High-risk early breast cancer in patients under 40 years of age: Improved clinical outcome with total estrogen blockade and tailored chemotherapy

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Abstract. This multicenter prospective trial assessed the outcome in 63 patients, 40 years of age or younger, with high-risk early breast cancer (HREBC), included in an ovarian protection study. The patients were treated with a luteinizing hormone-releasing hormone (LH-RH) analogue administered for 5 years, tailored chemotherapy and an aromatase inhibitor, in estrogen receptor-positive (ER^+) patients. T-regulatory cells (T-regs) and vascular endothelial growth factor (VEGF) were measured at baseline and yearly. The mean age of the patients was 36 years (range 26-40). Sixty-five percent had ER^+ tumors, 24% had negative axillary nodes with tumors >1 cm and high histological grade with lymphovascular invasion, while 76% had a mean of 3.6 positive axillary nodes (range 1-21). Serum estradiol was maintained at values <40 pg/ml in all of the patients. A statistically significant decrease in VEGF ($P<0.0001$) and T-regs ($P<0.0001$), with respect to baseline values, was observed after LH-RH administration. After a median follow-up of 110 months, the 10-year progression-free and overall survival rates were 86.1 and 89.7%, respectively. These data revealed that the administration of an LH-RH analogue to HREBC patients, followed by chemotherapy and hormonal therapy, decreased VEGF and T-regs and improved the expected clinical outcome.

Introduction

Despite the earlier diagnosis due to improved screening procedures and the progress regarding adjuvant treatments, young breast cancer patients still have a poorer prognosis compared to their older counterparts (1,2). A comprehensive genomic analysis of 784 young patients with early-stage breast cancers established that age at breast cancer diagnosis remains the most important variable in determining outcome (3). However, the fundamental causes of the aggressive behavior of breast cancer in young patients with locoregional and distant recurrence have not yet been elucidated (4). Estrogen plays a key role in the pathogenesis and development of breast cancer, and the risk for this disease is associated with the length of exposure to endogenous and exogenous estrogens (5). In addition, 17β-estradiol modulates vascular endothelial growth factor (VEGF) expression in breast cancer cells (6) and is a physiological regulatory factor for the peripheral development of T-regulatory cells (T-regs) ($CD4^+CD25^+$ T-regs) (7). It has been suggested that the tumor cytosolic content of VEGF is a possible predictor of survival in patients with node-positive breast cancer (8), and T-regs may be dominantly responsible for the immunosuppression in tumor immunity and a potential predictor of the poor prognosis of young breast cancer patients (9).

Current standard options for the adjuvant treatment of pre-menopausal women include chemotherapy followed by anti-estrogen therapy, predominantly with tamoxifen, and ovarian ablation by surgery, radiotherapy or by luteinizing hormone-releasing hormone (LH-RH) agonists (10). Moreover, the benefits of chemotherapy in pre-menopausal breast cancer patients have been attributed, in part, to the phenomenon of chemotherapy-related amenorrhea (11).

To date, the data have shown that there is a trend towards improved recurrence-free and overall survival (OS) in pre-menopausal patients who receive an LH-RH agonist plus chemotherapy and tamoxifen combination in comparison to chemotherapy alone (12,13).

In recent years, an important change in the adjuvant treatment of postmenopausal patients has occurred, after the publication of the results of adjuvant trials that used third-generation aromatase inhibitors (14-17). In addition, one study demonstrated that an aromatase inhibitor added to an LH-RH analogue was more effective than tamoxifen for the inhibition

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of estradiol secretion (18). This suggests that aromatase inhibitors are more effective than tamoxifen as adjuvant hormonal treatment for pre-menopausal patients with endocrine-responsive breast cancer.

Since September 1993, over 200 pre-menopausal women with high-risk early breast carcinoma were treated by our group with a gonadotropin-releasing hormone (Gn-RH) analogue for ovarian protection before and during adjuvant chemotherapy, which was tailored to the specific biological characteristrics of each patient tumor (19,20). The present study prospectively evaluated 63 female patients under 40 years of age treated with an LH-RH analogue for 5 years, tailored chemotherapy and by an aromatase inhibitor for estrogen receptor (ER)-positive tumors.

