if the site is accessible						x
Disease Progression Status	x		x		x	x

a. Demographics include patient's age, gender and race. Medical history includes primary breast cancer pathological diagnosis, ER/PR/HER2 status, prior therapies, procedures for breast cancer, other medical problems present, and review of systems.
b. Complete physical exam during the screening period. This will not be repeated if done within 30 (+/- 3) days before the start of treatment. During treatment Physical Exam as clinically indicated prior to trial treatment administration. Adverse events and ECOG performance status assessment can be done within 30 (+/- 3) days of the treatment.
c. Serum or urine pregnancy test must be performed per standard of care only for women of reproductive potential. A woman is considered to be of "reproductive potential" if she has had menses at any time in the preceding 12 consecutive months. A source document must be available for post-menopausal subjects (i.e., no menses for 12 months without an alternative medical cause), women with permanent sterilization (e.g., hysterectomy, bilateral salpingectomy and bilateral oophorectomy, or tubal ligation) and/or age over 55.

d. Hematologic and biochemical profiles (CBC, albumin, alkaline phosphatase, ALT, AST, LDH, fasting blood sugar, phosphorus, potassium, sodium, total bilirubin, total protein, BUN, creatinine) as standard of care (will not be repeated if done within 30 (+/- 3 days) days before the start of treatment). During treatment, an earlier evaluation will be performed if clinically indicated.
e. HDL, LDL, VLDL, triglycerides, total cholesterol. (Refer to 6.1.1 in details.). Baseline lipid panel will be done after xeloda for patients receiving xeloda while on study.
f. Peripheral blood and serum for correlative studies (Refer to 6.1.1 in details). Correlative studies blood collection during radiation will be done 2 weeks (+/- 5 days) after starting the radiation. Participating Cancer Network sites will refer to the lab manual for blood and tumor sample collection, storage, package, and shipment instructions.
g. Cardiology consult will be done to determine whether patients are recommended to be on high- or intermediate dose of statin based on ASCVD criteria (Figures 3 and 4)
h. For patients who agree to the optional blood collection before xeloda, the blood will be drawn prior to beginning therapy. Participating Cancer Network sites will refer to the lab manual for blood and tumor sample collection, storage, package, and shipment instructions.

6.0 CORRELATIVE STUDIES BLOOD COLLECTION AT PRE-XELODA TIMEPOINT IS AN OPTIONAL PROCEDURE IF APPLICABLE. COLLATERAL RESEARCH

Correlative research will be performed on biologic specimens from patients enrolled. Peripheral blood will be collected prior to beginning statin therapy as a baseline, at 3 months (+/- 4 weeks), 6 months (+/- 6 weeks), 12 months (+/- 6 weeks), 18 months (+/- 6 weeks), and 24 months (+/- 6 weeks) and/or at the time of disease recurrence, whichever occurs first. For patients who agree to the optional blood collection before xeloda, the blood will also be drawn prior to beginning therapy.

Original tumor tissue's block or unstained slides (archived initial diagnostic breast biopsy tissue and/or surgical specimens) prior to treatment (5 to 20 formalin fixed paraffin embedded slides from mastectomy) will be collected and banked for future studies if available. If available, up to twenty slides from formalin fixed paraffin embedded samples will also be collected and banked for future studies from time of progression if tumor biopsied as part of routine clinical care. These samples will be used to perform correlative studies aimed at identifying predictive markers and to enhance understanding of disease biology.

6.1 Biomarker assessments

Tumor tissue and peripheral blood will be collected prior to therapy and at selected time points on treatment. Residual sample material available after completion of the designated analyses may be used in the future for identification of additional predictive markers or to enhance understanding of disease biology. If biomarker samples are drawn but study drug is not administered, samples will be retained. A description of each assay system is described below.

6.1.1 Blood samples

<u>Collection and processing</u>

All blood tubes will be labeled only with the patients' unique study number. Samples #1 collected at all facilities will be sent to Epic; samples #2 and #4 will be sent to local diagnostic lab as SOC; sample #3, #5, #6 will be sent to Dr. Reuben's Lab in MDACC. See details in section 13.

