A Prospective, Randomized, Placebo Controlled Study of a Combination of Simvastatin and Chemotherapy in Metastatic Breast Cancer

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Abstract

Background: Preclinical studies support anticancer activity of statins, however, the existing clinical evidence are inconsistent and not definitive. Our study aimed at evaluating a postulated cancer chemo sensitizing effect of statin (simvastatin) in a cohort of metastatic breast cancer (MBC) patients.

Methods: We designed a prospective, single-centered, randomized, double blinded, and placebo controlled trial that encompassed MBC patients with an ECOG Performance Status scale ≤2 and undergoing a chemotherapy course consisting of carboplatin and vinorelbine at Al-Baironi Hospital, Damascus, Syria. Patients were enrolled between August 2011 and July 2012, and randomly allocated to receive a 15-day course of either simvastatin (40 mg) or placebo at the day -7 of each chemotherapy cycle. Primary endpoints were objective response rate (ORR) and toxicity, and secondary endpoint was overall survival (OS).

Results: Eighty-Two patients met the inclusion criteria and consented. ORR (35% vs. 32.5%) and predominant toxicity; grade≥3 neutropenia (occurred in 30% vs. 40% of the patients) were not significantly different between simvastatin and placebo groups, respectively. Over a median follow-up of 44 months (range, 10-60), median OS was 15 months in simvastatin group and 17 in placebo group (Hazard ratio (HR)=1.16, 95% CI (0.70-1.91), P =0.57). Elevated baseline values of high-sensitivity C-reactive protein (hsCRP >10 mg/l), lactate dehydrogenase (LDH >480 U/L) and chemotherapy being ≥2 nd line significantly associated with shorter OS for the total cohort in both univariate and multivariate analyses.

Conclusions: Our data prove a safe profile of simvastatin at 40 mg per day combined with carboplatin and vinorelbine in MBC patients, but without any beneficial increase of tumor sensitivity to chemotherapy. Moreover, we demonstrated a strong clinical advantage of baseline values of hsCRP and LDH as useful prognostic tools in MBC patients. Trial registration: Damascus University / 15073/. Registered on December 28, 2010.

Background

Breast cancer is a major public health problem for women worldwide (1). Early diagnosis and advances in treatments (i.e., chemotherapy, hormone therapy, and targeted therapies) have led to remarkable increases in survival rates. Nevertheless, a substantial number of patients will still develop metastases during the course of their disease (2). Based on the currently available therapeutic options, cure of metastatic breast cancer (MBC) is a rather elusive goal, and palliative care can only help maintain quality of life while possibly prolonging survival (3). Developing new therapeutic approaches for MBC is a time, patience and diligence demanding research area (4). Identifying new uses for established multi-modes of action drugs (i.e., statins, metformin, and aspirin), also known as “drug repositioning”, represents a less costly and time sparing evolving approach (5).

Statins, or 3-hydroxy–3-methylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors, are well established cholesterol-lowering agents that commonly used in the primary and secondary prevention of cardiovascular disease (5). Moreover, statins also inhibit the synthesis of essential isoprenoid intermediates required for activation of various intracellular signaling proteins known to play indispensable role(s) in multiple cellular processes, suggesting a pleiotropic nature of statins' effects. Beyond their lipid lowering properties, statins possess anti-inflammatory, antioxidant, and anti-proliferative effects, which fed the growing interest in the therapeutic potential of statins in multiple treatment areas including oncology (6). "Statin repositioning" was supported by in vitro and in vivo studies that have shown a wide range of anticancer activities, including induction of apoptosis, inhibition of tumor cell proliferation, and reduction of invasiveness and metastasis (6, 7, 8). In addition, observational studies have provided substantial evidence that statins use is associated with a reduction in cancer incidence and mortality in several cancer types including breast cancer (9, 10, 11). A few clinical studies has shown a positive role of lipophilic statins both as neo-adjuvant therapy (i.e., before surgery) (12, 13) and in secondary prevention in breast cancer survivors (14). Nevertheless, despite the growing evidence of synergistic effects of statins with chemotherapeutic drugs in other cancer types (15), no clinical studies investigated combination(s) of statins with standard treatment protocols in breast cancer.

