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Phase I biomarker modulation study of atorvastatin in women at increased risk for breast cancer

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Abstract

Selective estrogen receptor modulators (SERMs), tamoxifen, and raloxifene that reduce the risk of breast cancer are limited to only estrogen receptor-positive (ER$^{?}$) breast cancer. In addition, patient acceptance of SERMs is low due to toxicity and intolerability. New agents with improved toxicity profile that reduce risk of ER-negative breast cancer are urgently needed. Observational studies show that statins can reduce breast cancer incidence and recurrence. The objective of this prospective short-term prevention study was to evaluate the effect of a lipophilic statin, atorvastatin, on biomarkers in breast tissue and serum of women at increased risk. Eligible participants included women with previous history of carcinoma in situ, or atypical hyperplasia, or 5 year breast cancer projected Gail risk >1.67 %, or lifetime breast cancer risk >20 % calculated by models including Claus, Tyrer-Cuzick, Boadicea, or BRCAPRO. Patients underwent baseline fine needle aspiration (FNA) of the breast, blood collection for biomarker analysis, and were randomized to either no treatment or atorvastatin at 10, 20, or 40 mg/day dose for 3 months. At 3 months, blood collection and breast FNA were repeated. Biomarkers included C-reactive protein (CRP), lipid profile, atorvastatin, and its metabolites, Ki-67, bcl-2, EGFR, and pEGFR. Baseline genotype for 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR) was also

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Compliance with ethical standards

Conflict of interest

The authors declare no conflict of interest.

measured. Among 60 patients evaluated, a significant reduction in serum CRP, cholesterol and low-density lipoprotein (LDL), and increase in atorvastatin metabolites in serum and breast FNAs was demonstrated. No changes were observed in other tissue biomarkers. This study shows that atorvastatin and its metabolites are detectable in breast samples and may lower serum CRP among women without hyperlipidemia.

Keywords

Breast cancer risk; Statins; Atorvastatin; Breast cancer biomarkers

Introduction

Prospective chemoprevention studies have shown that selective estrogen receptor (ER) modulators Tamoxifen, Raloxifene, and aromatase inhibitors can reduce the risk of breast cancer anywhere from 30 to 65 % [1, 2]. However, studies indicate that the acceptance to take these agents is low [3]. While side effects might be a concern, but an even major issue is that these agents are not effective in reducing the risk of ER-negative breast cancer. Therefore, agents with a favorable toxicity profile that reduce incidence of both ER-positive and ER-negative breast cancer are urgently needed.

Large-scale randomized prevention trials require a large cohort, are expensive, and take a long time to complete. Short-term phase I and II biomarker modulation prevention trials offer a convenient method to study potential preventive agents [4]. Potential preventive agents to consider are the statins. Atorvastatin is a statin that inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR). Observational studies have demonstrated a decreased incidence of breast cancer among users of HMG CoA reductase inhibitors, while some others did not [5, 6]. Furthermore, a reduced risk of recurrence among statin users diagnosed with early stage breast cancer was reported [7]. Three other studies reported on the biomodulatory effect of statins in high-risk women, showing that some breast cancer risk biomarkers are modulated by these agents [8–10].

Because of the cumulative evidence on statins as potential preventive agents, we conducted a prospective short-term biomarker modulation study of atorvastatin in high-risk women to evaluate atorvastatin’s potential chemopreventive effect by demonstrating changes in breast cancer risk biomarkers in serum and in breast tissue. A phase I design was chosen in an effort to try to find the lowest dose that would induce modulation in biomarkers. The primary objective was to evaluate changes in Ki-67 and secondary objective included changes in C-reactive protein (CRP), lipid markers, and markers of apoptosis.

Materials and methods

Eligibility and study design

Women at increased risk for breast cancer were accrued prospectively at UTMDACC, Breast Medical Oncology High Risk Clinic. The study was approved by the IRB and all study participants signed informed consent.

High risk was defined as having a previous history of ductal carcinoma in situ (DCIS), lobular carcinoma in situ (LCIS), or atypical hyperplasia, 5-year breast cancer projected Gail risk >1.67 %, or lifetime breast cancer risk greater than 20 % calculated by models including Claus, Tyrer-Cuzick, Boadicea, or BRCAPRO. Other eligibility criteria included adequate organ function, no current use of statins and no hypercholesterolemia or hypertriglyceridemia. After signing informed consent, eligible participants underwent baseline blood draw and random periareolar fine needle aspiration (FNA). For women with previous history of LCIS or DCIS, FNA was performed in the contralateral breast. Patients were randomized in a 1:1:1:1 fashion to no treatment or daily 10, 20, or 40 mg atorvastatin for 3 months (arms A, B, C, and D respectively). At the end of 3 months blood draw and FNA were repeated. Participants were evaluable if they took 75 % of their assigned treatments. Fasting blood was collected at baseline and at completion of study (3 months), and serum was frozen at −80°C.