Samples and Assays

1) For circulating tumor cell analysis, blood samples will be collected in 10mL Cell-Free DNA BCT tubes (Streck, Inc.). For detailed SOP, please see the section 13 for blood collection, amount, type of tube and packaging and shipping information.

2) Lipid profile (including HDL, LDL, VLDL, triglycerides, total cholesterol) sent via standard of care testing of each respective institutional site laboratory. For lipid panel, Mint top tube will be used and 3.5mL of blood will be needed.

3) Circulating tumor DNA: Blood samples will be collected in **10mL Streck tubes** at the time points shown in the Study Calendar for analysis of hotspot mutations via ddPCR.

4) Systemic markers of inflammation: Erythrocyte sedimentation rate, high sensitivity C-reactive protein assays will be ordered as standard of care through the diagnostic lab. For ESR, lavender top tube will be used and 3.0mL of blood will be needed. For CRP, gold top tube will be used and 5.0 mL of blood will be needed.

5) Soluble factors such as cytokines and chemokines present in serum collected at baseline, during, and after treatment will be quantified using a multiplex assay which has the capability to simultaneously measure multiple analytes from a single sample. The primary cytokine of interest is IL-6 but additional cytokines in the assay such as IL-2 and TNFa may be assessed. Red top tube with 10 ml of blood will need to be drawn and frozen at 0.5 ml aliquots and stores at - 80 °C.

6) Peripheral blood mononuclear cells:

   The proportion of specific lymphocyte subsets to include T cell subsets including T-regulatory cells, B cells, and the proportion of memory and effector T cell subsets will be quantified by flow cytometry. EDTA tube with 10 mL of blood will need to be drawn and stored at room temperature (for MD Anderson patients) or shipped overnight at 4°C. Prior to processing the cells for flow cytometry, plasma will be collected first to measure proteins using OLINK method that has panels for Inflammation, Oncology and Immune-Oncology (IO).

Remaining serum and peripheral blood mononuclear cell component will be banked for future correlative studies that may include peripheral blood assessment of mevalonate and cancer signaling pathway components previously shown to mediate effects of statin therapy, additional immune profiling, and measurement of miR-33a/b to examine radiosensitization and toxicity.

6.1.2 Tumor samples

Archived initial diagnostic breast biopsy tissue and/or surgical specimens (block or unstained slides) obtained at any time prior to treatment will be collected from all consenting subjects and 5 to 20 formalin fixed paraffin embedded slides will be banked. 10 to 20 formalin fixed paraffin embedded slides will also be collected and banked for future studies from time of progression if tumor biopsied as part of routine clinical care. Up to 5 of these banked slides will be used for proposed multiplexed imaging. Remaining slides and tissues will be banked until future time point for additional correlative studies, which may include assessment of HMG CoA-reductase pathway, signaling pathways previously shown to mediate statin effects, and immune profiling.

Assays

At the time of sample processing, each Tissue-Tek-OCT-frozen punch biopsy specimen will be sectioned via cryostat (at 4 degrees). Four 5 um section slides will cut with the cryostat--two for H&E staining, two for immunofluorescence analysis of gamma-H2aX foci (indicative of DNA double-stranded damage), two for immunofluorescence analysis of pFOXO3a (indicative of signaling through EGFR and essential transcription factor in radiosensitization)

The remainder of the specimen will be fixed with formalin and stored for future immunohistochemical or immunofluorescence studies by interested investigators.

7.0 SAFETY MONITORING AND REPORTING

7.1 Adverse Event

Adverse events will be assessed according to the CTCAE version 4.0 from baseline onwards. All study patients who have received any dose of atorvastatin will be evaluable for safety. Unexpected adverse events including laboratory adverse events deemed clinically significant by the investigator will be graded and recorded in study database - Prometheus.

The ongoing review of safety data will include review of clinical AEs and SAEs. The NCI-CTC version 4.0 will be used to grade all AEs.

AEs (≥2 non-hematological and ≥3 hematological AEs) occurring after informed consent signing observed by the investigator or reported by the subject (whether or not attributed to atorvastatin), will be documented in the medical record or an appropriate source and recorded in Prometheus database. Abnormal laboratory values will not be reported as AEs; however, any clinical consequences of the abnormality should be reported as AEs and recorded in Prometheus.

7.2 Serious Adverse Event Reporting (SAE)

7.2.1 Internal SAE reporting

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator.