Therefore, we designed this study to investigate the chemo-sensitizing effects of short-term treatment of simvastatin, at clinically relevant doses (40 mg), in MBC patients receiving a combination of carboplatin and vinorelbine. Although not universally accepted as the standard treatment for MBC (16), vinorelbine in combination with carboplatin is adapted in the clinical practice for MBC patients in "Al-Baironi" the major oncology Hospital in Syria. Since inflammation is a critical component of tumor progression, and due to the well-established anti-inflammatory properties of statins (17), we sought to investigate the impact of statins on some inflammatory markers (high-sensitivity C-reactive protein (hsCRP), lactate dehydrogenase (LDH)) and their prognostic potential in predicting therapeutic outcomes in MBC.

Methods

Trial Design and Eligibility

This was a prospective, single-centered, randomized, double blinded, placebo controlled study. The study protocol was approved by the scientific research ethics committee at the Faculty of Pharmacy, Damascus University. The eligibility criteria were as follows: female patients attending the breast cancer unit at Al-Baironi Hospital, with confirmed diagnosis of metastases (stage IV) prior to commencing chemotherapy course consisting of carboplatin and vinorelbine; age between 20 and 75 years; adequate function of major organs (including cardiac, hepatic and renal functions); and an ECOG Performance Status score $\leq$2. Pregnant patients and those with previous treatment with statins or carboplatin and vinorelbine within 30 days of the study entry were excluded. All patients provided written informed consent and enrolled between August 2011 and July 2012. All treatments were double blinded to assure that neither oncologists involved the study nor do participants know which type of preparation is administered. The

follow-up lasted until death or the cutoff date of July 2017. Primary endpoints were objective response rate (ORR) and toxicity, and secondary endpoint was overall survival (OS) over the follow-up period.

Treatment Protocol

Statin or placebo was provided in outpatient setting. Patients were assigned (1:1 or 2:2 ratio) to each treatment group (carboplatin and vinorelbine plus simvastatin or placebo) using randomization with metastasis sites as stratification factors. Simvastatin 40 mg and placebo (provided in boxes of identical shape and sequentially numbered) were generous gifts from ALFARES Pharmaceuticals Co (Damascus, Syria). Chemotherapy regimen was conducted every 3 weeks according to the hospital protocol as follows; carboplatin (Carboplatin "Ebewe"), Area under the curve (AUC) 4, intravenously on day 1 and vinorelbine (Navelbine®) intravenously (25 mg/m^2) or orally (60 mg/m^2) on days 1 and 8 of each cycle. Simvastatin 40 mg or placebo administered orally once daily for 15 days starting at the day −7 of each chemotherapy cycle. Additionally, patients with bone metastases were treated with zoledronic acid via intravenous infusion (4 mg every 4 weeks), and palliative radiotherapy was allowed for brain metastases if needed. Study treatment was supposed to be continued in the absence of progression or until another termination criterion was met, including unacceptable toxicity, consent withdrawal, loss to follow-up or death.

Response and Toxicity Assessment

Each patient underwent an initial evaluation within one week prior to commencing treatment. Evaluation encompassed full medical history, physical examination, chest X-ray, computed tomography (CT) scan, bone scan, magnetic resonance image (MRI) scan and laboratory analyses; including complete blood counts (CBC), creatine kinase (CK), creatinine, alanine aminotransferase (ALT), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), hsCRP, LDH and tumor markers; carcinoembryonic antigen (CEA) and cancer antigen 15–3 (CA15–3). With the exception of tumor markers, laboratory tests were performed at each cycle before chemotherapy administration on day 1, whereas TC, LDL-C and HDL-C were repeated on day 8. To assess tumor progression, physical examination, tumor markers and radiological studies were conducted at baseline and every three cycles, and bone scan was repeated by the end of the sixth cycle. Patients' response was classified according to the response evaluation criteria in solid tumor (RECIST) (version 1.1) as follows; complete response (CR), complete disappearance of clinical evidence of disease for a minimum of 8 weeks; partial response (PR), decreased in tumor burden ≥30%; stable disease (SD), decreased by <30% or increased by <20%; progressive disease (PD), increased in tumor burden by ≥20%; and non-evaluable response, due to specific reasons (e.g., early death or toxicity). ORR was calculated based on both CR + PR. Treatment related-toxicity was graded according to the Common Terminology Criteria for Adverse Events, version 4. In case of chemotherapy-related toxicity, including grade 2 neutropenia before the start of each cycle, treatment was delayed for 1 week. For grade 3/4 neutropenia, granulocyte colony-stimulating factor (G-CSF) was administered subcutaneously for 3 days at a dose of 5 μg/kg. For simvastatin toxicity,

treatment was planned to be discontinued if the serum transaminase was of more than 3 times the upper limit of the reference range or if the CK concentration was more than 5 times the upper limit of the reference range. OS was defined as time from study entry to death from any cause.

