CLINICAL TRIAL

Targeting HMG-CoA reductase with statins in a window-of-opportunity breast cancer trial

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Abstract Lipophilic statins purportedly exert anti-tumoral effects on breast cancer by decreasing proliferation and increasing apoptosis. HMG-CoA reductase (HMGCR), the rate-limiting enzyme of the mevalonate pathway, is the target of statins. However, data on statin-induced effects on HMGCR activity in cancer are limited. Thus, this pre-operative study investigated statin-induced effects on tumor proliferation and HMGCR expression while analyzing HMGCR as a predictive marker for statin response in breast cancer treatment. The study was designed as a window-of-opportunity trial and included 50 patients with primary invasive breast cancer. High-dose atorvastatin (i.e., 80 mg/day) was prescribed to patients for 2 weeks before surgery. Pre- and post-statin paired tumor samples were analyzed for Ki67 and HMGCR immunohistochemical expression. Changes in the Ki67 expression and HMGCR activity following statin treatment were the primary and secondary endpoints, respectively. Up-regulation of HMGCR following atorvastatin treatment was observed in 68 % of the paired samples with evaluable HMGCR expression ($P = 0.0005$). The average relative decrease in Ki67 expression following atorvastatin treatment was 7.6 % ($P = 0.39$) in all paired samples, whereas the corresponding decrease in Ki67 expression in tumors expressing HMGCR in the pre-treatment sample was 24 % ($P = 0.02$). Furthermore, post-treatment Ki67 expression was inversely correlated to post-treatment HMGCR expression (rs $= -0.42$; $P = 0.03$). Findings from this study suggest that HMGCR is targeted by statins in breast cancer cells in vivo, and that statins may have an anti-proliferative effect in HMGCR-positive tumors. Future studies are needed to evaluate HMGCR as a predictive marker for the selection of breast cancer patients who may benefit from statin treatment.

Keywords HMGCR · Ki67 · Statins · Breast cancer · Mevalonate pathway

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Introduction

Statins are peroral drugs that historically have typically been prescribed as cholesterol-lowering agents. However, a growing body of literature has addressed their cholesterol-independent pleiotropic effects and suggested favorable preventive effects independent of cholesterol levels on both cardiovascular diseases [32, 41, 42] and cancer [1, 9, 14, 31].

Epidemiological support for the anti-neoplastic properties of statins has been mixed. Several studies have suggested a lower cancer incidence among statin users [9, 13, 24], whereas others have failed to confirm a decreased cancer risk [3, 6, 19, 45]. Recently, a reduced cancer mortality of 15 % was demonstrated among statin users [38]. However, prospective trials are warranted to clarify the impact of statins as an anti-cancer drug [10, 27, 44].

Lipophilic statins purportedly exert anti-tumoral effects on breast cancer by decreasing proliferation and increasing apoptosis [8, 11, 12, 22]. Although the biologic mechanisms for these actions are not fully elucidated, hydroxy-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase or HMGCR) is the well-recognized target of statins [20, 21, 26, 29]. HMGCR acts as the rate-limiting enzyme of the mevalonate pathway, which produces cholesterol, steroid-based hormones, and non-sterol isoprenoids [23, 35]. The isoprenoids demonstrate tumor-suppressive properties as regulators of important hallmarks of cancer, such as proliferation, migration, and angiogenesis [35, 37, 46]. In normal cells with a well-regulated mevalonate pathway, statin-induced HMGCR inhibition triggers a homeostatic feedback response that restores the mevalonate pathway [23]. In tumor cells, the mevalonate pathway may be deregulated by the deficient feedback regulation of HMGCR or increased HMGCR activity [11, 12]. Previous studies have demonstrated intertumoral variation of HMGCR protein expression in human breast cancer [4, 5, 7], thereby suggesting that HMGCR may be a positive prognostic marker and a potential predictive marker for tamoxifen response [5, 7]. Moreover, in response to statin treatment, the HMGCR activity revealed an adaptive induction of HMGCR expression in MCF7 breast cancer cells [18], lung cancer cells [2], and leukemia cells [47]. Currently, no in vivo statin-induced effects on HMGCR activity have been reported.