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices".

- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to MDACC IRB.

- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the MDA IRB office. This may include the development of a secondary malignancy.

7.2.1.2 Guidelines for participating Cancer Network Sites to report SAEs

- Participating Cancer Network Sites are required to report SAEs within 24 hours after becoming aware of the event. The SAE Form created specifically by the MD Anderson Cancer Center will be provided to each site. Follow-up information on the SAE event may be requested by MD Anderson Cancer Center. SAE report information must be consistent with the data provided on Prometheus.

- Participating Cancer Network Sites should also report SAEs, any unanticipated death or adverse event occurring after a patient has discontinued or terminated study participation that may reasonably be related to the study to their respective IRB according to the local IRB's policies and procedures and a copy of the submitted institutional SAE form should be uploaded in to the institutionally approved cloud-based storage folder.

7.2.2 Evaluating Adverse Events

An investigator or an assigned designee will evaluate and record adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0 as standard practice per institution guidelines.

8.0 STATISTICAL CONSIDERATION AND ANALYSIS PLAN

We plan to enroll a total 80 stage IIb/III TNBC patients who do not achieve pCR after neoadjuvant systemic chemotherapy. We will have an interim analysis after 30 of enrolled patients are treated with atorvastatin. The study will continue enrollment if within the first 30 treated patients, ≥6 patients demonstrate positive outcome in CTC at 6 months, i.e., CTC remains non-detected if baseline CTC is not detected or CTC count has decreased if the baseline CTC is detected, we will then enroll up to an additional 50 patients to complete the study.

With a total of up to 80 patients, we expect about 48 patients will have received the statin and 32 will not receive the statin, we will be able to estimate the percentage of patients with negative CTC, i.e, no CTC present in blood, at six months after surgery with a standard error not larger than 7% and 9%, respectively. At the end of the study, besides estimating the proportion of patients with negative CTC at 6 months with 95% confidence interval, we will also describe the patterns of the change in CTC (from negative to positive [any CTC count in blood], positive to negative, positive to positive or negative to negative) in those who receive statin and those who do not receive statin, separately. We will also explore any pattern of CTC counts at baseline and at follow-ups in patient subgroup, including patients treated with different adjuvant therapies.

Variables of interest including age, stage, IBC status, CTC counts and the change of CTC counts, lipid profile levels, ctDNA, ESR, CRP, serum IL-6 and other cytokines, adverse events, and imaging biomarkers will be summarized for each group (statin vs no statin) using standard descriptive statistics, such as mean, standard deviation, median, and range for continuous

variables, and frequency and proportion for categorical variables. We will evaluate the association between CTC counts or the change of CTC counts and other variables. Correlation between continuous variables will be assessed using Pearson or Spearman correlation coefficient, whichever is appropriate. We will define CTC response as either remaining negative CTCs at 6 months when the baseline CTC is negative or becoming negative when baseline CTC is positive. The difference between the two treatment groups (statin vs no statin) or between the CTC response groups will be tested using Wilcoxon rank sum test for continuous variables and Chi-squared test/Fisher's exact test. Recurrence-free survival (RFS) and overall survival (OS) times from the time of surgery will be estimated with 95% confidence intervals using the Kaplan-Meier method and compared between the treatment groups or CTC response groups using log-rank test. The Cox regression models may be applied to assess the effect of covariates of interest, including the treatment groups or CTC response groups, on survival outcomes. Adverse events, grade and relationship will be tabulated by treatment arms. Other statistical methods may be applied when appropriate. All analysis is exploratory and the results will serve for hypothesis generating purpose.

Inclusion of Minorities

The study will be available to all eligible patients regardless of race, or ethnic origin. There is no information currently available regarding differential effects of this regimen in subsets defined by race or ethnicity.

9.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

9.1 Product used in the study

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of product in accordance with the protocol and any applicable laws and regulations. The use of the Atorvastatin is standard of care.

Table 1 Product Descriptions

Product Name & Potency	**Dosage Form**
Atorvastatin 20 mg or 40 mg daily	Tablet to be administered by mouth

9.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

9.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

9.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.
Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site per their institutional guidelines.
Clinical supplies may not be used for any purpose other than that stated in the protocol.