Statistical Analysis

Statistical analysis was performed using Graphpad Prism® (version 5) except for Cox proportional hazard regression models that were performed using SPSS® (version 22) to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for both univariate and multivariate analyses. Between group comparisons were performed using Chi-square test for categorical variables, unpaired T test for normally distributed data and Mann-Whitney test for data that were not normally distributed (hsCRP and LDH). Within each group, comparisons for lipid values (pre- vs. post-chemotherapy) were performed using paired T test. Median overall survival was estimated using Kaplan-Meier analysis. Statistical significance was tested using the Log-rank test, and two-tailed *P* value < 0.05 was considered significant. The median follow-up was estimated using reverse censoring for overall survival.

Results

Patient Characteristics

The eighty-two MBC patients who enrolled were randomly assigned to treatment groups; 41 patients to the carboplatin and vinorelbine plus simvastatin and 41 patients to the carboplatin and vinorelbine plus placebo. The two treatment groups were well balanced in terms of their baseline characteristics as shown in Table 1.

The median age of all patients was 47.5 years (range 24–74 years). The ECOG-performance status was 1 in the majority of patients (73.17%). Fifty-Six patients (68.29%) were positive for HER2 (human epidermal growth factor receptor–2), and 39 patients (47.56%) were ER/PR negative (estrogen receptor/progesterone receptor). Forty-two patients (51.22%) had two or more sites of metastases. The chemotherapy regimen was the first line in 37 patients (45.12%) and the second in 36 (43.9%).

Treatment Outcomes

Of the 82 patients enrolled in our study, 80 patients (97.6%) received at least 1 cycle of chemotherapy (median 4.5 cycles; range, 1–12 cycles). The remaining 2 patients (2.4%) withdrew consent. Only 77 patients were assessable for response by the end of the chemotherapy course; as 3 patients were classified not evaluable for response due to early death (n = 2) and chemotherapy toxicity (n = 1), as illustrated in Figure 1.

In the simvastatin group, one patient had a complete response, 13 patients had a partial response, and the ORR was 35%. Similarly, four patients had a complete response, nine patients had a partial response,

and the ORR was 32.5% in the placebo group. No significant differences were found between the two treatment groups with regard to the response assessment results (P = 0.57), Table 2.

All patients who received at least one dose of therapy were assessable for toxicity. Most common grade 3 or higher adverse events were neutropenia (30% and 40% in the simvastatin and placebo group, respectively), and anemia (20% in both groups). The addition of simvastatin did not result in clinically significant increase in chemotherapy related toxicities (no cases of CK elevated ≥five times the upper limit of the normal range or ALT ≥three times the upper limit of the normal range), as shown in Table 3.

By the end of a median follow-up period of 44 months (range, 10–60), an overall 65 fatalities were recorded. Median OS was not significantly different between the simvastatin group (15 months) and the placebo group (17 months) (HR = 1.16, 95% CI (0.70–1.91), p = 0.57),as depicted in Figure 2.

We analyzed the difference(s) of overall survival outcomes between subgroups classified according to baseline characteristics including age, ECOG-PS, hormone receptors status, HER2 status, number of metastatic sites, chemotherapy line's gradeand baseline levels of CEA, CA15–3, hsCRP and LDH. Univariate Cox models of survival revealed significantly shorter survival of MBC patients when they were <50 years old (P = 0.026), having two metastatic sites or more (P = 0.02), elevated baseline levels of hsCRP (P = 0.002), LDH (P = 0.002), or CEA (P = 0.016), or chemotherapy being ≥2^{nd} line (P = 0.04). CA15–3 levels, ECOG-PS, HER2 and hormone receptors status did not significantly impact survival (P>0.05). After adjusting for other factors, multivariate Cox models proved that only elevated baseline levels of hsCRP (HR = 2.168, 95% CI (1.299- 3.616), P = 0.003) or LDH (HR = 2.213, 95% CI (1.273- 3.845), P = 0.005) and chemotherapy being ≥2^{nd} line (HR = 1.766, 95% CI (1.067- 2.923), P = 0.027) were still significantly predictive for shorter survival. Table 4. Demonstrates detailed results for univariate and multivariate analyses.

