**Simvastatin Radiosensitizes Differentiated and Stem-Like Breast Cancer Cell Lines and Is Associated With Improved Local Control in Inflammatory Breast Cancer Patients Treated With Postmastectomy Radiation**

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**Key Words.** Statins • Inflammatory breast cancer • Local recurrence • Radiation

**ABSTRACT**

Reported rates of local failure after adjuvant radiation for women with inflammatory breast cancer (IBC) and triple-negative non-IBC are higher than those of women with receptor-expressing non-IBC. These high rates of locoregional recurrence are potentially influenced by the contribution of radiation-resistant cancer stem cells to these cancers. Statins have been shown to target stem cells and improve disease-free survival among IBC patients. We examined simvastatin radiosensitization of multiple subtypes of breast cancer cell lines in vitro in monolayer and mammosphere-based clonogenic assays and examined the therapeutic benefit of statin use on local control after postmastectomy radiation (PMRT) among IBC patients. We found that simvastatin radiosensitizes mammosphere-initiating cells (MICs) of IBC cell lines (MDA-IBC3, SUM149, SUM190) and of the metaplastic, non-IBC triple-negative receptor cell line (SUM159). However, simvastatin radioprotects MICs of non-IBC cell lines MCF-7 and SKBR3. In a retrospective clinical study of 519 IBC patients treated with PMRT, 53 patients used a statin. On univariate analysis, actuarial 3-year local recurrence-free survival (LRFS) was higher among statin users, and on multivariate analysis, triple negative breast cancer, absence of lymphatic invasion, neoadjuvant pathological tumor response to preoperative chemotherapy, and statin use were independently associated with higher LRFS. In conclusion, patients with IBC and triple-negative non-IBC breast cancer have the highest rates of local failure, and there are no available known radiosensitizers. We report significant improvement in local control after PMRT among statin users with IBC and significant radiosensitization across triple-negative and IBC cell lines of multiple subtypes using simvastatin. These data suggest that simvastatin should be justified as a radiosensitizing agent by a prospective clinical trial.

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population with CD44\textsuperscript{high}/CD24\textsuperscript{low} surface markers, one putative marker of breast cancer stem/progenitor cells [11, 12]. Because of the therapy-resistant character of cancer stem/progenitor cells, significant effort has been made to identify drugs targeting these types of cells. We adapted the three-dimensional (3D) in vitro mammosphere-based self-renewal assay [13] to screen cancer stem/progenitor cells radiosensitizers [14] and demonstrated that drugs that radiosensitize differentiated cells in standard monolayer clonogenic assays can promote the survival and resistance of cancer stem/progenitor cells in 3D assays [15].

3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors or statins have been associated with breast cancer incidence [16–18] and with reduced cancer-related mortality [19]. Interestingly, lipophilic statins were associated with a 10% reduced risk of breast cancer recurrence in a nationwide, population-based prospective cohort study of Danish women with invasive breast cancer [20]. The ability of statins to inhibit tumor growth, angiogenesis, and metastasis has been attributed to their effects on inhibition of G-proteins Ras and Rho [21], reduction of metalloproteinases [22], decreased synthesis of inflammatory cytokines [23, 24], decreased circulating vascular endothelial growth factor (VEGF) levels and VEGF-induced signaling [25–27], and very recently, inhibition of lymphangiogenesis [28]. More important, statins have been shown to reduce the “stemness” of cancer cells by shifting colorectal cancer cells from a stem-like state to a more differentiated state [29] and, in breast cancer cells, by decreasing the expression of CD44 protein [30] and by inhibiting the protein geranylgeranylation [31].

Taking together the well-known characteristics of IBC and the described effects of statins on biology associated with IBC, we hypothesized that statins can inhibit local recurrence after PMRT in IBC by sensitizing differentiated cancer cells and cancer stem cells to radiation. We studied the radiosensitization of breast cancer stem-like cells in vitro after treatment with the most commonly used statin, simvastatin, and examined the influence on local control after PMRT among IBC patients taking statins. This work provides new insight on combination regimens for breast cancer treatment and radiosensitization of this clinically radioresistant disease.

