Adjuvant Ovarian Suppression, High-dose Chemotherapy and Immunotherapy for Premenopausal Patients with High-risk Breast Cancer

FRANCESCO RECCHIA[1,3], GIAMPIERO CANDELORO[1], MICHELE ROSSELLI[2,3], MASSIMO BRATTA[3,4], VITTORIO PASTA[5], VALERIO D'ORAZI[5], LUCA A. FUMAGALLI[6] and SILVIO REA[3,4]

[1]*Oncology Department, Civilian Hospital, Avezzano, Italy;*
[2]*Oncology Department, Civilian Hospital, Frascati, Italy;*
[3]*Carlo Ferri Foundation, Monterotondo, Rome, Italy;*
[4]*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Aquila, Italy;*
[5]*Department of Surgical Sciences, Sapienza University of Rome, Rome, Italy;*
[6]*Department of Surgical Sciences, Lecco Hospital, Lecco, Italy*

Abstract. *Background: Premenopausal patients with breast cancer and more than 10 positive axillary nodes (BC>10) have a poor prognosis: In these patients the best adjuvant therapy (CT) has not yet been established. Patients and Methods: Forty-two BC>10 received, in sequence, the following adjuvant treatments: luteinizing hormone releasing hormone (LH-RH) analog for 5 years; anthracycline-based induction chemotherapy; radiation therapy; platinum-based high-dose CT, with autologous bone marrow transplantation; immunotherapy with interleukin 2 (IL2) and 13-cis retinoic acid (RA); anastrazole given 5 years to estrogen receptor-positive patients. Primary endpoints of the study were disease-free survival (DFS) and overall (OS) survival. A secondary endpoint was toxicity. Results. The median age of patients was 41 years, and the mean number of positive axillary nodes was 14. Estrogen and progesterone receptors were positive in 57% and 29% of patients respectively, while 14% of patients had triple-negative disease. With a median follow-up of 120 months for patients remaining alive at the end of study, median DFS and OS, had not yet been reached. The 20-year DFS and OS rates were 63.8%, and 81.6%, respectively. One to two years after the end of the therapy, three patients had had four full-term pregnancies. Conclusion. Treatment with LH-RH analog, high-dose CT, peripheral blood progenitor cells and IL2 with RA for patients with BC>10 is feasible, has moderate toxicity, while preserving ovarian function, seems to improve the expected DFS and OS for these high-risk patients.*

Correspondence to: Francesco Recchia, MD, Via Del Castagneto n. 15, 67056 Luco dei Marsi (AQ), Italy. Tel/Fax: +39 086352119, e-mail: frecchia1946@libero.it

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Breast cancer in younger patients is a relatively rare disease and has pathological features associated with a worse prognosis compared to older patients (1). In recent years, considerable progress has been achieved in the treatment of premenopausal women with high-risk breast cancer with more than 10 involved axillary nodes.

In the era of cyclophosphamide-methotrexate-5 fluorouracil (CMF) chemotherapy (2), the probability of remaining free of disease at 5 years in these patients was in the range of 20-30%. These figures have improved to 50% with the introduction of anthracycline–CMF sequential chemotherapy regimens (3, 4). With the use of hematopoietic growth factors and high-dose chemotherapy with autologous bone marrow transplantation, the 5-year disease-free survival (DFS) rate of both pre- and postmenopausal patients was improved to 65% and overall survival (OS) to 77% (2).

Estrogens play a fundamental role in the initiation and progressive growth of human breast cancer (5). They have two critical functions that contribute to cancer growth and invasion: secretion of vascular endothelial growth factor (VEGF) (6), and regulation of peripheral development of $CD4^+CD25^+$ T-regulatory cells (T-regs) (7). VEGF, the fundamental mediator of angiogenesis (8), is associated with poor prognosis when overexpressed in breast cancer (9) and changes in circulating VEGF levels have been related to clinical outcome (10). In addition, estrogens, by modulating T-reg numbers, have a detrimental effect on the immune response against tumor antigens (11).

The easiest and least toxic way to reduce circulating estrogens is the use of luteinizing hormone releasing

hormone (LH-RH) analogs. In a recent meta-analysis, it was shown that there is a trend towards improved DFS and OS in patients who received an LHRH agonist plus chemotherapy in comparison to those who received chemotherapy alone (12). Ovarian suppression plus chemotherapy was also shown to improve OS in premenopausal patients with estrogen receptor-positive (ER^+) breast cancer (13). In another study, patients with relatively low risk factors and estrogen receptor negative (ER^-) breast cancer were randomized to chemotherapy with or without LH-RH analog goserelin. The patients randomized to chemotherapy plus the LH-RH analog had a statistically significant improvement in DFS and OS with respect to the patients randomized to chemotherapy alone (14).