In total, the literature on statins and cancer indicates the likelihood of an association mediated by the mevalonate pathway with HMGCR as a key player. The aim of this window-of-opportunity study was to investigate the anti-proliferative impact of a 2-week, high-dose statin therapy in patients with invasive breast cancer while assessing the potential of HMGCR as a predictive marker for statin-induced alterations in tumor proliferation.

Materials and methods

Trial design

The trial was designed as a phase II study using the "window-of-opportunity" design in which the treatment-free window between breast cancer diagnosis and surgical tumor resection is used to study the biologic effects of a certain drug. In this study, atorvastatin, a lipophilic statin, was prescribed to the participants for 2 weeks pre-operatively. As a non-randomized trial, all patients received an equal daily dose of 80 mg of atorvastatin for 2 weeks. The trial was conducted as a single center study at Skåne University Hospital in Lund, Sweden. A power calculation showed that a sample size of 43 patients is sufficient to achieve 90 % power to detect a 0.5 standard deviation geometric mean Ki67-difference with a two-sided test at the alpha-level of 0.05. To safeguard against a power drop due to non-evaluable patients, a sample size of 50 was chosen. The Ethical Committee at Lund University and the Swedish Medical Products Agency approved this trial. The study has been registered at ClinicalTrials.gov (i.e., ID number: NCT00816244, NIH). The study adheres to the REMARK criteria [36].

Patients

Women diagnosed with primary invasive breast cancer who had a tumor measuring at least 15 mm and were candidates for radical surgery were eligible for participation in this study. Moreover, a performance status below two according to the European Cooperative Oncology Group (ECOG) and normal liver function as evidenced by normal levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were required at the beginning of the study for eligibility. All patients signed an informed consent form. The exclusion criteria included pregnancy, on-going hormonal replacement therapy, cholesterol-lowering therapy (i.e., including statins, fibrates, and ezetimibe), a medical history of allergic reactions attributed to compounds with a similar biologic composition to that of atorvastatin, and a history of hemorrhagic stroke. The study was opened for recruitment in February of 2009, and the pre-planned number of 50 patients was achieved in March of 2012.

Of the 50 patients enrolled in the study, a total of 42 patients completed all portions of the study. Two of the 50 patients discontinued their participation for personal reasons. One patient was excluded due to elevated levels of serum ALT before treatment initiation, and another patient was excluded because her serum ALT increased beyond the maximum reference levels following 1 week of statin treatment. Another two patients could not complete the pre-planned 2 weeks of statin treatment because their date of surgery was rescheduled to earlier dates. One patient was excluded because the diagnosis of invasive breast cancer was questioned; thus, further investigations were warranted. Finally, one patient left the study due to side effects from the treatment, i.e., nausea and dizziness.

Endpoints and tumor evaluation

The primary endpoint was a statin-induced tumor response measured by the change in tumor proliferation (i.e., Ki67 expression). The secondary endpoints were to study the potential predictive role of HMGCR expression before statin treatment evaluated by change in proliferation as well as the change in HMGCR expression after the administration of pre-surgical atorvastatin during a 2 week "window-of-opportunity" [16, 17]. Following inclusion, the participants underwent a study specific core biopsy before statin treatment initiation. Core biopsies were formalin-fixed immediately. Subsequent to the 2-week statin treatment, breast surgery was performed according to standard surgical procedures, and tumor tissue was retrieved from the primary tumor at the Department of Pathology at Skåne University Hospital, Lund, Sweden.

Formalin-fixed and paraffin-embedded tumor tissue from core biopsies and surgical samples were cut into 3–4 μm sections and transferred to glass slides (Menzel Super Frost Plus), dried at room temperature, and baked in a heated chamber for 2 h at 60°C. Deparaffinization and antigen retrieval were performed using PT Link (Dako Denmark A/S) and a high pH buffer. Staining was performed in an Autostainer *Plus* Dako Denmark A/S) using a di-amino-benzidine (DAB)-based visualization kit (K801021-2, Dako Denmark A/S). Counterstaining was performed using Mayer's hematoxylin with antibodies against Ki67 (MIB1, Cat. No M7240, Dako Denmark A/S, diluted 1:500) and HMGCR (Cat. No HPA008338, Atlas Antibodies AB, Stockholm, Sweden, diluted 1:150). All slides were stained in one batch. Western blot experiments using HPA008338 and UT-1 cell line extracts demonstrated that this antibody recognized a band migrating to ∼90 kDa, which is the expected molecular weight of HMGCR (data not shown).