Patients and methods

Study design and patient selection. Sixty-three women with histologically confirmed breast cancer who had undergone complete or segmental mastectomy plus axillary node dissection or sentinel node biopsy were recruited for the study. All patients were pre-menopausal, between 26 and 40 years of age, with normal menstruation and normal gonadotropins, estradiol and progesterone values. Patients with pathological T1-4, N0-2 and M0 tumors were eligible. Women with high-risk node-negative tumors (≥1 cm in diameter with a high histological grade, lymphovascular invasion or both) were also included. The levels of hormone receptors were determined by an immunohistochemical assay, with a cutoff point of 10%. Patients were excluded if they had distant metastases, residual disease in the breast or axilla, other serious medical illnesses or a previous occurrence of cancer. Women considering pregnancy in a period of 5 years or using hormones were excluded.

The following laboratory parameters were required: granulocyte count ≥2,000/μl, platelet count ≥100,000/μl, hematocrit ≥30%, total bilirubin and AST levels ≤1.5 times the upper limit of normal, serum creatinine concentration ≤1.8 mg/dl and a left ventricular ejection fraction ≥50%. Bilateral bone marrow aspirates and biopsies immunostained for cytokeratin were performed routinely in patients with >5 positive axillary nodes and having radiographic or scintigraphic pelvic bone abnormalities present. VEGF was determined by the Human VEGF Colorimetric ELISA kit (Pierce Endogen) as previously described (21), while flow cytometric analysis was used to measure T-regs.

Treatment plan. The study was performed according to the Declaration of Helsinki following local ethics committee approval, and the patients provided their written informed consent. Staging assessment included patient history, physical examination, complete blood count and liver function tests before chemotherapy, and chest radiograph and bone scan within 8 weeks of starting chemotherapy. The patients were required to start chemotherapy within 4-6 weeks after the first histological diagnosis of breast cancer. They received 11.25 mg of the LH-RH analogue subcutaneously 1 week before starting chemotherapy, and every 84 days for 5 years. All patients received cyclophosphamide 600 mg/m^2, epirubicin 75 mg/m^2 on day 1 and 5-fluorouracil 600 mg/m^2 on days 1 and 8 for four courses. Forty-four patients were treated with segmental mastectomies and 10 patients with modified radical mastectomy; patients with more than 10 positive axillary nodes received radiation therapy to the breast and supraclavicular nodes, with concurrent cyclophosphamide 600 mg/m^2, 5-fluorouracil 600 mg/m^2 and methotrexate 40 mg/m^2 (CMF) on day 1, every 3 weeks, for four courses. In addition, the patients received further adjuvant chemotherapy with the appropriate regimen being selected according to the biologically relevant characteristics of the tumor subpopulation.

Ten patients with more than 10 positive axillary nodes and 8 patients with triple negative tumors received a high-dose platinum-based chemotherapy with (22) or without (23) bone marrow (PBPC) transplantation, respectively. Fifteen patients with CERB-2$^+$ tumors received four courses of 100 mg/m^2 docetaxel, after the end of the anthracycline-based chemotherapy, and/or CMF and radiation therapy. All 43 patients with ER$^+$ tumors received an aromatase inhibitor daily for 5 years following the completion of chemotherapy. During the study, all patients were supplemented with vitamin D, calcium and bisphosphonates and received continuous psychological support.

Table I. Patients and tumor characteristics.

Characteristics	No.	%
No. of patients	63	100
Age (years)		
Median	36	
Range	26-40	
Hormone receptor status		
ER$^+$	41	65
ER$^-$	22	35
Tumor histology		
Ductal infiltrating	51	81
Lobular infiltrating	5	8
Medullary	5	8
Other	2	3
Grading		
G2	25	40
G3	38	60
Clinical stage		
IIA	43	68
IIB	7	11
IIIA	1	2
IIIB	4	6
IIIC	8	13
Nodes		
0	15	24
1-3	33	52
4-9	7	11
>10	8	13
Type of primary surgery		
Mastectomy	19	30
Quadrantectomy	44	70

Table II. Vascular endothelial growth factor (VEGF) and T-regulatory cell (T-reg) measurement at baseline and at 1, 5 and 6 years.