The impact of simvastatin on lipids and inflammatory markers

Neither baseline TC nor LDL-C levels were significantly different between the two groups. However, simvastatin clearly induced a substantial drop in TC and LDL-C levels throughout the chemotherapy cycles in comparison with placebo (P<<0.05) (see additional file 1). Thorough follow up of serum TC, LDL-C and HDL-C levels, pre- and post-chemotherapy administration at each cycle revealed a significant decrease in TC and LDL-C levels at day 8 of each chemotherapy cycle, except for the sixth, in both simvastatin and placebo groups (summarized in Table 5). Noteworthy, following the one-week drug break, all patients recovered TC and LDL-C levels to within at least 77% of their baseline by the start of the next cycle, and this pattern persisted over all treatment cycles. Serum HDL-C levels did not differ significantly between the two groups at any cycle post simvastatin exposure.

No significant changes were observed in the levels of inflammatory markers (hsCRP and LDH) during the study in any of the treatment

groups (see additional file 2).

Discussion

Recently, the cholesterol lowering-independent or “pleiotropic” effects of statins have gained greater recognition, particularly in the area of cancer therapeutics (7). Cumulative *in vivo* evidence as well as observational clinical studies suggest a therapeutic potential of statins in different cancer models (17). Nevertheless, translating these findings into clinical studies faces multi-dimensional challenges comprising; which statin to use, the timing (when or at what stage of cancer progression), the temporal frame (short-term versus long-term), and what cytotoxic agents/chemotherapy line, hormonal, or radiotherapy with which should a particular type of statin is co-administered. To our knowledge, this study represents the first clinical trial to utilize short-term simvastatin at therapeutically relevant dose (40 mg) in combination with carboplatin and vinorelbine in metastatic breast cancer patients.

The combination of carboplatin and vinorelbine is a one of the palliative treatments for MBC in Al-Baironi Hospital. Our study confirms the effectiveness of carboplatin plus vinorelbine with an ORR of 33.75%, a median OS of 16 months and 35% grade ≥3 neutropenia for all patients. Laffaioli et al reported their experience with a same therapy regimen, the ORR was 41%, median OS was 16 months and the principal toxicity was myelotoxicity; grade 3/4 leukopenia in 46% of advanced breast cancer patients (18).

Expectedly, the lipid lowering effect of simvastatin was evident by a significant decrease in total cholesterol and LDL-C levels compared with placebo. These findings suggest good compliance with the study intervention and confirm the effectiveness of simvastatin in lowering lipids within a short-term (14 days) exposure frame.

Surprisingly, a significant drop in total cholesterol (range –3.26% to –9.43%, $p<<0.05$) and LDL-C (range –2.82% to –12.73%, $p<<0.05$) levels was observed between the first and eighth day of each of the treatment cycles in patients who received chemotherapy plus placebo. These observed simvastatin-independent changes in cholesterol levels are consistent with *in vitro* experiments, where in acute myeloid leukemia (AML) samples exhibited abnormally increased demands for cholesterol following exposure to cytotoxic agents (daunorubicin or cytarabine), a phenomenon described as “defensive adaptation” of cancerous cells to increase chemo-resistance (19).

Within the 14-day simvastatin exposure in each cycle, no changes in hsCRP level was observed, suggesting that this short-term duration may not have been sufficient to exhibit anti-inflammatory benefit in MBC patients. These findings contradict others in coronary artery disease (CAD) patients, where short term exposure of simvastatin lowered hsCRP(20). We should note here that the hsCRP baseline levels are rather different between the two patient populations, (median [lower-upper quartile]; 8.18 [3.93–22.11] in MBC vs. 2.8 [1.3–4.8] mg/l or 1.1 [0.8–2.5] in female and male CAD patients, respectively). A significant reduction in serum CRP in breast cancer patients may demand a longer duration of statin therapy (e.g. at least 3 months) to demonstrate a similar effect to that observed in CAD patients (21).

Despite the promising *in vitro* and *in vivo* evidence that provided support to anti-tumor effect(s) of statins in a variety of human malignancies (15), our findings prove that clinically relevant dose of simvastatin, added to carboplatin and vinorelbine course has no clinical benefit in terms of outcome (i.e. ORR and median OS) in MBC patients. However, treatment with simvastatin in MBC proved to be very well tolerated, as no significant chemotherapy toxicity or simvastatin adverse effects were recorded.

These findings are in agreement with a study by kim and colleagues that investigated a combination of statins with capecitabine and Cisplatin in advanced gastric cancer, as no increase in progression free survival was reported (22). Similarly, gemcitabine-simvastatin at 40mg daily vs. gemcitabine-placebo resulted in no significant difference in time to progression in advanced pancreatic cancer patients (23). The overall null results in our study and others may stem from statins' conflicting properties; on one hand, they have anti-proliferative effects, but on another hand, they exhibit immune tolerance-promoting properties during tumor development. Therefore, statins might be concurrently inhibiting and promoting tumor growth (24). Another explanation may arise from the pulsatile administration of statin, which may not be enough in terms of cholesterol deprivation of cancerous cells.