Tumor tissue evaluation for Ki67 was performed via manual counting by one senior breast pathologist (DG), who was blinded to other tumor data on the same specimen and to the corresponding Ki67 staining in the sample pair. A fixed number of 400 tumor cells in both core biopsies and surgical samples were counted from representative areas of the tumor. In a similarly blinded manner, HMGCR expression was evaluated via cytoplasmic intensity using a four-grade scale (i.e., negative, weak, moderate, or strong) as previously described [4, 5, 7]. Two observers simultaneously performed the HMGCR evaluation (OB and SB).

From the 42 patients who completed all portions of the study, paired tumor samples were available from 38 patients because tumor tissue was not found in the core biopsies of four cases. For the analyses of Ki67, a minimum of 400 invasive tumor cells in both the core needle biopsies and surgical specimens were required, which was the case for the samples from 26 patients (Fig. 1).

Statistical analysis

Changes in tumor proliferation following statin treatment were evaluated on both the linear scale (i.e., absolute change) and the log scale (i.e., relative change). Analysis on the linear scale was performed by direct comparison of changes in proportions using a paired t test. After log transformation of the proportions, the same test was used also in the latter case. The average relative change was defined as the geometric mean of the Ki67 ratios. To test for differences in the ordered categorical variable, i.e., the HMGCR intensity before and after statin treatment, the McNemar-Bowker test was used. Logistic regression was used in an analysis comparing the odds of proliferation reduction in HMGCR-negative versus HMGCR-positive cases. Subgroup differences in the distribution of the ordered categorical HMGCR intensity scores were evaluated with the Mann–Whitney U test (i.e., for two groups) or with the Kruskal–Wallis test (i.e., for three groups). Spearman correlation (r_s) was used for quantification of the correlation between Ki67 and HMGCR. All tests were two-sided. For the primary and secondary aim, differences with p-values below 5 % were considered significant, whereas a more stringent cut-off is appropriate for the exploratory subgroup analyses presented in the tables. No adjustment for multiple testing was, however, performed. Two software packages, i.e., Stata version 12.1 (StataCorp LP, College Station, TX, 2012) and IBM SPSS Statistics Version 19, were used for the data analysis.
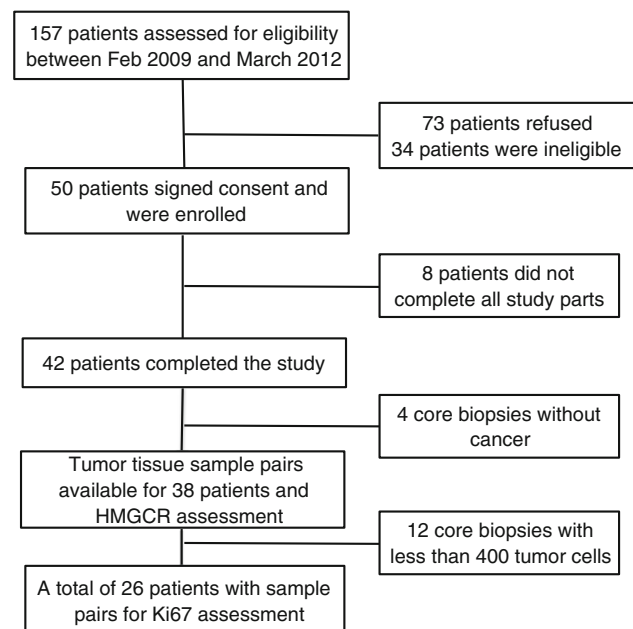