10.0 DATA MANAGEMENT

The Principal Investigator is responsible for assuring that the data entered into the database are complete and accurate and that data entry is performed in a timely manner. A single Prometheus database for all sites will be used.

10.1 Data collection for this study including:

1) demographic information (sex, race, and date of birth),

2) date of initial breast cancer diagnosis, pathology report of primary breast cancer, biomarker status, and date and location of distant metastases at disease progression;

3) history of breast cancer surgery, adjuvant xeloda and radiation therapy, if applicable;

4) All AEs will be collected, however only >/=2 non-hematologic AEs and >/=3 hematological AEs (whether or not attributed to atorvastatin) will be recorded in Prometheus. Other abnormal laboratory values will not be reported as AEs; however, any clinical consequences of abnormality should be reported as AEs and recorded in Prometheus.

5) Concomitant medication will be recorded per standard of care in clinic database, and will not be recorded in the study database. Participating Cancer Network Sites will upload a copy of the concomitant medication list(s) into the assigned cloud-folder as specified on the time points in the study schema.

10.2 Data confidentiality plan

All laboratory and clinical data gathered in this protocol will be stored in a password-protected database. All patient information will be handled using anonymous identifiers. Linkage to patient identity will be possible only after accessing a password-protected database. Access to the database will be available only to individuals directly involved in the study.

Information gathered for this study will not be reused or disclosed to any other person or entity, or for other research. Once the research has been completed, identifiers will be retained for as long as is required by law and by institutional regulations, and at that point will be destroyed.

11.0 MULTICENTER STUDY MANAGEMENT PLAN

This study is collective effort of centers including MD Anderson Cancer Center, and participating Cancer Network Sites. All treatments and procedures proposed in the study are standard of care. There is no research treatment and procedures involved. We will only collect research samples and clinical data, so this is a low risk study.

Participating Cancer Network Sites will adhere to the protocol, Data Quality Management Plan (DQMP), and conduct the study in accordance with the Institutional policies and procedures.

11.1 RESEARCH SAMPLE DISTRIBUTION

Blood samples collected in 10mL Cell-Free DNA BCT tubes (Streck, Inc.) will be transported at ambient temperature to the Epic Sciences central laboratory (San Diego, CA) using of a standardized ISO-certified shipping process as instructed in section 13.0.

All other biological samples, including blood, tissue and/or slides will be sent to MDACC IBC lab at the below address except the blood for CTC analysis which should be sent directly to Epic Sciences separately. Archival tissue may be sent in batches;

Lily Villarreal / IBC Lab6565 MD Anderson Blvd. Z12.4031 B
Houston TX 77030
Phone: 713-792-4925
Email: BMO_BMOCTRLab_Users@mdanderson.org

Participating Cancer Network Sites will refer to the Lab Manual for Correlative studies collection details including how to request the lab kits, air bill, sample collection, storage, package, and shipping information.

12.0 REFERENCES

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13.0 SAMPLE COLLECTION INSTRUCTION – EPIC SCIENCE CTC SAMPLE

Blood samples will be collected in 10mL Cell-Free DNA BCT tubes (Streck, Inc.) and transported at ambient temperature to the Epic Sciences central laboratory (San Diego, CA) using of a standardized ISO-certified shipping process. Samples will be de-identified and labelled with Subject ID and Draw Date/Time for blood age.

The instruction for sample collection and shipping are as below:

Epic Sciences Study Logistics

CTC Sample Collection in Streck Cell-Free DNA Blood Collection Tubes (BCT)

CTC Samples should be collected in the 10mL Streck Cell-Free DNA BCT. These tubes are commercially available through Streck (Omaha, NE).

IMPORTANT: The first 5 mL of blood collected from the fresh venipuncture **cannot** be used for the collection into the Streck tubes due to possibility of contaminating epithelial cells during venipuncture. **Please ensure that at least one blood tube of 5 mL or more is collected prior to collection of the CTC sample to avoid adversely affecting the test results.**

Prevention of Backflow:

Since Streck Cell-Free DNA BCT tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, observe the following precautions:

- Keep patient's arm in the downward position during the collection procedure.
- Hold the tube with the stopper uppermost.
- Release tourniquet once the blood starts to flow into the tube, or within 2 minutes of application.
- Tube contents should not touch stopper or the end of the needle during the collection procedure.