**MATERIALS AND METHODS**

**Cell Culture**

Six different breast cancer cell lines of multiple subtypes were used in our studies [32]. IBC cell lines SUM149 and SUM190 were obtained from Asterand (Detroit, MI, https://www.asterand.com), and MDA-IBC3 was generated in our laboratory [33]. Non-IBC cell line MCF-7 was obtained from ATCC (Manassas, VA, http://www.atcc.org); SKBR3 was a generous gift from Dr. Jennifer Mourtada of Christiana Care’s Helen F. Graham Cancer Center in Newark, Delaware; and SUM159 was obtained from Asterand. All IBC cell lines and SUM159 were cultured as monolayers in Ham’s F-12 media supplemented with 10% fetal bovine serum (FBS), 1 μg/ml hydrocortisone, 5 mg/ml insulin, and 1% antibiotic-antimycotic. MCF-7 cells were cultured as monolayer in modified Eagle’s medium (MEM) supplemented with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 5 μg/ml insulin, 1 μg/ml hydrocortisone, and 1% antibiotic-antimycotic. SKBR3 cells were cultured as monolayer in Dulbecco’s MEM supplemented with 10% FBS and 1% penicillin-streptomycin. All cell lines were also propagated in serum-free MEM supplemented with 20 ng/ml basic fibroblast growth factor, 20 ng/ml epidermal growth factor, and 827 in ultra-low attachment plates to enrich for cancer stem/ progenitor cell populations [13, 34, 35]. These conditions allow mammosphere formation in a liquid medium (3D culture) of all cell lines used in our studies.

**Radiosensitivity Studies**

Radiosensitivity of all cell lines was evaluated in both types of culture, monolayer (two-dimensional [2D]) and mammosphere (3D), as described previously [15]. Briefly, cells were trypsinized, counted, and seeded into six-well plates (ultra-low attachment for 3D cultures) with or without simvastatin. Following a short recovery incubation period of 4 hours at 37°C with 5% CO\textsubscript{2}, cells were exposed to increasing doses of γ-radiation (2 Gy, 4 Gy, and 6 Gy) using a Shepherd Irradiator (J.L. Shepherd and Associates, San Fernando, CA, http://www.jlshepherd.com). Monolayer cultures were incubated between 10 days and 30 days, depending on cell line. Next, colonies were fixed with methanol, stained with crystal violet, and counted. Mammosphere cultures were incubated for 7 days, after which mammospheres were counted with an automated colony counter (Oxford Optronix, Oxford, U.K., http://www.oxford-optronix.com), following addition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to increase the contrast and allow automatic detection of mammospheres. Fresh stock solutions of simvastatin (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com) were prepared weekly with dimethyl sulfoxide at a concentration of 2 mg/ml and stored at 4°C. Final concentration of simvastatin used to treat the cells varied between 0.5 μM and 2.5 μM, according to cell line sensitivity, such that formation of colonies occurred in control wells. Simvastatin was added to cell cultures in a single dose at seeding time, and culture media was not changed until the experiment finished. All conditions were tested in triplicate in two independent experiments. Survival curves were generated using SigmaPlot version 8.0 (Systat Software Inc., Richmond, CA, http://www.systat.com), and t test was used to compare surviving fractions of groups.

**Source Population and Data Collection**

In our studies, the IBC database constructed and maintained by the Breast Cancer Management System at MD Anderson Cancer Center was examined. This database includes 1,177 patients diagnosed with IBC between February 24, 1970, and January 27, 2011. We excluded stage IV IBC patients because these patients were previously shown to have no benefit from statin use [36]. Other exclusion criteria include patients diagnosed prior to 1995, patients who did not receive adjuvant postmastectomy radiotherapy, and patients who had a locoregional recurrence prior to radiation. Consequently, 519 patients were included in the final analysis.

The following variables were included in the analysis: age; body mass index (BMI); menopausal status; race (white vs. black/others); clinical/pathologic nodal status; pathologic stage; nuclear grade; status of estrogen receptor (ER) and progesterone receptor (PR); HER2 status; lymphatic/vascular invasion; and use of neoadjuvant, adjuvant, or hormonal therapy. Final HER2 status was determined based on both immunohistochemistry and
fluorescence in situ hybridization. Triple-negative breast cancer (TNBC) status was determined based on proven ER/PR status and final HER2 status.