	VEGF (pg/ml[a])	T-regs (mm^3 ± SD)
Baseline	533.7±258	101±20
1 year	359.6±174	58±13
5 years	272.6±146	33±21
6 years	216.6±92	90±11

[a]picograms/milliliter; SD, standard deviation.

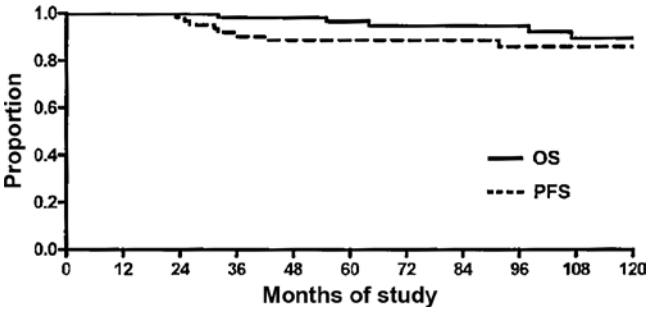

Figure 1. Ten-year progression-free survival (PFS): 55 censored patients [87.3% (95% CI 76-94)] were progression-free; 8 events of progression [12.7% (95% CI 6-23)]. Overall survival (OS): 58 censored patients [92% (95% CI 82-97)]; 5 death events [7.9% (95% CI 3-17)].

Statistical considerations. The study was designed to test the hypothesis that the administered regimen decreases VEGF and T-regs. A positive response was defined as a ≥50% decrease from baseline values of VEGF and T-regs in the absence of any clinical or radiological evidence of disease progression for at least 12 months. Simon's optimal two-stage design was used (24). The first stage required that 8 or more patients out of 24 had a confirmed ≥50% decrease in VEGF and T-regs to rule out an undesirably low response probability of 0.30 (P0), in favor of a desirable response probability of 0.50 (P1), with a 5% probability of accepting a poor agent (α=0.05) and a 10% probability of rejecting a good agent (β=0.1) before proceeding to the second stage. In the second stage, accrual of a total of 63 assessable patients provided the desired outcome when a total of 10 or more patients showed a confirmed decrease in VEGF and T-regs, then the primary endpoint was met.

Secondary end points were the determination of disease-free survival (DFS), OS and fertility preservation rate. The results were expressed as the mean ± standard deviation of determinations made in three different experiments. *Post hoc* comparisons were performed by Turkey's honestly significant difference test. For variables not normally distributed ($CD4^+$/$CD8^+$), the Friedman repeated measures ANOVA by ranks was used. *Post hoc* comparisons were performed using the Wilcoxon's rank sign test, with a downward adjustment of a level to compensate for multiple comparisons. The time to relapse was defined as the time between the start of therapy and any relapse or any appearance of a second primary cancer or death, whichever occurred first. OS was measured from study entry to death, or study entry to February 2010, for censored patients. Statistical analysis was performed with SAS Statistical software (version 8.12, 2000; SAS Institute Inc., Cary, NC, USA). Progression-free survival (PFS) and OS were determined using the Kaplan-Meier method (25). Adverse events were evaluated using Standard World Health Organization criteria (26). Analysis of data was performed in February 2010.

Results

Patient characteristics. The baseline demographics and tumor characteristics of the patients are shown in Table I. Sixty-three consecutive patients diagnosed with unilateral adenocarcinoma of the breast, stage PT2-3a, N-/+, M0, who had undergone modified radical mastectomy or breast conserving surgery plus full axillary node dissection or sentinel node biopsy, were recruited into the study. These patients were also fully evaluated for ovarian function protection. All patients had a good performance status, with a median age of 36 years (range 26-40). Treatment compliance was satisfactory. All patients completed chemotherapy and treatment with an LH-RH analogue after a median follow-up of 110 months.

Biomarker results. Serial assessment of plasma biomarkers was available for 44 patients at baseline, for 40 patients after 1 year and for 38 patients after 5 and 6 years. After 1 year, there was a 67% relative decrease in serum VEGF levels (95% CI 63-71; P<0.0001) and a 56% relative decrease in Fox-P3 T-regs (95% CI 46-66; P<0.0001) from baseline. The measurements performed yearly showed a progressive decrease in VEGF and Fox-P3 values (Table II). One year after the discontinuation of LH-RH analogues, there was a 59% decrease in VEGF (95% CI 53-65), with respect to the 5th year value and a 36% increase in T-regs (95% CI 27-46) with respect to the values obtained at the end of the 5th year. In addition, changes in VEGF could not be attributed to chemotherapy-induced thrombocytopenia and successive rebound increase in platelet count (27). In fact, VEGF was measured at baseline, before chemotherapy and 1 year after, when chemotherapy was completed.