Fig. 1 Flow-chart showing study enrollment

Results

The average age of all 50 patients at the time of inclusion was 63 years with a range from 35 to 89 years, and a similar age distribution was seen among the 42 patients who fulfilled all portions of the study. All the 42 tumors that were examined were indeed invasive breast cancers with an average pathological tumor size (pT) of 21 mm and ranged from 6 to 33 mm. A vast majority of the tumors were estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2) normal, and histologic grade II or III; moreover, most had a low mitotic index. The tumor characteristics were similar for the cohort of 42 patients who completed all portions of the study and the cohort of the 26 patients for whom Ki67 was evaluable (Table 1). For the 26 complete Ki67 pairs, the mean Ki67-index at baseline was positively and significantly associated with both tumor grade and mitotic index (i.e., $P = 0.003$ and $P < 0.001$, respectively) (Table 2). Furthermore, baseline Ki67 was significantly higher in ER negative, progesterone receptor (PgR) negative, HER2 positive, and triple-negative samples. The change in Ki67 following treatment was not associated with the baseline tumor characteristics. The associations between tumor characteristics and HMGCR expression at baseline, HMGCR expression at surgery, and the change in HMGCR expression are shown in Table 3. Baseline HMGCR expression and the change in HMGCR expression were not associated with the tumor characteristics, whereas HMGCR expression in post-atorvastatin samples was positively associated with hormone receptor status.

The primary endpoint in the study, i.e., a change in the Ki67 index following 2 weeks of atorvastatin treatment, was adequately evaluated in 26 paired tumor samples. The Ki67 index had declined in the post-treatment surgical samples in 15 cases and increased in 11 cases as compared to the pre-treatment biopsy samples (Fig. 2a). In the core biopsies, the Ki67 index showed an average of 24.0 % (i.e., with a range of 4.5–87.3 %); in comparison, the average Ki67 index in the surgical samples was 21.9 % (i.e., with a range of 3.0–80.3 %). Therefore, the average absolute reduction was 2.1 percentage points ($P = 0.24$), and the average relative reduction was 7.6 % ($P = 0.39$).

The expression of the target enzyme of statins, i.e., HMGCR, and the potential statin-induced change in expression was the secondary end-point in this study. A total of 38 sample pairs were sufficiently stained and evaluable for scoring of HMGCR intensity. Among the core biopsies collected before statin treatment, HMGCR was not expressed in 37 % of the 38 evaluated samples, weakly expressed in 29 %, moderately in 26 %, and strongly in 8 % of the samples. In contrast, HMGCR expression in surgical samples from the corresponding post-statin treatment tumors was absent in 3 %, weakly expressed in 18 %, moderately expressed in 53 %,

and strongly expressed in 26 %. Out of the 38 evaluated cases, the HMGCR scores remained unchanged for nine patients; in contrast, 29 cases were discordant between the core biopsies and surgical samples, and 26 cases demonstrated an increased intensity following statin treatment (Fig. 2b). This change in HMGCR intensity score was highly statistically significant ($P = 0.0005$).

The treatment predictive value of HMGCR was tested in the analyses of tumors with any HMGCR expression in the pre-treatment biopsy samples (Fig. 3a). In this subset of patients (i.e., $n = 24$), the average absolute reduction in the Ki67 index following statin treatment was 4.6 % ($P = 0.03$), and the average relative reduction was 24 % ($P = 0.02$). Cases with absent HMGCR in the pre-treatment biopsy samples (i.e., $n = 14$) had a non-significant, slight average increase in the Ki67 index corresponding to 0.9 % ($P = 0.77$) and a non-significant 15 % increase on the relative scale ($P = 0.33$; Fig. 3b). The change in the Ki67 index in the two HMGCR subgroups was significantly different on the relative scale ($P = 0.02$) but not on the absolute scale ($P = 0.12$). Ignoring the size of the change in the Ki67 index, the odds of a reduction in the Ki67 index was 7.3 times higher in the HMGCR-positive tumors as compared to the HMGCR-negative tumors (OR = 7.3, 95 % CI: 1.3–42, $P = 0.03$). Assuming a linear trend in the Ki67 index changes over the four HMGCR categories (i.e., negative, weak, moderate, or strong), the average decrease was found to be 4.0 % ($P = 0.04$) per category, and the corresponding average relative decrease was 20 % per category ($P = 0.02$). Furthermore, post-treatment Ki67 expression was inversely correlated to post-treatment HMGCR expression (rs = –0.42; $P = 0.03$).

Analyses stratified for histologic grade (i.e., grade I/II vs grade III) and irrespective of HMGCR status showed no statin-induced change in the Ki67 index for grade I/II tumors ($P = 0.95$) and a non-significant absolute reduction of 5.7 % ($P = 0.10$) and a non-significant average relative reduction of 19 % ($P = 0.17$) for grade III tumors (Fig. 3c, d).