The FNA procedure was performed as previously described [11]. All of the FNA samples were pooled in 5 mL of Cytolyte solution for preparing Thin-Prep slides as previously described [11]. One slide from each case was stained with Papanicolaou stain to assess cellularity and cytomorphology. Depending on the cellularity, additional 2–6 Thin-Prep slides were made and were saved in the tissue bank for biomarker study.

Biomarkers

CRP, total cholesterol, LDL, and HDL were measured by the UTMDACC Hospital Clinical Laboratory. Atorvastatin and its 2 hydroxylated metabolites (o-hydroxyatorvastatin and p-hydroxyatorvastatin) were measured in both serum and FNA samples using tandem mass spectrometers at the UTMDACC Pharmacology and Analytical CORE Laboratory as previously described [12].

Since certain HMG-CoAR genotypes can affect response to statins, we evaluated the rs12654264 gene for the A/A, A/W, or T/T genotypes and correlated with biomarker changes. The genotyping was carried at our institution’s DNA Analysis Core Facility.

Proliferation and apoptosis markers were immunostained in pre- and post-treatment FNA samples using unstained Thin-Prep slides. Staining for Ki-67 (ab66155, 1:100, Abcam), BCL-2 (ab692, 1:100, Abcam), and EGFR (CM063, 1:100, Biocare Medical). Phospho-EGFR (Tyrosine 1068) (2234, 1:50, Cell Signaling) was performed in the Pathology Immunohistochemistry Core Laboratory, according to the manufacturer’s instruction. Scoring of individual biomarker expression levels was performed by our 2 cytopathology collaborators (NS and YG), who recorded the percentage of positive-stained ductal cells.

Statistical analysis

We planned to enroll 66 patients so that after attrition we would have at least 60 evaluable patients, with 15 patients on each arm (arm A: no treatment; arm B: 10 mg/day; arm C: 20 mg/day; arm D: 40 mg/day). Evaluable was defined as having paired pre- and post-serum and/or FNA samples for markers analysis. The standard deviation is about 10 % for a single Ki-67 measure at the baseline or after treatment. Under a conservative assumption that the correlation coefficient between the before and after treatment Ki-67 is 0.5, the standard

deviation of the Ki-67 modulation is also 10 %. Therefore, an effect size of 1 corresponds to a Ki-67 change of 10 %. A one-way design with the sample size of 15 in each group can achieve any-pair power of 0.85, assuming the effect size of 1 and the significance level of 0.05. The any-pair power is defined as the probability of detecting the significant difference between any treatment groups and the control group. The effect size is the standardized mean difference between the treatment group and the control group, which is the ratio of detectable difference between the two groups and the common standard deviation within groups. The difference in biomarkers before and after atorvastatin treatment for each patient was summarized and compared among study arms using a Kruskal–Wallis test. The difference in biomarkers was also compared among genotype groups.

Results

Patient characteristics and tolerability

Sixty-six patients were enrolled to this prospective study and out of these 61 patients could be evaluated (Fig. 1). Five patients did not complete the study (1 did not have enough cells on FNA, 1 was found ineligible due to concomitant medication, and 3 had withdrawn consent). Patient characteristics of 61 patients are shown in Table 1. Median age was 52 years, lifetime breast cancer risk by the Claus model for the entire cohort was 29 %.

All 61 patients were compliant in taking more than 75 % of their assigned doses. Overall, atorvastatin was well tolerated and compliance was good with a total of 16 possibly ($n = 15$) or probably ($n = 1$) related grade 1 or 2 toxicity in nine patients was observed. No drug-related grade 3 and 4 toxicity was observed (Table 2). All grade 2 side effects resolved within the study defined acceptable time frame; 5 grade 1 side effects (possible) did not resolve.

Proliferation and apoptosis markers

Pre- and post-treatment Ki-67, EGFR, pEGFR, and bcl-2 expressions were evaluated in FNA samples via immunohistochemistry (see Table 3, top panel). Overall, there was no significant change in Ki-67 between treatment arms and the control group; however, as shown in Fig. 2, there was some variability in terms of decrease/increase among patients, especially in the 40 mg treatment arm. As an exploratory analysis, we have also analyzed changes in Ki-67 by menopause status and there was no difference in biomarkers in pre- and post-treatment samples in any of the treatment groups (Supplementary Fig. 1A and 1B). However, it appeared that in postmenopausal women a decrease of Ki-67 was seen in all groups, including the control arm. In the premenopausal group there were baseline variations among patients, especially in the 40 mg treatment arm with 3 patients having a high proliferation which might be due to their menstrual cycle or represents a small focus of hyperplasia. However, since core biopsies were not performed this could not be evaluated histologically (Supplementary Fig. 1A and 1B). Changes in EGFR, pEGFR, and bcl-2 expression were also evaluated and there were no significant changes in any of these tissue markers (Table 3, top panel).

Serum and lipid markers

We then examined changes in serum markers including lipid markers, CRP, and Atorvastatin metabolites. Pre- and post-serum cholesterol and LDL levels are shown in Table 3, bottom panel. At 3 months, a significant decrease was seen in cholesterol and LDL in atorvastatin groups compared to control ($p = 0.0001$). No change was observed in HDL levels in any of the study arms.