Fertility outcome. One year after the last dose of the LH-RH analogue, 91% of the patients exhibited normal gender steroid hormones and menses, including 5 women treated with high-dose chemotherapy and PBPC transplantation. One of the patients that had been treated with high-dose chemotherapy and PBPC transplantation became pregnant and had a voluntary abortion. Two patients completed a normal pregnancy, which resulted in the birth of 3 healthy children at term, 5 years after the completion of chemotherapy and radiotherapy.

Outcome: Disease-free survival and overall survival. At data cut-off for this analysis, February 2010, median follow-up for living patients was 110 months (range 60-120). The 10-year PFS was 86.1%, with 8 patients exhibiting disease progression (12.7%, 95% CI 6-23) and 55 patients being disease progression-free (87.3%, 95% CI 76-94) (Fig. 1). The 10-year OS rate was 89.7%, with 5 death events (7.9%, 95% CI 3-17) and 58 censored patients (92%, 95% CI 82-97) (Fig. 1).

Table III. Toxicity.

	Type of therapy		
	LH-RH analogue (63 patients)[a]	Anthracycline-based CT (63 patients)[a]	HD-CT (18 patients)[a]
Hematologic			
Leukopenia	0	40 (63)	18 (100)
Thrombocytopenia	0	18 (29)	18 (100)
Anemia	0	17 (27)	7 (39)
Gastrointestinal			
Nausea-vomiting	0	16 (25)	6 (33)
Diarrhea	0	15 (24)	2 (11)
Mucositis	0	22 (35)	7 (39)
Infection	0	9 (14)	3 (17)
Neurotoxicity	0	2 (3)	2 (11)
Alopecia	0	63 (100)	18 (100)
Hot flushes	40 (63)	0	0

[a]All values are expressed as the no. (%). CT, chemotherapy; HD-CT, high-dose chemotherapy.

The effect of this therapy was explored in subgroups defined by overall hormone receptor status. As expected by this total estrogen blockade, there was a statistically significant difference ($P<0.05$) in DFS, favoring the 41 patients with ER^+ with respect to the 22 patients with ER^-. OS did not show any statistically significant difference between the two groups ($P=0.9$).

Toxicity. Adverse events reported during the chemotherapeutic treatment are shown in Table III. Forty patients (63%) complained of hot flushes. No unexpected toxicity occurred during the administration of anthracyclne and taxane-based chemotherapy, and all 63 patients completed the scheduled treatment. Hematological toxicity occurred in all paients treated with high-dose chemotherapy with or without PBPC transplantation. Three of these patients, with a platelet count $<20x10^4/mm^3$ required a platelet transfusion (median 2 units). Diarrhea occurred in 15 patients (24%), while 16 patients (25%) reported nausea and vomiting. An infection was reported in 3 patients (17%). Grade 3 alopecia was universal. No significant reduction in the left ventricular ejection fraction was observed in any patient. Anemia, which was infrequent due to the use of erythropoietin, occurred in 17 patients (27%). Mucositis occurred in 22 patients (35%). Bone pain with median duration of 2 days was reported by 4 patients after PBPC transplantation. There were no chemotherapy-related deaths.

Discussion

No consensus exists regarding the causes and treatment of the aggressive behavior of breast cancer in patients younger than 40 years of age. In these patients, the role of chemotherapy, tamoxifen and LH-RH analogues, alone or in various combinations, has been the subject of intense investigation. A meta-analysis of randomized adjuvant trials of LH-RH analogues as adjuvant treatment in pre-menopausal patients with hormone receptor-positive breast cancer, combined with chemotherapy and tamoxifen, demonstrated a significant 32.3% reduction in the odds of recurrence in patients 40 years of age or less (13). To the best of our knowledge, no study has been conducted using the three modalities used in our study in the following sequence: ovarian suppression for 5 years, tailored chemotherapy and hormonal therapy with an aromatase inhibitor. After surgery, all of the patients received the LH-RH analogue with the aim of 'putting to sleep' the ovaries and thus protecting rapidly proliferating oocytes from the damage of chemotherapy. The objective of the ovarian protection was partially achieved; only 9% (95% CI 3-19) of the patients had chemotherapy-related amenorrhea (CRA). These findings were favorable when compared to data reported from the literature that described a 40% CRA (95% CI 36-44) for patients less than 40 years of age treated with chemotherapy (28).