There have been only a few reports on prognostic factors in patients with metastatic disease (25). Notably, not every study identifies the same set of risk factors explaining variation in prognosis following breast cancer metastasis (26). Some studies have shown that age, number of metastatic sites, ER/PR and HER2 status, ECOG-PS and baseline values of CEA and CA15–3 are valuable prognostic factors (25, 26, 27, 28). Nevertheless, our study did not provide support for a significant influence of any of these factors on survival in multivariate analysis. Variations of prognostic factors in terms of the patients' selection, availability of clinical covariates, patients' lost follow-up, lines of chemotherapy and statistical method for analysis (26, 29) may explain the differences between our results and these from previous ones.

Nevertheless, our findings prove that increased baseline serum concentrations of hs-CRP may serve as a predictor for poor prognosis among MBC patients. This result is consistent with Albuquerque et al study (30), Al Murri et al study (31) and Petekkaya et al study (32).

On another hand, Swenerton et al. was the first to report the clinical importance of serum lactate dehydrogenase in predicting survival of MBC patients (29). Our results reinforced that elevated serum LDH levels significantly correlate with poorer survival among MBC patients (32, 33).

No global consensus exists regarding the ideal treatment strategy for MBC. Thus, once first line failed, second and later lines are adapted, reflecting the clinically challenging picture of progressive disease (2). Our data showed that the grade of chemotherapy line had significant impact on survival of MBC patients.

Conclusions

The present study is the first, to the best of our knowledge, to investigate the proposed chemo-sensitizing effect of simvastatin added-on to carboplatin and vinorelbine in metastatic breast cancer. Unfortunately,

adding simvastatin to this combination did not seem to provide any additional clinical benefit but also did not result in any significant increase in toxicity. We were able to show that inflammatory markers, such as CRP and LDH, can be used to predict prognosis in patients with metastatic breast cancer. We recognize our study's limitations concerning the generalization of the conclusions based on data originated from a small sized sample of MBC patients receiving one line of chemotherapy. Larger population-based studies on different chemotherapy agents are needed to confirm or refute

any prognostic significance in MBC patients.

Abbreviations

ALT: alanine aminotransferase;*AML:* acute myeloid leukemia;*AUC:* Area under the curve; *CA15–3:* cancer antigen 15–3; *CAD:* coronary artery disease; *CBC:* complete blood counts; *CEA:* carcinoembryonic antigen;*CIs:* confidence intervals; *CK:* creatine kinase; *CR:* complete response; *CT:* computed tomography; *ER/PR:* estrogen receptor/progesterone receptor; *G-CSF:* granulocyte colony-stimulating factor; *HDL-C:* high-density lipoprotein cholesterol; *HER2:* human epidermal growth factor receptor–2; *HMG-CoA:* 3-hydroxy–3-methylglutaryl-Coenzyme A; *HRs:* hazard ratios; *hsCRP:* high-sensitivity C-reactive protein; *LDH:* lactate dehydrogenase; *LDL-C:* low-density lipoprotein cholesterol; *MBC:* metastatic breast cancer; *MRI:* magnetic resonance image; *ORR:* objective response rate; *OS:* overall survival; *PD:* progressive disease; *PR:* partial response; *RECIST:* response evaluation criteria in solid tumor; *SD:* stable disease; *TC:* total cholesterol.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Scientific Research Ethics Committee at the Faculty of Pharmacy, Damascus University (Damascus, Syria; Number: 10, Date: November 26, 2013) and performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Our study adhered to CONSORT guidelines. All eligible patients gave signed informed consent.

Consent for Publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Authors' Contributions

Individual contributions are as follows: LAY: Concept and design; data interpretation; co-writing and revision of manuscript. HA: Eligibility assessment; data acquisition and interpretation; co-writing of manuscript. MS: Eligibility assessment; treatment supervision; follow-up of enrolled patients. All authors read the final manuscript and gave their approval.