Inflammation biomarker: CRP

When baseline and 3 months serum CRP levels were evaluated, a significant reduction in the inflammation marker CRP was seen in the atorvastatin treated patients compared to the control group ($p = 0.04$), (Table 3, bottom panel). The 20 mg atorvastatin arm showed significant reduction.

Atorvastatin and metabolites in serum and breast tissue

In order to evaluate whether atorvastatin was present in serum and actually reached the target organ (breast), we evaluated atorvastatin, lactone, and 2 metabolites (o-hydroxyatorvastatin and p-hydroxyatorvastatin) in serum as well as in FNA samples obtained from the breast tissue. Compared to the baseline levels, atorvastatin and its metabolites were significantly increased in serum and FNA in the treatment arms, but not in controls (Fig. 3), confirming that the drug reached its target.

HMG-CoAR genotype

Single nucleotide analysis of the HMG-CoA Reductase gene (rs12654264) showed that 50 % of patients were AA genotype, 33.9 % AW, and 16.1 % AT genotype. We further evaluated changes in biomarkers by these different genotypes. Genotype was not associated with changes in Ki-67 and the rest of the tissue biomarkers. However, we did observe significant changes in serum biomarkers by genotype. Specifically, significant decrease in cholesterol was seen overall and in the AA genotype ($p = 0.0153$) but not in AT and AW (Fig. 4a). A significant decrease in LDL was also seen overall and in the AA group ($p = 0.0078$), but not AT and AW (Fig. 4b). Overall, there was a reduction in CRP ($p = 0.045$) for the whole cohort that was not pronounced in any specific genotype group (Fig. 4c).

Discussion

In this prospective clinical biomarker modulation study with 3 months of atorvastatin treatment, we have shown a significant favorable modulation in CRP (reduction), Cholesterol, and LDL (reduction) in women who are at an increased risk for developing breast cancer. Furthermore, we demonstrate that atorvastatin is well tolerated and the drug reaches its target organ as the active metabolites of atorvastatin were observed in the breast tissue. To the best of our knowledge, this finding has not been reported previously.

Previous short-term biomarker modulation studies have evaluated statins. In the study by Higgins et al., the biomodulatory effect of simvastatin was evaluated in women with previous ER-negative breast cancer. Fifty women received 40 mg per day simvastatin for 24–28 weeks. As in our study, there was a decrease in serum cholesterol, triglyceride, and

CRP; however, no modulation was seen in their other endpoints estradiol and estrone levels and no change was seen in mammographic density. No changes in these biomarkers related to HMG-CoAR genotype were observed and breast tissue biomarkers were not evaluated in that study [8]. In a recently published study by Ji et al. 47 premenopausal women at increased risk for breast cancer received 40 mg of atorvastatin or placebo for a year. The biomarkers they evaluated were mammographic density (MD) and insulin growth factor1 (IGF-1). The statin group demonstrated a significant decrease in cholesterol and LDL similar to our study. After accounting for BMI, their study reported no change in MD but there was a significant increase in serum IGF-1 in the statin group [10].

Another study by Vinayak and colleagues was designed as a single arm study in which lovastatin was evaluated at 40 mg daily for 6 months in women with previous ER-negative breast cancer or who have a deleterious BRCA1/2 or p53 germline mutation, or lifetime breast cancer risk greater than 20 %. In 26 evaluable patients, there was no change in the primary endpoint which was change in cytology in FNA samples. There was also no modulation in secondary endpoints including serum lipid levels, CRP, IGF-1, IGFBP-3, and mammographic density. No change in biomarkers by HMG-CoAR genotype was observed [9]. The difference in results could be due to several factors including variability in patient cohorts. For example, in the Higgins et al. and Vinayak et al. studies, patients were already diagnosed with invasive breast cancer. It is possible that the biomodulatory efficacy of statins is different in contralateral ‘‘high-risk’’ breast tissue of women who have a previous history of breast cancer. The type of statins and the doses are also different in these studies, which might have contributed to different outcomes. Furthermore, it is acknowledged that such phase I and II studies with small sample size, make it difficult to compare and draw meaningful conclusions.

In our study we have evaluated Ki-67 as a potential biomarker, and we did not observe any significant modulation. Ki-67 was previously used in biomarker modulation studies that have revealed conflicting data [13–16]. Fabian et al. found reduction in Ki-67 with 6 months of letrozole therapy, whereas another study by Harper-Wynne did not show any change with 6 months of letrozole therapy [13, 14]. The difference could be due to the fact that in the study by Fabian et al., eligible high-risk women had to have baseline cytologic atypia or borderline atypia with 500 or 1000 epithelial cells on the slides processed for Ki-67 staining. In a study of arzoxifene, no changes in Ki-67 were seen; this study included pre- and post-menopausal women [17]. In a separate study on premenopausal women using 12 months of a plant compound, lignans, and reduction in Ki-67 was observed [16]. The conflicting results may be due to baseline patients’ characteristics and perhaps it would be important to select patients with a higher baseline Ki-67, if this biomarker is indeed intended as a study endpoint. In our study, patient population was mainly postmenopausal and therefore had a lower baseline Ki-67. In the remaining premenopausal participants, the timing of FNA was not performed by menstrual cycle status; therefore, this could have confounded our results.