Blood Collection Instructions:

***Schedule courier for same-day sample pick-up prior to collection*

- Confirm blood tube is not expired. Expired tubes should not be used for blood collection.
- Draw whole blood sample into 10 mL Streck Cell-Free DNA BCT tube (*see note regarding prevention of backflow). Fill tube until blood flow stops. NOTE: Epic requires a minimum of 4 mL blood per sample, but a full 10 mL tube of blood should be provided when possible.
- Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Tube inversion prevents clotting. Inadequate or delayed mixing may result in inaccurate test results.

Blood samples will be packaged in the Epic Sciences Ambient kit and the instructions are as below:

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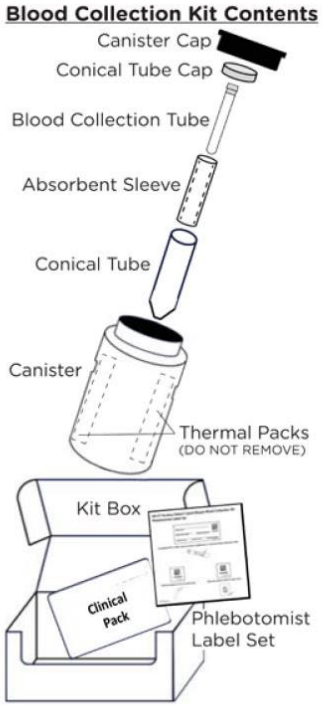
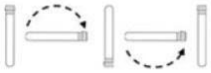


STEP 1: Open collection kit to access blood collection tube and inspect for broken or expired items. **DO NOT REMOVE THERMAL PACKS OR DISCARD ANY ITEMS.**

STEP 2: Complete the **CTC sample requisition form**, fill out the blood tube label and apply all (3) phlebotomist labels as specified.

STEP 3: Using the included blood collection tube, collect at least 8ml peripheral blood. Immediately **INVERT** tube ten **(10x)** times after blood draw to prevent clotting.



STEP 4: Upon completion of blood collection:
- Place blood collection tube into absorbent sleeve and conical tube.
- Replace conical tube cap.
- Place conical tube into center of canister and replace canister cap.

STEP 5: Fold and put the completed requisition form into the kit box.

STEP 6: Place the collection kit box directly into the pre-addressed **Clinical Pack**, seal the Pack, and **STORE** at **ROOM TEMPERATURE (15-30°C)** pending shipment.

STEP 7: ENSURE COURIER PICKUP is scheduled for the **SAME DAY** sample is collected. Ship via Overnight service.

DO NOT REFRIGERATE OR FREEZE KIT OR CONTENTS LBL-00064 Rev A

To order kits, please email: partners@epicsciences.com and cc' lincy.chu@epicsciences.com

Sample Shipment Instructions. . .

- **Whole blood should be collected on weekdays (Monday - Friday) and shipped on the day of collection at ambient temperature for overnight delivery to Epic**
  - Epic is open for sample receipt and accessioning Monday – Saturday
- **All shipments should include completed requisition forms, labeled Streck tubes and ambient gel packs (or similar)**
  - Blood tubes should have at least 2 unique identifiers that match the req form, such as Protocol ID and Patient ID
- **Epic recommends "Saturday Delivery" to be checked on air bill for pickups on Fridays**
- **Do not place "Infectious Substance" sticker on shipper, as this may result in a delay of shipment**

- **Notice of shipment and tracking information from the clinical site will be provided at the time of shipment**
  - Sample email notification will contain:
    - ✓ "2018-0550/MN-017" in email subject
    - ✓ Patient ID and time point (if applicable)
    - ✓ Courier tracking number
    - ✓ Attach requisition form if possible

Samples and completed CTC Sample requisition form are sent via Overnight carrier with advanced notification:
Ship to:
Epic Sciences Attn: 2018-0550/MN-017
9381 Judicial Drive, STE 200
San Diego, CA 92121
Contact Phone; +1-858-356-6610

Send Email to:
partners@epicsciences.com
Include:
1. Tracking number
2. Number of samples being shipped
3. Date and time of each blood draw
4. Case report from including white blood cell count of patient(s)