Discussion

Herein, we evaluated changes in tumor proliferation following a pre-operative, short-term administration of high-dose atorvastatin and observed a significant, however modest, decrease in proliferation in HMGCR-positive breast cancer. Statin effects were limited to patients with the pre-treatment expression of HMGCR, i.e., the target enzyme for statins. This study indicates that HMGCR may be a predictive marker for statin therapy as the anti-proliferative effect was insignificant in the non-stratified analyses of all tumors.

The potential to use statins as anti-cancer agents in breast cancer has been addressed in previous publications both from an epidemiological point of view [1, 3],

Table 1 Patient and tumor characteristics

	Completed all study portions $n = 42$	HMGCR/Ki67 complete pairs $n = 26$
Mean age (range)	63 (35–89)	63 (35–82)
Tumor size, mm (range)	21 (6–33)	22 (13–32)
Positive nodal status	17 (41 %)	14 (54 %)
Tumor grade (NHG)		
I	9 (21 %)	5 (19 %)
II	17 (41 %)	10 (39 %)
III	16 (38 %)	11 (42 %)
Mitotic index		
1	23 (55 %)	14 (54 %)
2	5 (12 %)	3 (12 %)
3	14 (33 %)	9 (35 %)
ER positive	37 (88 %)	23 (89 %)
PgR positive	33 (79 %)	20 (77 %)
HER2 amplified	7 (17 %)	5 (19 %)
Triple-negative	4 (10 %)	2 (8 %)

Mitotic index according to Nottingham criteria

Triple-negative if ER negative, PgR negative, and HER2 negative

NHG Nottingham histologic grade I-III, *ER* estrogen receptor, positive if >10 %, *PgR* progesterone receptor, positive if >10 %, *HER2* human epidermal growth factor receptor 2

in vitro/in vivo models [8], and in one previous human study [22]. Considering these results in conjunction with recent reviews, the need for prospective trials that consider the anti-cancer potential of HMGCR inhibitors is emerging [10, 12, 44]. As previously demonstrated, HMGCR is differentially expressed showing an intertumoral heterogeneity in human breast cancer [4, 5, 7]. These findings led to the hypothesis that statins may serve as a potential-targeted therapy in breast cancer. This study was designed as a window-of-opportunity study that allowed for the evaluation of the tumor-biologic response following an interventional therapy [16, 17]. In accordance with previous window trials, tumor response as indicated by the change in tumor proliferation measured by the Ki67 index was the primary endpoint [17, 22, 39]. Ki67 is the most widely used marker of tumor proliferation; however, several controversies regarding the counting strategies used with this marker have been raised and were recently addressed in a consensus report for Ki67 assessment [15]. In line with the recommendations from the International Ki67 in Breast Cancer Working Group, this study applied a counting strategy that is applicable for both pre-operative core biopsies and surgical samples. More specifically, we applied a strategy designed to count the average proliferation from across the entire tumor sample, not just the periphery, which is likely to be a highly proliferative zone [15]. In all surgical samples and in 26 out of 42 core

biopsies, the objective of counting 400 tumor cells was achieved. However, the number of counted tumor cells might be questioned. Previously reported data have indicated that counting a total of 400 tumor cells is sufficient for the establishment of a valid proliferation index [40]. In our previous report using tumor samples from an untreated cohort, the Ki67 indices in core biopsies and surgical samples were analyzed. The results revealed an absolute higher mean proliferation value of 3.9 % in core biopsies as compared to surgical samples. However, no consistent pattern emerged; i.e., in some cases, the Ki67 index in surgical samples would exceed the index in core biopsies. Consequently, a "correction factor" could not be developed [40]. In our previous study, Ki67 was first evaluated in hotspots. However, the Ki67 consensus report, which was published shortly after our previous study, recommended that Ki67 should be scored as an overall average score for the purpose of consistency while awaiting more robust data from the International Ki67 in Breast Cancer Working Group. In this study, that recommendation was followed, thus making any comparison to our previous hotspot-based counting method difficult. Comparing different sample types for treatment evaluation may not be optimal, and the preferable approach is to compare core biopsies taken at the time of surgery to pre-surgical core biopsies [15]. This study does not have access to core biopsies from surgery; therefore, we applied the recommendation from the consensus report, i.e., with the intention of scoring the surgical sample from fields across the entire tumor [15].