CRP is an interesting inflammation marker that has gained recent interest also as a breast cancer risk biomarker. In a recent case–control study, Ollberding et al. [18] evaluated factors associated with postmenopausal breast cancer risk and showed that systemic inflammation (as measured by CRP) is an independent risk factor, adding to the growing literature related

to systemic inflammation and breast cancer risk. It is logical to think that statins might reduce inflammation as shown by the reduction of CRP and thereby reduce the risk of breast cancer. Therefore, it is intriguing to see a decrease in CRP in our study; CRP indeed could serve as a surrogate marker for future short-term prevention trials.

While some observational studies have shown that statins can reduce risk of breast cancer, some others have not confirmed these findings [5, 6]. One of the explanations for this observation could be that patients' baseline breast cancer risk levels are different in different studies. It also needs to be recognized that none of these studies are prospective studies with breast cancer being the primary endpoint.

Currently, it is not known how statins exert potential anticancer and preventive effects: whether it is by reducing cholesterol levels or via cholesterol independent pathways, which remains unclear. In regards to cholesterol independent pathways, several preclinical studies have shown that statins can inhibit breast cancer cell growth by inhibiting NFkB and PTEN [19], or by inducing p21 and p27 [20], inhibit proliferation, and increase apoptosis [21, 22]. Studies have also shown that ER-negative cell lines were more sensitive to statins [23], and that statins could induce suppression of TNBC via PI3 K pathway activation [24]. However, statins may achieve their cancer preventive effects by both mechanisms.

Hypercholesterolemia has been associated with an increased breast cancer risk in several studies [25, 26]. It appears that its metabolite 27-hydroxycholesterol is especially involved in promoting ER-positive breast cancer growth [27, 28]. Furthermore, a recent study has shown that cholesterol can mask membrane glycosphingolipid (GSL) tumor-associated antigens and reduce their immune detection in several cancers, such as breast, colon, and prostate cancer [29]. Therefore, reduction of cholesterol by statins (and exercise) can ‘‘unmask’’ GSL and thereby increase immune surveillance and detection of pre-cancer or cancer cells.

Another suggested anticancer or prevention benefit from lowering cholesterol comes from data showing that a reduction in cholesterol downregulates matrix metalloproteinase which could play a role in decreased initiation and progression of breast cancer [30]. Indeed, preclinical studies have shown that cholesterol sulfate can promote the proteolytic activity of matrix metalloproteinase [31]. Decrease in cholesterol levels could reduce matrix metalloproteinase activity, thereby affecting cancer risk.

Another question is how statins modulate HMG-CoAR expression in breast tissue and whether this could have any clinical preventive implications. One interesting study showed that HMG-CoAR expression in 511 breast cancer tumors was associated with favorable factors such as smaller tumor size, low grade, low-Ki 67, and high p27 expression [32]. In a subsequent study, which was a presurgical window of opportunity study in 50 women with breast cancer, atorvastatin at 80 mg/day was evaluated. In that study, 2 weeks of atorvastatin upregulated HMG-CoAR levels significantly and reduced Ki-67 in tumors that had a higher HMG-CoAR expression [33]. Whether an increase in HMG-CoAR levels is a marker of statin benefit or whether it has an independent antitumor, or potential preventive effect, needs to be evaluated in future studies.

We did not show a modulation in breast tissue biomarkers by HMG-CoA Reductase genotype (rs12654264, AA, AT, and AW). There is very limited data about this genotype within the context of breast cancer prevention trials and only 2 studies have evaluated the association with this genotype and modulation of biomarkers and have shown no correlation [8, 9]. However, in our study we did show that change in cholesterol and LDL was more pronounced in the AA genotype. This was not reported in previous breast prevention trials. One study evaluated the association between statin use and colorectal cancer, and identified that cancer risk was lower in individuals with the AA genotype of this gene and that this genotype was associated with lower LDL levels [34]. It is intriguing to think that, based on our results, further studies could evaluate whether certain patients with this genotype could benefit more from statin prevention than other genotypes and whether high-risk patients could be selected for certain drugs based on their genotype.