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Tables

Table 1. Baseline patients' characteristics

Total (n=82)	*P* value	Chemotherapy + placebo (n=41)	Chemotherapy + simvastatin (n=41)	Characteristics
n (%)		n (%)		
	0.73			Age (years)
47.5		49	47	Median
24-74		24-71	28-74	Range
	0.46			BMI (kg/m²)
1 (1.21)		0 (0)	1 (2.44)	<18.5
20 (24.39)		11 (26.83)	9 (21.95)	18.5-24.9
34 (41.46)		19 (46.34)	15 (36.59)	25-29.9
27 (32.92)		11 (26.83)	16 (39.02)	≥30
				No. of Metastatic Sites
40 (48.78)	0.23	17 (41.46)	23 (56.10)	1
37 (45.12)		20 (48.78)	17 (41.46)	2
5 (6.09)		4 (9.75)	1 (2.44)	3
	0.54			Site of Metastases
11 (13.41)		6 (14.63)	5 (12.20)	Bone
11 (13.41)		3 (7.32)	8 (19.51)	Liver
7 (8.54)		3 (7.32)	4 (9.76)	Lung
1 (1.22)		1 (2.44)	0 (0)	Brain
4 (4.88)		2 (4.88)	2 (4.88)	Skin/chest wall
6 (7.32)		2 (4.88)	4 (9.76)	Lymph-node
42 (51.22)		24 (58.54)	18 (43.9)	Multiple sites
	0.84			ECOG-PS
8 (9.75)		4 (9.75)	4 (9.75)	0
60 (73.17)		31 (75.61)	29 (70.73)	1
14 (17.07)		6 (14.63)	8 (19.50)	2
	0.46			Hormone Receptor
27 (32.92)		13 (31.71)	14 (34.15)	ER+ PR+
39 (47.56)		21 (51.22)	18 (43.90)	ER- PR-
6 (7.32)		4 (9.76)	2 (4.88)	ER+ PR-
6 (7.32)		1 (2.44)	5 (12.20)	ER- PR+
4 (4.88)		2 (4.88)	2 (4.88)	Unknown
	0.6			HER 2
56 (68.29)		26 (63.41)	30 (73.17)	HER+
22 (26.82)		13 (31.71)	9 (21.95)	HER-
4 (4.88)		2 (4.88)	2 (4.88)	Unknown
	0.37			Chemotherapy line
37 (45.12)		19 (46.34)	18 (43.90)	1 st line
36 (43.90)		19 (46.34)	17 (41.46)	2 nd line
9 (10.97)		3 (7.32)	6 (14.63)	≥3 rd line

Table 2. Efficacy outcomes

Best response*	Chemotherapy+ simvastatin (n=40)	Chemotherapy+ placebo (n=40)	*P* value
	n (%)		
Complete response	1 (2.5)	4 (10)	0.57
Partial response	13 (32.5)	9 (22.5)	
Stable disease	10 (25)	11 (27.5)	
Progressive disease	14 (35)	15 (37.5)	
Not evaluable	2 (5)	1 (2.5)	

* The assessment did not include the withdrawn consent patients (n=2)

Table 3. Most common grade 1 to 4 adverse events of chemotherapy and adverse events of special interest to simvastatin

Adverse event [a] n (%)	Chemotherapy+ simvastatin (n=40)		Chemotherapy+ placebo (n=40)	
	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Anemia	34 (85)	8 (20)	32 (80)	8 (20)
Thrombopenia	5 (12.5)	1 (2.5)	4 (10)	2 (5)
Neutropenia	22 (55)	12 (30)	18 (45)	16 (40)
Clinical hemorrhage	-	-	1 (2.5)	-
Injection site reaction	7 (17.5)	-	5 (12.5)	-
Rash	-	-	1 (2.5)	-
Left ventricle function	1 (2.5)	-	-	1 (2.5)
Stomatitis	-	-	1 (2.5)	-
Creatinine elevation	6 (15)	-	5 (12.5)	-
Simvastatin special adverse events				
ALT elevation*	12 (30)	-	13 (32.5)	-
CK elevation[#]	1 (2.5)	-	4 (10)	-

* None of the elevated levels of ALT exceed more than 3 times the upper limit of the reference range

[#] None of the elevated levels of CK exceed more than 5 times the upper limit of the reference range