We hypothesized that immunologic-mediated mechanisms, the decrease in VEGF and T-regs, could explain the improved clinical outcome observed in our patients. Both of these factors play a fundamental role in reproduction and are mediated by estrogens, which also regulate the expression of the oncogenic signaling pathways that characterize pre-menopausal breast cancer.

Estrogens mediate several reproductive functions through the secretion of VEGF (29); inducing neoangyogenesis at the implantation site, VEGF creates an environment necessary for the embryo survival (30). In the regulation of normal menstruation, inhibition of VEGF at the time of follicle recruitment or selection prevents endothelial cell proliferation, leading to the inhibition of follicular development (29).

Apart from physiological conditions, VEGF also has a fundamental role in the development of breast cancer. VEGF is a target gene for the ER and contributes to breast cancer

progression (31). Estrogen induction of free extracellular VEGF may be one mechanism involved in gender steroid-dependent breast carcinogenesis; tumor development may be facilitated by a deregulation of angiogenic factors (32). Estradiol modulates VEGF expression in breast cancer cells, involving transcriptional activation through the ER (33). Other *in vivo* findings showed that overexpression of VEGF significantly increased tumor growth and angiogenesis in a murine model of breast cancer (31). In addition, patients with early-stage breast cancer who have tumors with elevated levels of VEGF have a higher likelihood of recurrence or death compared to patients with low-angiogenic tumors, even when treated with conventional adjuvant therapy (34).

Another function of estrogens, directly related to reproduction, is the increase in T-regs in order to allow tolerance of the eterologous embryo. In fact, estradiol modulates the function of human T-reg cells by regulating their numbers (35). Previous studies in mouse models showed that T-regs are essential for maternal tolerance of the conceptus and that the expansion of the T-reg cell pool through antigen-specific and antigen non-specific pathways allows their suppressive actions to be exerted in the critical peri-implantation phase of pregnancy (30). It has been observed that estradiol, at physiological doses, not only expanded T-reg cells in different tissues, but also increased the expression of the Foxp3 gene, a hallmark for $CD4^+CD25^+$ T-reg cell function, and the IL-10 gene as well (36). In fertile non-pregnant women, an expansion in T-regs is tightly correlated with serum levels of estradiol, and reproductive failure may result from the inability of T-regs to expand during the pre-implantatory phase (37).

The suppressive effect of T-reg cells facilitates breast cancer development; in fact, T-regs prevent effector T-cell activation, facilitating immune escape and, ultimately, tumor progression (38).

One study suggested that T-regs are reversely correlated with the survival of patients with breast cancer; a new subset of tumor-resident T-regs, CCR6(+) T-regs, may be dominantly responsible for the immunosuppression in tumor immunity and a potential predictor of poor prognosis in breast cancer (9).

All of these estrogen-mediated functions correspond to patterns exhibited by breast cancer for its progression. In the present study, patients treated with the LH-RH analogue exhibited an early decrease in VEGF and T-regs. This decrease was stable over time, and the values of both VEGF and T-regs were maintained at low values over a 5-year period. After the discontinuation of the LH-RH analogue a normal ovarian function was observed in 91% of the patients who enjoyed a normal sexual and reproductive life. Our 9-year DFS and OS for patients with ER^+ and positive lymph nodes were both 92%. Our data compared favorably to data from the literature that report a PFS and OS of 64 and 69%, respectively, in the same category of patients (39).

In conclusion, the administration of an LH-RH analogue for 5 years, followed by tailored chemotherapy and an aromatase inhibitor for ER^+ patients seems to be feasible, well tolerated and appears to improve the expected outcome of patients less than 40 years of age with high-risk early breast cancer.

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