In conclusion, in the current study we found that atorvastatin and its metabolites were detectable in breast fine needle aspiration specimens and atorvastatin use was associated with a decrease in CRP. However, strong modulations of other biomarkers were not found. To the best of our knowledge, successful measurement of atorvastatin and metabolites in serum and breast tissue have not been reported previously in short-term breast prevention trials using. Atorvastatin should be further studied in phase II breast cancer prevention studies as a single agent or in combination with other potential agents including an evaluation of inflammation and lipid pathway biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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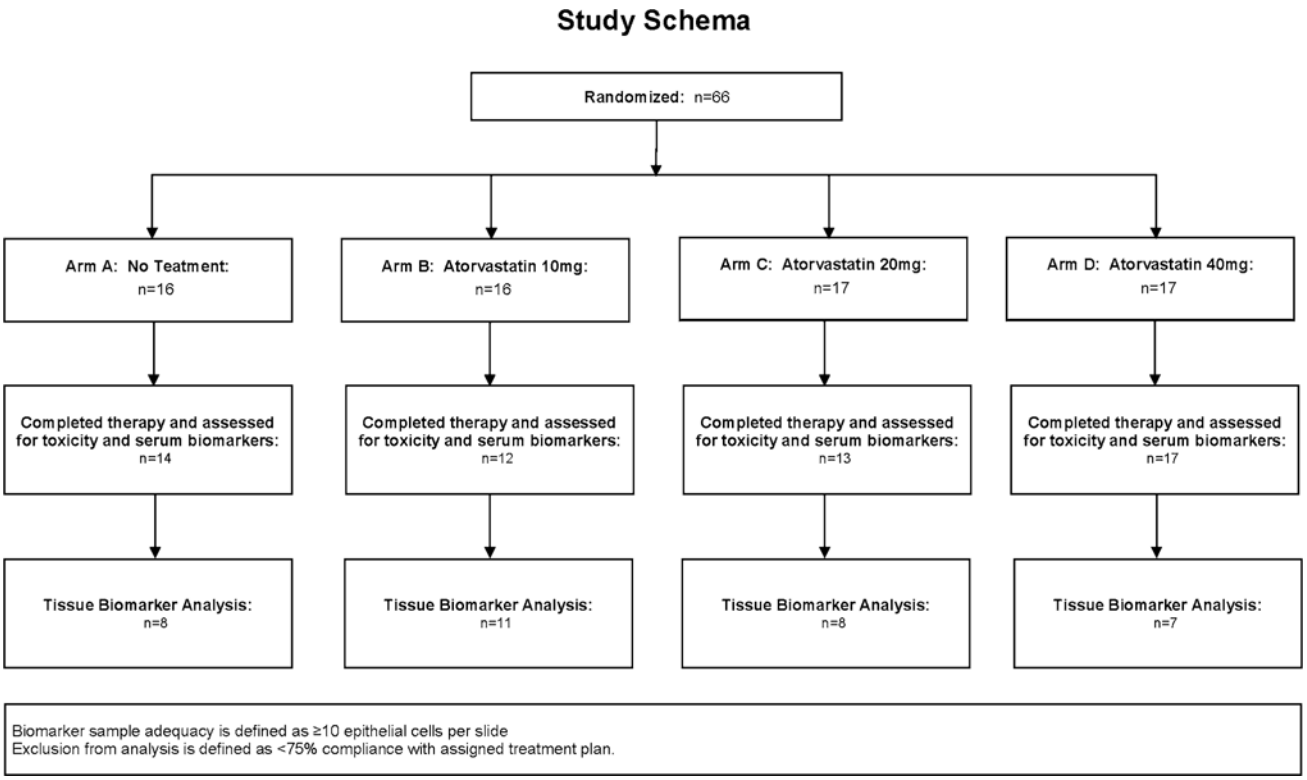

Fig. 1.

Phase I study schema designed to identify the lowest dose of atorvastatin that would induce modulation in cancer risk biomarkers in breast tissue and serum