In this study, all patients received an equal dose of the lipophilic statin atorvastatin at the maximum recommended dose to optimize the chances of drug delivery into the breast cancer cells. High-dose atorvastatin was well-tolerated during the two-week administration as evidenced by the fact that only one patient withdrew from the study due to side effects. No serious adverse events were observed. In a previous window-of-opportunity trial on lipophilic statins in breast cancer, a randomized trial design in which patients received either 20 or 80 mg of fluvastatin during a period ranging from 21 to 50 days was applied [22]. All patients in the present study were treated for a period of 2 weeks. The results from the fluvastatin trial and this present study cannot be used to determine whether the duration of statin treatment influences the tumor proliferation results or not. Nevertheless, the results of the two studies were similar despite differences in statin dose and duration. Garwood et al. [22] reported a significant reduction in the Ki67 index in grade III tumors, whereas no significant reduction was demonstrated in the remaining analyses, including all of the 29 sample-pairs. The latter finding corresponds with our results. Regarding the results for the grade III tumors in the present study, we

Table 2 Association of tumor characteristics and baseline Ki67 and change in Ki67

	n	Ki67, % mean (SD) pre-atorvastatin	P	Change in Ki67 % (SD) post–pre atorvastatin	P
Age[a]	26	–	(−0.37) 0.06	–	(0.54) 0.005
Tumor size					
≤20 mm	12	25.8 (27.1)	0.64	−3.1 (7.5)	0.54
>20 mm	14	22.4 (13.3)	–	−1.2 (10.1)	–
Nodal status					
Positive	14	19.1 (13.3)	0.29	−1.6 (9.3)	0.92
Negative	12	29.7 (26.0)	–	−2.6 (8.6)	–
Tumor grade (NHG)					
I	5	13.8 (7.9)	0.003	−2.1 (3.8)	0.25
II	10	12.0 (5.7)	–	1.9 (7.5)	
III	11	39.5 (23.0)	–	−5.7 (10.5)	
Mitotic index					
1	14	12.7 (6.5)	<0.001	0.3 (6.8)	0.20
2	3	21.9 (11.8)	–	−6.3 (12.2)	–
3	9	42.3 (24.5)	–	−4.4 (10.6)	–
ER					
Positive	23	18.8 (12.1)	0.02	−1.3 (8.6)	0.40
Negative	3	63.8 (30.9)	–	−7.4 (11.1)	–
PgR					
Positive	20	15.9 (8.6)	<0.001	−0.8 (8.2)	0.30
Negative	6	51.0 (24.3)	–	−6.2 (10.6)	–
HER2 amplified					
Yes	5	33.8 (10.6)	0.03	−6.8 (10.6)	0.20
No	21	21.7 (21.7)	–	−0.9 (8.3)	–
Triple-negative					
Yes	2	81.2 (8.5)	0.02	−5.8 (15.2)	0.70
No	24	19.2 (12.0)	–	−1.8 (8.6)	–

Test of linear trend for variables with three ordered categories and Mann–Whitney U test for variables with two categories

Triple-negative if ER negative, PgR negative, and HER2 negative

Mitotic index according to Nottingham criteria

NHG Nottingham histologic grade I-III, *ER* estrogen receptor positive if >10 %, *PgR* progesterone receptor positive if >10 %, *HER2* human epidermal growth factor receptor 2

[a] Spearmans Rho

demonstrated a non-significant 19 % relative reduction in proliferation. However, grade III tumors were significantly associated with high Ki67 expression, which is in agreement with other previous studies [15, 43].

HMGCR is the rate-limiting enzyme in the mevalonate pathway, which is a pathway required for generating a number of fundamental end-products, including cholesterol, isoprenoids, isopentenyladenine, dolichol, and ubiquinone [23]. Deficient feedback control of HMGCR and increased HMGCR expression and activity in tumor cells has been reported in other studies [11], and in this study 2 weeks of statin treatment, resulted in a significant increase in tumor-specific HMGCR expression. This is interpreted as the activation of the negative feedback loop controlling cholesterol synthesis within the mevalonate pathway [11, 12] and corresponds with findings from

previous in vitro studies [18]. Furthermore, the demonstrated increase in HMGCR expression subsequent to statin treatment indicates sufficient drug delivery to the breast cancer cells despite atorvastatin's high first-pass metabolism in the gut wall and the liver with an oral bioavailability of 14 % [34].