**Treatment**

A total of 491 patients (94.6%) received neoadjuvant chemotherapy, the specific regimens of which have been described previously [36]. All patients in the examined cohort received PMRT.

**Definition of Outcomes and Statistical Methods**

Patient characteristics data were first summarized using descriptive statistics and frequency tabulation. Specific traits were further analyzed and compared between statin usage groups using \( \chi^2 \) and Fisher’s exact test when appropriate. The primary endpoint of this analysis was local recurrence-free survival (LRFS), which was calculated from the date of definitive surgery to the date of local recurrence or last follow-up date. The Kaplan-Meier method was used to assess time to recurrence, and log-rank tests were used to compare patient characteristic groups. Both univariate and multivariate Cox proportional hazard models were used to assess time to recurrence, and log-rank tests were used to compare patient characteristic groups. Both univariate and multivariate Cox proportional hazard models were used to assess the effects of covariates of interest on time to LRR. All \( p \) values < .05 were considered to be significant. Statistical analyses were conducted using either SAS version 9.2 (SAS Institute, Inc., Cary, NC, http://www.sas.com) and S-PLUS 8.0 (TIBCO Software Inc., Palo Alto, CA, http://www.tibco.com) or SPSS version 15 (IBM Corp., Armonk, NY, http://www-01.ibm.com/software/analytics/spss/).

**RESULTS**

**Simvastatin Radiosensitizes Mammosphere-Initiating Cells of IBC Cell Lines**

In order to evaluate the effects of simvastatin on in vitro treatment of cancer cells with radiation, we examined the ability to form 2D colonies of six different breast cancer cell lines following treatment with radiation only and in combination with simvastatin. Using standard monolayer clonogenic assays, we demonstrated that simvastatin promoted radiosensitization of IBC and non-IBC cell lines of multiple subtypes (Fig. 1). Among IBC cell lines, the HER2-positive IBC cell line SUM190 had the best response to combined treatment regardless of the radiation dose used (\( p = .001 \); Fig. 1A). In contrast, among non-IBC cell lines, HER2-negative cell lines MCF-7 and SUM159 had greater responses to combined treatment regardless of the radiation dose used (\( p = .02 \) and \( p < .001 \), respectively; Fig. 2B) than HER2-positive cell line SKBR3.

Next, we investigated the effects of simvastatin on 3D mammosphere-based clonogenic assays. Because mammospheres are enriched with mammosphere-initiating cells (MICs) and some drugs that radiosensitize differentiated cells in monolayer cultures promote the resistance of MICs in mammosphere cultures, we hypothesized that simvastatin might be a MIC radiosensitizer. As can be appreciated in Figure 2, simvastatin had a significant effect in all cell lines tested. Concerning IBC cell lines, all had a significantly greater response to combined treatment than to radiation alone, regardless of subtype (MDA-IBC3: \( p < .0001 \); SUM190: \( p < .0001 \); SUM149: \( p = .006 \); Fig. 2A). However, among non-IBC cell lines, simvastatin radioprotected the ER-positive cell line MCF-7 and the HER2-negative cell line SKBR3 (\( p < .0001 \) in both cell lines; Fig. 2B) and only radiosensitized the triple-negative cell line SUM159 (\( p = .01 \); Fig. 2B).

**Patient Characteristics of the Cohort**

After exclusionary criteria were applied, a total of 519 patients with stage III IBC who received postmastectomy radiotherapy were analyzed. In this cohort, 53 patients (10.2%) used statins, whereas 466 patients (89.8%) did not. Median follow-up time for the entire cohort was 2.5 years, and median
age was 49 years (range: 23–78 years). Table 1 summarizes the baseline patient characteristics stratified by statin usage. Overall, 83% of statin users were older patients (defined as older than 50 years of age) compared with no-statin users, of which only 44% were older than 50 years of age ($p < .0001$). Consequently, most statin users were postmenopausal (84.6%) in the statin-user group, whereas the percentage of postmenopausal women was significantly smaller in the no-statin-user group (47.2%, $p < .0001$). As expected, statin users also tended to be obese (63%) compared with no-statin users (41.4%, $p = .01$).