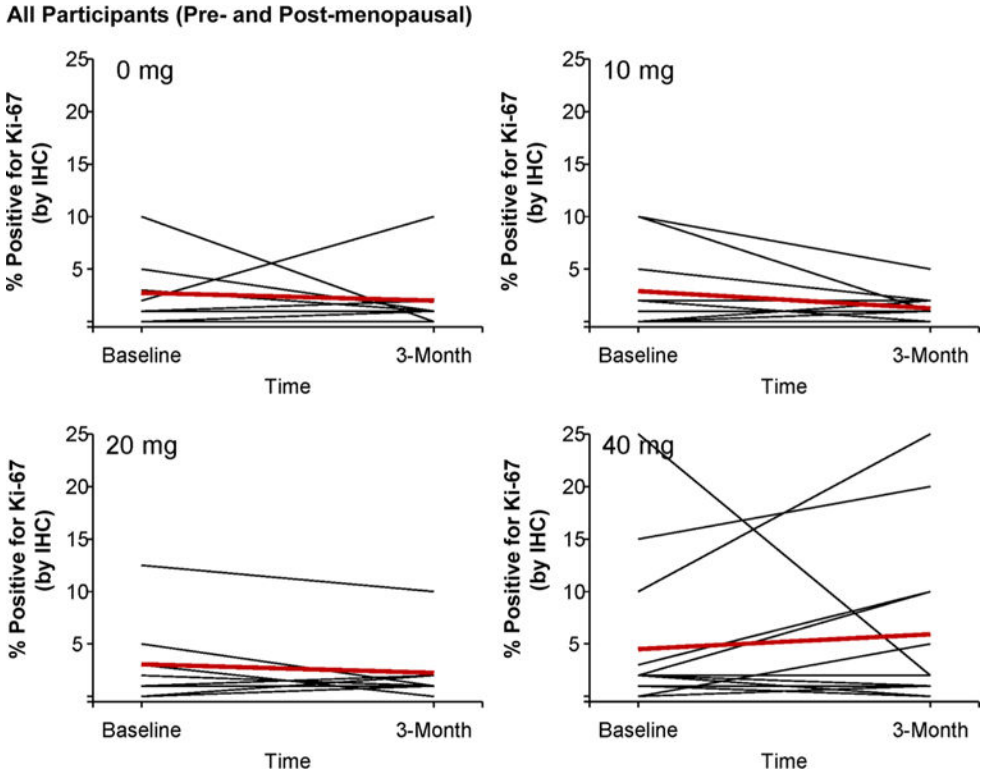

Fig. 2.

Modulation in Ki-67 expression induced by pre-and post-atorvastatin treatment. Patients who signed the informed consent were randomized into four arms: no treatment or 10 mg/20 mg/40 mg atorvastatin for 3 months followed by blood draw and FNA. Proliferation and apoptosis markers were immunostained in pre- and post-treatment FNA samples using unstained Thin-Prep slides. Staining for Ki-67 was performed in the pathology immunohistochemistry core laboratory and scoring of individual biomarker expression levels were recorded. No significant change in Ki-67 between treatment arms and the control group was observed. There was some variability in terms of decrease/increase in Ki-67 levels among patients in the 40 mg treatment arm

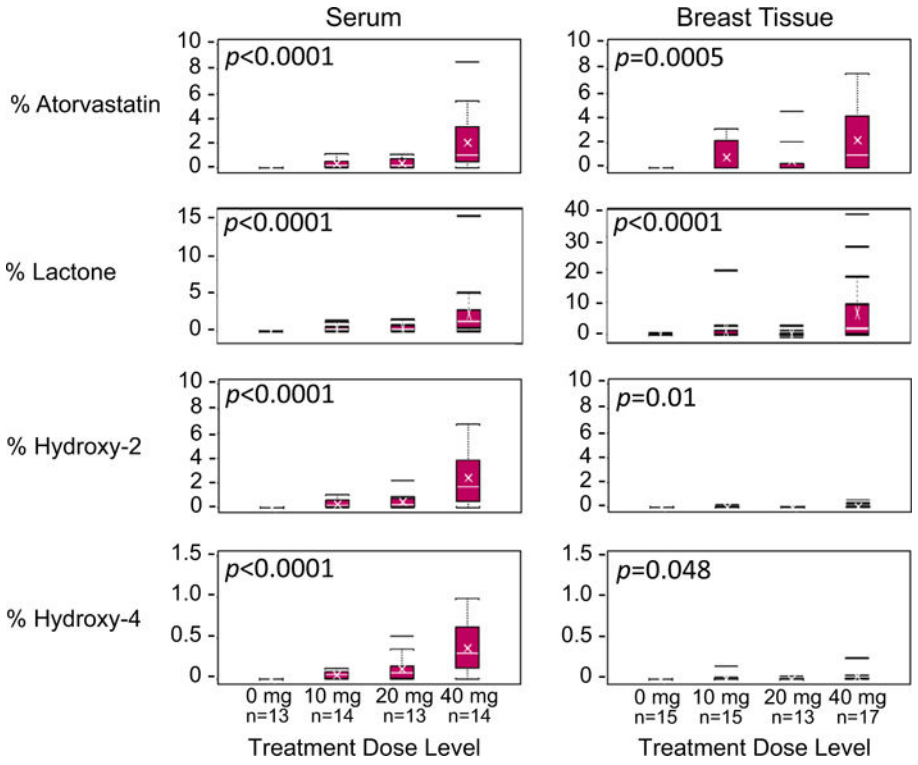

Fig. 3.

Target engagement of atorvastatin and other metabolites in serum and breast tissue. Atorvastatin, its metabolites (2-hydroxyatorvastatin and 4-hydroxyatorvastatin), and lactone were significantly increased both in serum and breast tissue in all the treatment arms with the maximum in 40 mg arm. No trace of atorvastatin and its metabolites were seen in the control arm indicating that the drug reached its target