Interestingly, a recent review by Thurnher et al. [44] addressed the role of statins as an anti-tumor agent through altered protein prenylation from the isoprenoids produced by the mevalonate pathway. Statin-induced inhibition of HMGCR blocks down-stream products in the mevalonate pathway, including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Both products are central for protein prenylation [44]. Inhibition of protein prenylation may induce a cellular stress response, thereby generating danger signals and subsequently an
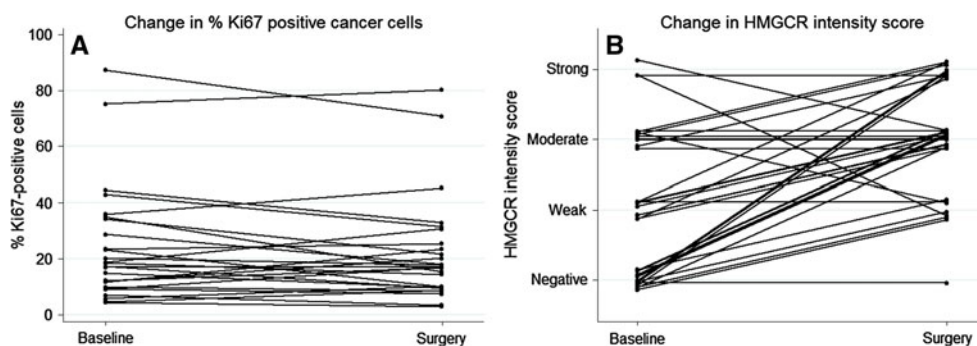
Table 3 Tumor characteristics in relation to HMGCR pre- and post-atorvastatin and change in HMGCR expression

n = 38	HMGCR pre-atorvastatin					HMGCR post-atorvastatin					Change in HMGCR			
	Negative	Weak	Moderate	Strong	P	Negative	Weak	Moderate	Strong	P	Decreased	Unchanged	Increased	P
Age[a]	–	–	–	–	(−0.38) 0.02	–	–	–	–	(0.09) 0.58	–	–	–	(−0.08) 0.62
Tumor size														
≤20 mm	5	5	6	2	0.21	1	2	10	5	0.72	2	5	11	0.90
>20 mm	9	6	4	1	–	0	5	10	5	–	1	4	15	–
Nodal status														
Positive	8	4	4	0	0.15	0	3	8	5	0.59	0	3	13	0.90
Negative	6	7	6	3	–	1	4	12	5	–	3	6	13	–
Tumor grade (NHG)														
I	1	4	3	0	0.38	0	0	7	1	0.40	0	2	6	0.16
II	7	3	4	1	–	0	3	7	5	–	2	2	11	–
III	6	4	3	2	–	1	4	6	4	–	1	5	9	–
Mitotic index														
1	7	7	5	1	0.72	0	2	13	5	0.17	1	4	15	0.53
2	2	0	3	0	–	0	1	2	2	–	1	1	3	–
3	5	4	2	2	–	1	4	5	3	–	1	4	8	–
ER														
Positive	12	9	10	2	0.83	0	5	18	10	0.03	2	7	24	0.83
Negative	2	2	0	1	–	1	2	2	0	–	1	2	2	–
PgR														
Positive	9	9	9	2	0.31	0	3	16	10	0.01	2	6	21	0.71
Negative	5	2	1	1	–	1	4	4	0	–	1	3	5	–
HER2 amplified														
Yes	3	1	2	0	0.60	0	3	2	1	0.21	0	1	5	0.98
No	11	10	8	3	–	1	4	18	9	–	3	8	21	–
Triple-negative														
Yes	2	1	0	1	0.80	1	2	1	0	0.02	1	2	1	0.70
No	12	10	10	2	–	0	5	19	10	–	2	7	25	–

Mitotic index according to Nottingham criteria

Triple-negative if ER negative, PgR negative and HER2 negative. P-values from exact Mann–Whitney test (two categories) or Kruskal–Wallis test (three categories)