Statin users more commonly had pathologic N0 disease (39.6% vs. 24.8%, $p = .029$). However, race, clinical nodal status, pathologic stage, hormone receptor positivity, HER2 status, and triple-negative disease were found in similar proportions in both groups (Table 1).

Impact of Statin Usage on Time to Locoregional Recurrence

Among the entire 519-patient cohort with stage III IBC who underwent adjuvant radiotherapy, 120 patients (23.1%) experienced LRR. In the statin-usage group, 6 of 53 patients experienced a local recurrence (11.3%) compared with 114 of 466 patients in the no-statin-usage group (24.5%). The Kaplan-Meier estimate of LRR is shown in Figure 3. The actuarial 2- and 5-year local control rates for patients in the no-statin group are 76% and 69%, respectively, and for patients in the statin group are 92% and 85%, respectively.

The results of univariate and multivariate regression analyses are listed in Tables 2 and 3. Despite the fact that older, postmenopausal patients more frequently used statins, neither age (hazard ratio [HR]: 0.81; 95% confidence interval [CI]: 0.57–1.17; $p = .262$) nor menopausal status (HR: 1.03; 95% CI: 0.72–1.48; $p = .8569$) affected time to local recurrence on univariate analysis. Consistent with our recently published report [37], non-triple-negative IBC had a reduced risk of LRR on univariate analysis (HR: 0.44; 95% CI: 0.29–0.67; $p = .0001$). Consequently, lack of hormone therapy was associated with increased risk of LRR (HR: 1.87; 95% CI: 1.27–2.76; $p = .001$). Additional variables associated with reduced LRR on univariate analysis included absence of lymphatic or vascular invasion and response to neoadjuvant chemotherapy (Table 2), which are consistent with our previously published findings on IBC [37].

As hypothesized, the use of statins was associated with reduced LRR on multivariate analysis (HR: 0.40; 95% CI: 0.16–1.00; $p = .0499$). Additional factors associated with reduced LRR on multivariate analysis include non-TNBC (HR: 0.43; 95% CI: 0.28–0.67; $p = .0002$) and complete pathologic response to neoadjuvant chemotherapy (HR: 0.26; 95% CI: 0.10–0.72; $p = .0096$). The presence of vascular invasion, as expected, was associated with increased risk of LRR on multivariate analysis (HR: 2.54; 95% CI: 1.61–4.02; $p < .0001$). BMI was associated with increased LRR in multivariate analysis, but analysis for interaction between use of statins and BMI was negative (HR: 1.94; 95% CI: 1.28–2.95; $p = .0018$).

**DISCUSSION**

We have previously reported radioresistance of IBC cell lines and of stem cell surrogates [15] and comparatively higher rates of LRR among patients with IBC [37]. In this paper, we report for the first time that simvastatin promoted radiosensitization of monolayer cultures across IBC and non-IBC cell lines of multiple subtypes and radiosensitization of MICS of IBC and non-IBC triple-negative cell lines. Furthermore, statin use was independently associated with significant improvement in local control after PMRT among IBC patients.

Statins have a well-described safety and toxicity profile and have been used since the 1980s; very recently, large retrospective studies from Denmark [19, 20] reopened the interest of the
Table 1. Characteristics of patients with stage III inflammatory breast cancer who received adjuvant radiation