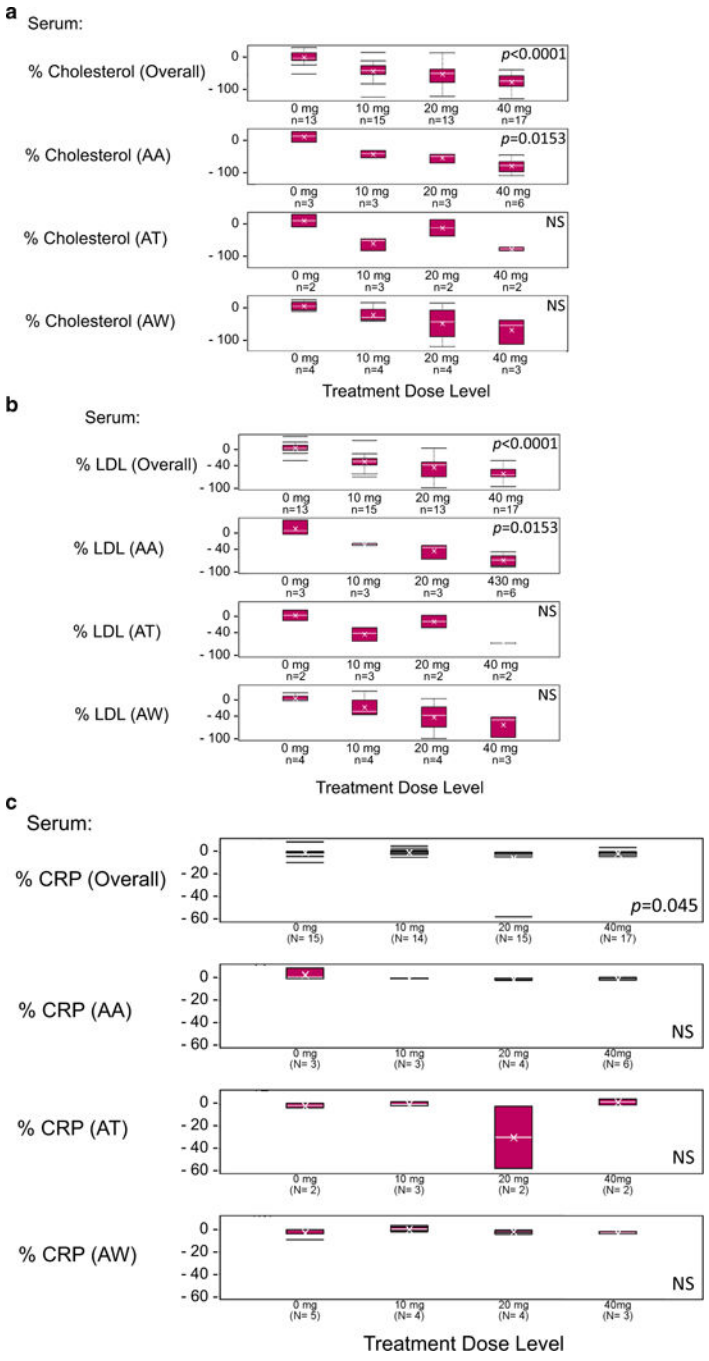

Fig. 4.

Modulation in breast tissue and serum biomarkers by HMG-CoA reductase genotype associated with atorvastatin treatment. Single nucleotide analysis of the HMG-CoA Reductase gene (rs12654264) showed that 50 % of patients were AA genotype, 33.9 % AW, and 16.1 % AT genotype. Evaluation of changes in biomarkers by these different genotypes was not associated with breast tissue biomarkers. Significant changes in serum biomarkers were observed by genotype. **a** Specifically, significant decrease in cholesterol was seen overall and in the AA genotype ($p = 0.0153$) but not in AT and AW. **b** A substantial decrease

in LDL was also seen overall and in the AA group ($p = 0.0078$), but not AT and AW. **c** Overall, there was a reduction in CRP ($p = 0.045$) for the whole cohort that was not pronounced in any specific genotype group

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Table 1

Demographics of the study population (all enrolled subjects, $n = 66$)

Patient Characteristics	Single No Treatment Arm		Three Atorvastatin Treatment Arms (3 months of treatment in each arm)						Total	
	A: 0 mg/day ($n = 16$; 24 %)		B: 10 mg/day ($n = 16$; 24 %)		C: 20 mg/day ($n = 17$; 26 %)		D: 40 mg/day ($n = 17$; 26 %)		Arms: A–D ($n = 66$; 100%)	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Demographics										
Age										
Mean	55		54		51		48		52	
Std dev		7		8		10		9		9
Range		42–69		36–64		27–64		39–63		27–69
Race										
White	14	(88)	16	(100)	15	(88)	15	(88)	60	(91)
Black	1	(6)	0	(0)	1	(6)	0	(0)	2	(3)
Asian	0	(0)	0	(0)	0	(0)	1	(6)	1	(1)
Unknown	1	(6)	0	(0)	1	(6)	1	(6)	3	(5)
Ethnicity										
Hispanic	2	(13)	2	(13)	3	(18)	1	(6)	8	(12)
Non-Hispanic	4	(87)	14	(87)	14	(82)	16	(94)	58	(88)
Menopausal status										
Pre	5	(31)	8	(50)	5	(29)	10	(59)	28	(42)
Post	11	(69)	8	(50)	12	(71)	7	(41)	38	(58)
Eligibility By										
History of										
DCIS	6	(38)	1	(6)	4	(24)	4	(23)	15	(23)
LCIS	1	(6)	2	(13)	1	(5)	2	(12)	6	(9)
ADH	3	(18)	2	(13)	8	(47)	3	(18)	16	(24)
High projected risk *	6	(38)	11	(68)	4	(24)	8	(47)	29	(44)