[a] P value >>0.05 for all comparisons

Table 4. Factors associated with overall survival for total cohort

Multivariate Analysis		Univariate Analysis		Variables
P value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	
				hsCRP (mg/l)
0.003	2.168 (1.299- 3.616)	**0.002**	2.210 (1.339-3.647)	>10 vs. ≤ 10
	2.213 (1.273- 3.845)			**LDH (U/l)**
0.005		**0.002**	2.335 (1.351-4.036)	> 480 vs. ≤ 480
				CEA (ng/ml)
		0.016	1.842 (1.119-3.033)	>5 vs. ≤ 5
				CA15-3 (U/ml)
		0.444	1.289 (0.673-2.469)	>30 vs. ≤ 30
				Age (years)
		0.026	0.555 (0.330-0.932)	≥50 vs. <50
				ECOG-PS
		0.068	2.352 (0.939-5.893)	1/2 vs. 0
				HER2
		0.973	1.009 (0.583-1.747)	(-) vs. (+)
				Hormone Receptors (HR)
		0.133	0.681 (0.412-1.124)	(-) vs. (+)
				No. of metastatic sites
		0.020	1.802 (1.095-2.965)	≥ 2 sites vs. 1 site
				Chemotherapy line
0.027	1.766 (1.067- 2.923)	**0.040**	1.692 (1.025-2.795)	≥ 2 nd line vs. 1 st line

Table 5. Differences in TC, LDL-C and HDL-C levels during each chemotherapy cycle

Variable	Simvastatin		Placebo	
	Differences between 1 st & 8 th day of each chemotherapy cycle			
	Mean (%)	*P* value	Mean (%)	*P* value
TC (mg/dl)				
Cycle 1	-15.67 (-9.54%)	**0.007**	-18.13 (-8.57%)	**0.003**
Cycle 2	-15.91 (-9.07%)	**0.013**	-20.47 (-9.43%)	**0.0002**
Cycle 3	-13.58 (-7.81%)	**0.018**	-13.52 (-6.6%)	**0.017**
Cycle 4	-16.05 (-9.01%)	**0.013**	-19.45 (-8.71%)	**0.002**
Cycle 5	-14.29 (-8.43%)	**0.016**	-20.13 (-9.16%)	**0.001**
Cycle 6	-8.17 (-4.85%)	0.3	-7 (-3.26%)	0.3
LDL-C (mg/dl)				
Cycle 1	-10.42 (-11.75%)	**0.006**	-16 (-12.73%)	**0.001**
Cycle 2	-16.85 (-16.77%)	**0.0006**	-8.71 (-6.7%)	**0.014**
Cycle 3	-10.02 (-9.84%)	**0.018**	-9.84 (-7.85%)	**0.006**
Cycle 4	-11.41 (-10.94%)	**0.008**	-15.59 (-11.4%)	**0.0002**
Cycle 5	-6.29 (-6.44%)	0.2	-7.31 (-5.53%)	**0.056**
Cycle 6	-7.17 (-7.31%)	0.29	-3.85 (-2.82%)	0.6
HDL-C (mg/dl)				
Cycle 1	0.52 (1.06%)	0.5	-3.47 (-6.77%)	0.1
Cycle 2	0.06 (0.12%)	0.9	-4.65 (-8.58%)	0.06
Cycle 3	0.03 (0.07%)	0.9	-0.55 (-1.12%)	0.8
Cycle 4	0.22 (0.46%)	0.9	0.23 (0.45%)	0.9
Cycle 5	-0.21 (-0.45%)	0.9	-5.31 (-9.38%)	**0.03**
Cycle 6	0.23 (0.48%)	0.9	-0.46 (-0.84%)	0.8

Figures

Figure 1.