| Variable                      | No statin use (n = 466, 89.8%) | Statin use (n = 53, 10.2%) | p value |
|-------------------------------|-------------------------------|---------------------------|---------|
| Age                           |                               |                           |         |
| <50                           | 261 (56)                      | 9 (17)                    | <.0001  |
| ≥50                           | 205 (44)                      | 4 (8)                     |         |
| Menopausal status             |                               |                           |         |
| Premenopausal                 | 244 (52.8)                    | 8 (15.4)                  | <.0001  |
| Postmenopausal                | 218 (47.2)                    | 44 (84.6)                 |         |
| Body mass index               |                               |                           |         |
| <25                           | 108 (24.4)                    | 10 (21.7)                 | .0099   |
| 25–29                         | 151 (34.2)                    | 7 (15.2)                  |         |
| ≥30                           | 183 (41.4)                    | 29 (63)                   |         |
| Race                          |                               |                           |         |
| White                         | 412 (88.4)                    | 49 (92.5)                 | .3249   |
| Black                         | 35 (7.5)                      | 4 (7.5)                   |         |
| Other                         | 19 (4.1)                      | 0 (0)                     |         |
| Nuclear grade                 |                               |                           |         |
| I                             | 5 (1.2)                       | 0 (0)                     | .1373   |
| II                            | 76 (17.9)                     | 14 (29.2)                 |         |
| III                           | 343 (80.9)                    | 34 (70.8)                 |         |
| Lymphatic invasion            |                               |                           |         |
| No                            | 169 (37.9)                    | 26 (51)                   | .0698   |
| Yes                           | 277 (62.1)                    | 25 (49)                   |         |
| Vascular invasion             |                               |                           |         |
| No                            | 211 (47.4)                    | 26 (51)                   | .6293   |
| Yes                           | 234 (52.6)                    | 25 (49)                   |         |
| N class (clinical)            |                               |                           |         |
| N0                            | 73 (16.4)                     | 9 (18.4)                  | .0570   |
| N1                            | 227 (51)                      | 17 (34.7)                 |         |
| N2                            | 45 (10.1)                     | 4 (8.2)                   |         |
| N3                            | 100 (22.5)                    | 19 (38.8)                 |         |
| N class (pathologic)          |                               |                           |         |
| N0                            | 111 (24.8)                    | 21 (39.6)                 | .0292   |
| N1                            | 188 (42.1)                    | 12 (22.6)                 |         |
| N2                            | 72 (16.1)                     | 11 (20.8)                 |         |
| N3                            | 76 (17)                       | 9 (17)                    |         |
| Stage (pathologic)            |                               |                           |         |
| 0                             | 61 (14.1)                     | 12 (23.1)                 | .2423   |
| I                             | 22 (5.1)                      | 4 (7.7)                   |         |
| II                            | 115 (26.6)                    | 10 (19.2)                 |         |
| III/IV                        | 234 (54.2)                    | 26 (50)                   |         |
| Estrogen receptor             |                               |                           |         |
| Negative                      | 232 (52.7)                    | 27 (52.9)                 | .9769   |
| Positive                      | 208 (47.3)                    | 24 (47.1)                 |         |
| Progesterone receptor         |                               |                           |         |
| Negative                      | 274 (63.4)                    | 36 (72)                   | .2309   |
| Positive                      | 158 (36.6)                    | 14 (28)                   |         |
| HER2                          |                               |                           |         |
| Negative                      | 229 (60.3)                    | 36 (75)                   | .0476   |
| Positive                      | 151 (39.7)                    | 12 (25)                   |         |
| Triple negative               |                               |                           |         |
| No                            | 326 (79.1)                    | 32 (66.7)                 | .0492   |
| Yes                           | 86 (20.9)                     | 16 (33.3)                 |         |
| Neoadjuvant chemotherapy      |                               |                           |         |
| No                            | 26 (5.6)                      | 2 (3.8)                   | .5814   |
| Yes                           | 440 (94.4)                    | 51 (96.2)                 |         |
| Neoadjuvant clinical response |                               |                           |         |
| Complete response             | 55 (12.9)                     | 7 (14)                    | .4986   |
| Partial response              | 221 (51.6)                    | 30 (60)                   |         |
| Stable disease                | 144 (33.6)                    | 13 (26)                   |         |
| Progressive disease           | 8 (1.9)                       | 0 (0)                     |         |
| Neoadjuvant pathologic response|                             |                           |         |
| Complete response             | 61 (14.4)                     | 12 (23.5)                 | .0872   |
| Non-complete response         | 363 (85.6)                    | 39 (86.5)                 |         |
| Adjuvant chemotherapy         |                               |                           |         |
| No                            | 208 (44.6)                    | 33 (62.3)                 | .0148   |
| Yes                           | 258 (55.4)                    | 20 (37.7)                 |         |
| Adjuvant hormonal therapy     |                               |                           |         |
| No                            | 283 (60.7)                    | 28 (52.8)                 | .2661   |
| Yes                           | 183 (39.3)                    | 25 (47.2)                 |         |
Successfully using simvastatin and recent studies. Such kinds of cells have been targeted like cells have become the therapeutic focal point of numerous treatment-resistant breast cancer cells or breast cancer stem-cells have become the therapeutic focal point of numerous recent studies. Such kinds of cells have been targeted successfully using simvastatin and \( \gamma \)-tocotrienol combined therapies via inhibition of the mevalonate pathway [38]. Furthermore, the inhibition of the protein geranylgeranylation by simvastatin reduced the cancer stem-like cell populations in basal/mesenchymal mammospheres (including SUM149, SUM159, and SUM190) but not in luminal mammospheres (including MFC-7) [31].