Atypical lobular hyperplasia (ALH); Ductal carcinoma in situ (DCIS); Lobular carcinoma in situ (LCIS); Standard Deviation (Std Dev)

* High projected risk is defined as either a 5-year risk>1.67 % calculated by the Gail risk assessment model, or a lifetime risk>20 % calculated by the Claus, Tyrer-Cuzick, Boadicea or BRCAPRO breast cancer risk models

Table 2

Subject toxicities following treatment with atorvastatin versus no treatment

Characteristic and reported appearance	Three Atorvastatin Treatment Arms (3 months of treatment in each arm)											
	Arm B: 10 mg/day (*n* = 16; 24.2 %)				Arm C: 20 mg/day (*n* = 17; 25.8 %)				Arm D: 40 mg/day (*n* = 17; 25.8 %)			
	Grade I		Grade II		Grade I		Grade II		Grade I		Grade II	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Nausea	1	(6)	0	(0)	0	(0)	1	(6)	0	(0)	0	(0)
Fatigue	1	(6)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Rash	1	(6)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Swelling	0	(0)	1	(6)	1	(6)	0	(0)	1	(6)	0	(0)
Myalgia	0	(0)	1	(6)	1	(6)	1	(6)	0	(0)	0	(0)
Arthralgia	0	(0)	1	(6)	1	(6)	0	(0)	0	(0)	0	(0)
Cramping	1	(6)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Dry Eye	0	(0)	0	(0)	0	(0)	0	(0)	1	(6)	0	(0)
Dry Lip	0	(0)	0	(0)	0	(0)	0	(0)	1	(6)	0	(0)
Thirst	0	(0)	0	(0)	0	(0)	0	(0)	1	(6)	0	(0)

Table 3

The effects of atorvastatin treatment on biomarker expression

Biomarkers	Single No Treatment Arm		Three Atorvastatin Treatment Arms (3 months of treatment in each arm)					
	A: 0 mg/day		B: 10 mg/day		C: 20 mg/day		D: 40 mg/day	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Tissue biomarkers								
Proliferation								
Ki67								
*N**	8	8	11	11	8	8	14	14
Mean	2.2	2.0	2.4	2.0	3.0	2.0	4.5	5.3
Std dev	3.1	3.1	3.5	2.9	3.8	2.9	7.0	7.5
p value	0.19							
EGFR								
*N**	3	3	6	6	3	3	5	5
Mean	0	0	3.33	1.9	3.3	0.8	5.0	5.0
Std dev	0	0	5.2	3.8	5.8	2.0	11.2	8.7
p value	0.92							
pEGFR								
*N**	3	3	7	7	4	4	6	6
Mean	5	0	2.5	11.4	0	24	13.6	7.9
Std dev	8.66	0	3.8	15.7	0	32.9	19.7	12.2
p value	0.12							
Apoptosis								
Bcl-2								
*N**	8	8	10	10	4	4	7	7
Mean	15.6	3.7	10.8	5.4	1.6	3	8	7
Std dev	16.7		67	18	3.8	6.7	17.6	4.1
p value	0.8							
Serum biomarkers								
Lipid profile								
Cholesterol								

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Biomarkers	Single No Treatment Arm		Three Atorvastatin Treatment Arms (3 months of treatment in each arm)					
	A: 0 mg/day		B: 10 mg/day		C: 20 mg/day		D: 40 mg/day	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
*N**	13	13	15	15	13	13	17	17
Mean	227	232	200	161	220	169	205	134
Std dev	55	54	35	43	26	36	33	27
p value	\0.0							
LDL								
*N**	13	13	15	15	13	13	17	17
Mean	123	128	104	72	127	780	112	51
Std dev	34	40	24	34	25	27	32	22
p value	\0.0							
HDL								
*N**	13	13	15	15	10	10	17	17
Mean	59	59	73	73	68	67	67	66
Std dev	14	12	21	22	23	13	17	19
p value	0.48							
Inflammation								
CRP								
*N**	15	15	14	14	15	15	17	17
Mean	4.4	5	2.5	2.7	6.0	2.0	2.4	1.9
Std dev	3.8	4.4	3.4	3.2	14.3	2.1	2.2	1.8
p value	0.04							

N*: Number of samples analyzed; B cell CLL/lymphoma 2 (Bcl-2); C-reactive protein (CRP); Epidermal growth factor receptor (EGFR); high-density lipoprotein (HDL); antigen Ki-67 (Ki-67); low-density lipoprotein (LDL); phosphorylated-EGFR 4 (pEGFR); Pre-treatment (Pre); Post-treatment (Post); Standard deviation (Std Dev). For Ki67, EGFR, pEGFR, bcl-2 % of positive-stained epithelial cells were recorded