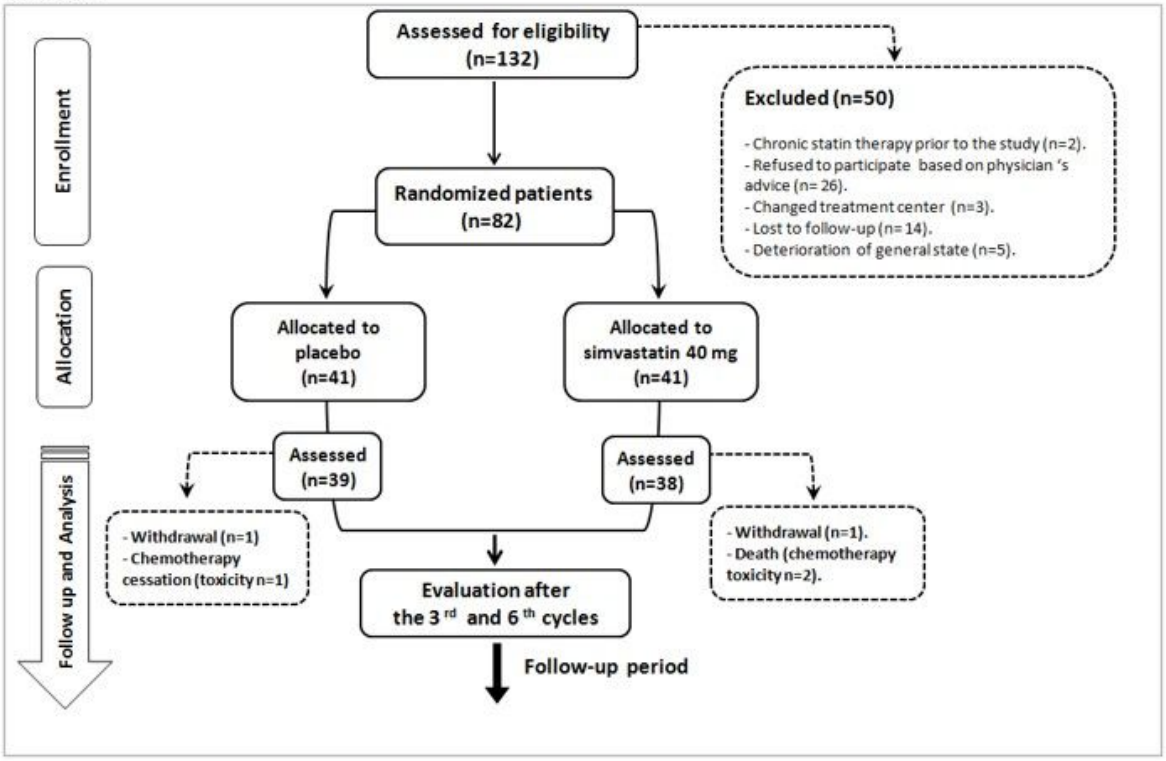

Figure 1

CONSORT flow chart

Figure 2.

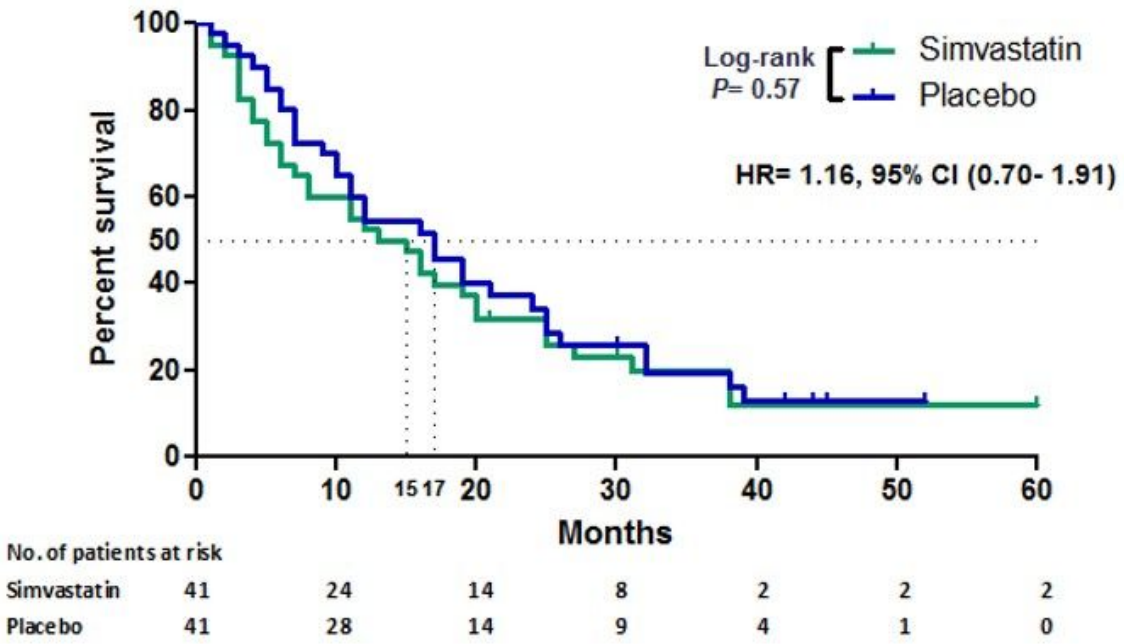

Figure 2

Survival for 60 months. Survival curve with reference to treatment groups (simvastatin vs. placebo) for MBC patients who were treated with palliative chemotherapy (carboplatin and vinorelbine). Median survival was estimated during a 60-month follow up period using Kaplan-Meier method. Statistical significance was assessed using the Log-rank test and two-tailed P value of <0.05 was considered significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CONSORT2010Checklist.doc
- additionalfile2.docx
- additionalfile1.docx