In the present study, we observed that combined treatment with simvastatin and radiation had distinct effects on IBC and non-IBC cell lines cultured as monolayer or mammospheres. The HER2-positive cell line SUM190 was the only IBC cell line that responded to combined treatment when cultured as monolayer; however, all IBC cell lines, regardless of subtype, cultured as mammospheres responded to combined therapy. In contrast, the non-IBC cell lines with greater response to combined therapy were HER2-negative MCF-7 and SUM159 when cultured as monolayer and only the triple-negative SUM159 when cultured as mammospheres. Given the lack of reduction in stem-like cells in luminal cell lines examined by Ginestier et al., the radioprotection offered by simvastatin to the cell lines MCF-7 and SKBR3 (both luminal) that we observed is possibly related to the lack of a specific signaling pathway associated with these cell lines when cultured as a monolayer or mammosphere [31].

It has been reported that cancer cells from different organs are more responsive to simvastatin than pravastatin [39] and that simvastatin induces death of HER2-overexpressing cell lines, such as MDA-MB-361, SK-Ov3, and SKBR3, and inhibits the activity of the HER2 promoter [40]. We did not observe this outcome with the HER2-positive cell lines used in this study, yet activation of different pathways by radiation treatment might be a reason for such outcome. Interestingly, the use of lipophilic statins has been associated with a reduction in the proportion of hormone receptor-negative breast cancers [41] and treatment of triple-negative cell lines with simvastatin has been reported to induce cell death through the PI3K pathway [42]. In our study, triple-negative cell lines did not consistently correlate with response to combined treatment when monolayer cultures were used; however, when mammosphere cultures were used, both TNBC cell lines (IBC SUM149 and non-IBC SUM159) were radiosensitized by simvastatin.

| Type of statin                  | Neutral vs. hydrophilic | Lipophilic vs. hydrophilic | Race             |
|--------------------------------|------------------------|---------------------------|------------------|
| Black vs. white                | 4.62                   | 3.92                      | Black vs. white  |
| Other vs. white                | 0.66                   | 0.72                      | Other vs. white  |
| Nuclear grade, I/II vs. III    | 0.62                   | 0.72                      | Nuclear grade    |
| Lymphatic invasion, no vs. yes | 0.31                   | 0.72                      | Lymphatic invasion|
| Vascular invasion, no vs. yes  | 0.32                   | 0.72                      | Vascular invasion|
| Stage (pathologic)             | 0.81                   | 0.72                      | Stage (pathologic)|
| 0 vs. III/IV                   | 1.17                   | 0.94                      | 0 vs. III/IV     |
| I vs. III/IV                   | 0.94                   | 0.94                      | I vs. III/IV     |
| II vs. III/IV                  | 0.56                   | 0.56                      | II vs. III/IV    |
| Estrogen receptor, negative vs. positive | 1.87 | 1.27 | 0.0014 |
| Progestrone receptor negative vs. positive | 1.40 | 1.27 | 0.016 |
| HER2, negative vs. positive    | 1.06                   | 1.27                      | HER2, negative vs. positive |
| Triple negative, no vs. yes    | 0.44                   | 1.27                      | Triple negative, no vs. yes |
| Neoadjuvant chemotherapy, no vs. yes | 1.35 | 1.27 | 0.3880 |
| Neoadjuvant clinical response  |                        |                           | Neoadjuvant clinical response |
| CR vs. Z.PD                    | 0.12                   | 0.40                      | CR vs. Z.PD      |
| Partial response vs. Z.PD      | 0.17                   | 0.40                      | Partial response vs. Z.PD |
| Stable disease vs. Z.PD        | 0.29                   | 0.40                      | Stable disease vs. Z.PD |
| Neoadjuvant pathologic response, CR vs. non-CR | 0.22 | 0.40 | 0.0011 |
| Adjuvant chemotherapy, no vs. yes | 0.81 | 0.40 | 0.2574 |
| Adjuvant hormone, no vs. yes   | 1.87                   | 0.40                      | Adjuvant hormone, no vs. yes |