NHG Nottingham histologic grade I-III, ER estrogen receptor, positive if >10 %, PgR progesterone receptor, positive if >10 %, HER2 human epidermal growth factor receptor 2, amplified if index with FISH ≥2

[a] Spearmans Rho

Fig. 2 Change in tumor expression of Ki67 and HMGCR from baseline (i.e., before atorvastatin treatment) to time of surgery (i.e., after atorvastatin treatment). **a** Ki67 ($n = 26$); **b** HMGCR ($n = 38$). Random noise, uniformly distributed over the interval -0.15 to $0.15x$,

where x is the arbitrary distance between adjacent categories of the HMGCR intensity scale, has been added to each pair of intensities to visually separate identical otherwise completely overlapping trend lines. This operation does not affect the slopes of the lines

Fig. 3 Change in the Ki67 index measured at baseline (i.e., before atorvastatin treatment) and at surgery (i.e., after atorvastatin treatment) stratified for HMGCR and grade. **a** HMGCR positive; **b** HMGCR negative; **c** grade I/II; **d** grade III
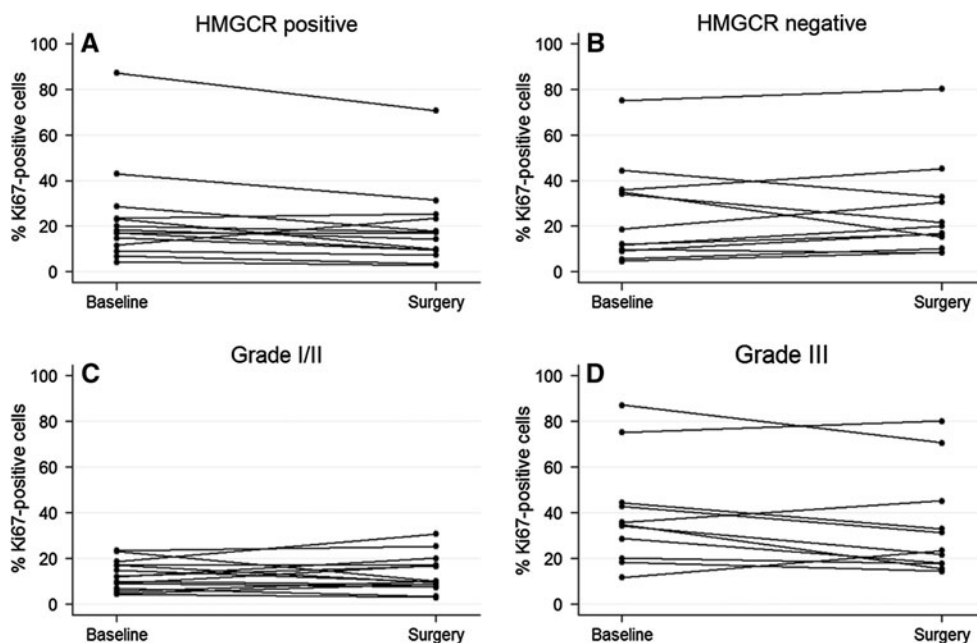
immunological response against the tumor cell [25]. As for the statin-induced anti-proliferative effects indicated in this study, geranylgeranylated proteins may play a central role because they are believed to be essential for cancer cell progression into S-phase [10]. Thus, the mechanisms behind the anti-proliferative effects of statins may depend upon a blockage of the transition of G1-S in the cell cycle [30], which could potentially be mediated by an upregulation of two cyclin-dependent kinase inhibitors, i.e., p21 and p27 [28, 33].

In conclusion, results from this window-of-opportunity trial suggest an upregulation of HMGCR in breast cancer samples following 2 weeks of atorvastatin treatment. The results indicate that HMGCR is targeted in the tumor, and consequently the HMGCR protein is over-expressed depending on feedback loop controlling cholesterol

synthesis within the mevalonate pathway. In tumors expressing HMGCR before treatment with atorvastatin, a modest decrease in tumor proliferation was observed. Future studies selecting HMGCR-positive breast cancers may shed further light on the potential anti-proliferative effects exerted by statins.

the treatment of breast cancer. The other authors disclosed no potential conflicts of interest.

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