Table 2. Univariate analysis of locoregional recurrence (stage III inflammatory breast cancer with adjuvant radiation)

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Table 3. Multivariate analysis of locoregional recurrence (stage III inflammatory breast cancer with adjuvant radiation)

| Variable                        | HR  | 95% CI    | p value |
|---------------------------------|-----|-----------|---------|
| Statin use, yes vs. no          | 0.40| 0.16–1.00 | .0499   |
| Vascular invasion, yes vs. no   | 2.54| 1.61–4.02 | <.0001  |
| Neoadjuvant pathologic response, CR vs. non-CR | 0.26| 0.10–0.72 | .0096   |
| Triple negative, no vs. yes     | 0.43| 0.28–0.67 | .0002   |
| BMI, ≤ 25 vs. >25               | 1.94| 1.28–2.95 | .0018   |

Abbreviations: BMI, body mass index; CI, confidence interval; CR, complete response; HR, hazard ratio.

IBC cells retain intracellular cholesterol esters, free cholesterol, and triglycerides in lipid-deficient environments [43]; increased angiogenesis and lymphangiogenesis are associated with IBC [44]; and VEGF-A, a lymphangiogenesis mediator, was recently shown to be a prognostic indicator in IBC [45]. All of these described characteristics of IBC may also contribute to the differences in radiosensitization that we found between mammospheres of IBC and non-IBC cell lines. Lymphangiogenesis is important for IBC invasion and metastasis, and very recently, a study on corneal and cutaneous lymphangiogenesis in vivo demonstrated that statins are potent inhibitors of lymphangiogenesis, with simvastatin showing the strongest effect [28].

This work includes a retrospective study and is limited by the biases inherent in all retrospective work. Statin use was primarily in women with hyperlipidemia as well as history of coronary artery disease, and other hidden biases may exist that are unaccountable for in the study. Statin use was extracted from the chart, and complete details regarding duration and dosage were not available in all cases. Patients who did not receive PMRT were excluded, thus representing a bias excluding those who progressed on chemotherapy without surgery or preoperative radiation and those who progressed after surgery and before chemotherapy. Nevertheless, considering all known, extractable variables, the findings are of considerable interest, are congruent with the preclinical findings, and are worthy of additional consideration in the prospective setting for patients with nonluminal IBC and triple-negative breast cancer.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest.

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**DISCUSSION**

Statins represent a potential new therapeutic strategy to reduce LRR among patients with IBC when used in combination with radiation. Specifically, simvastatin is approved worldwide by different U.S. Food and Drug Administration-equivalent organizations, has a safe toxicity profile, and is commercially available in generic forms. Randomized trials evaluating statins in this disease should test radiosensitization in their design.

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**AUTHOR CONTRIBUTIONS**

L. Lacerda: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing; J.P.R.: data analysis and interpretation, manuscript writing; D.L.: data analysis and interpretation; R.L., L. Li, B.G.D., W.X.: collection and assembly of data; H.M., T.B.: collection and assembly of data, provision of study material or patients; G.N.H., T.A.B.: financial and administrative support; N.T.U.: financial support, provision of study material or patients; W.A.W.: conception and design, financial support, provision of study material or patients, data analysis and interpretation, manuscript writing and final approval of manuscript.

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