Immunotherapy, shown to be able to give long-term response with interleukin-2 (IL2) in metastatic cancer (15), in the past few years has been tested in several malignancies. Remarkable responses have been obtained in erb-b2 receptor tyrosine kinase 2 (HER2)-positive metastatic breast cancer by combining immunotherapy with two monoclonal antibodies and chemotherapy (16).

Aromatase inhibitor, which is superior to tamoxifen in postmenopausal patients (17), may be administered to premenopausal patients if an LH-RH analog is given concurrently (18).

We have previously shown that a combination of the growth factors granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) was very active and safe in reducing the toxicity of high-dose chemotherapy with peripheral blood progenitor cell (PBPC) transplantation (19). With this background, in 1995 we started the present study, with the primary endpoint of investigating whether the administration of an LH-RH analog followed by high-dose chemotherapy, with PBPCT, radiation therapy, immunotherapy with IL-2 and an aromatase inhibitor would improve the expected DFS and OS of these premenopausal patients with high-risk breast cancer.

Patients and Methods

Patient selection. Premenopausal patients with histologically confirmed ER^+ or ER^- primary breast cancer were screened for the following inclusion criteria: Stage pT1-3 or pT4b, pN greater than nine, M0, and Ki-67 labeling index ≥30%. Women were considered premenopausal if they had normal menses with plasma estradiol level >40 pg/ml. Patients were enrolled either at the Civilian Hospital of Avezzano or the University of L'Aquila/Carlo Ferri Foundation, Monterotondo. Primary local treatment consisted of mastectomy or breast-conserving surgery with axillary dissection, with at least 10 identifiable and involved lymph nodes in the specimen. The following ranges for laboratory parameters served as additional inclusion criteria: creatinine <2 mg/dl, bilirubin <2 mg/dl, hemoglobin >10 g/dl, platelets >100,000/dl, and neutrophils >2000/dl. Participants were excluded if they had heart disease or reduced lung function, if they had received any prior hormonal treatment, if they were pregnant or lactating, if they had an active infection, or if they had a history of other active malignancies in the past five years except *in situ* cervical carcinoma or non-melanoma skin cancer.

Treatment. Three weeks after surgery, 11.25 mg leuprorelin was administered and repeated every 84 days, during chemotherapy and afterwards, for 5 years, to maintain serum estradiol levels below 40 pg/ml. Six courses of 90 mg/m^2 epirubicin and 75 mg/m^2 docetaxel on day 1 were administered every 21 days as induction chemotherapy. After the sixth course, G-CSF (7 μg/kg) was administered from days 2 to 10 and peripheral blood progenitor cells (PBPCs) were collected by aphaeresis. Radiation therapy was delivered to all patients at 50 Gy, 2 Gy/fraction with five fractions per week, to the chest wall after mastectomy or to the residual breast after breast-conserving surgery, at the apex of the axilla and supraclavicular lymph nodes. A boost of 10 Gy was delivered to the tumor bed. One to two months after the end of radiation therapy, when recovery from the toxicity was complete, patients received an intensification high-dose chemotherapy regimen consisting of carboplatin at an area under the curve (AUC) of 7, 9,000 mg/m^2 ifosfamide and 300 mg/m^2 etoposide, intravenously over three consecutive days, followed by PBPC transplantation and by hematopoietic growth factors (19). One month after high-dose chemotherapy, endocrine therapy was continued in patients with ER^+ disease with 1 mg/day anastrazole for 5 years. Immunotherapy consisted of a fixed dose of 13-*cis*-retinoic acid (RA; 0.5 mg/kg body weight), administered orally with meals for 5 days/week, and IL2, self-administered by subcutaneous injection at bedtime daily, 5 days/week for 3 weeks each month at a dose of 1.8×10^6 IU/day. These doses had been tested previously by our group for various tumor types, showing efficacy and low toxicity (20, 21). Antipyretic, antidiarrheal and antiemetic medications were prescribed as needed. After completion of 1 year of treatment, responding patients received IL2 and RA as maintenance therapy for 5 days/month. In order to improve compliance with therapy during this study period, all patients received psychological support.

Follow-up. At the beginning of the study, participants underwent history and physical examinations and assessment of performance status. Laboratory studies included: complete blood counts, liver and renal function tests, estradiol, progesterone, follicle-stimulating hormone (FSH), LH, carcinoembryonic antigen (CEA), and carbohydrate antigen CA15-3 determination. Bone scanning and computed tomography of the chest and liver were also performed. During the administration of chemotherapy and radiation therapy, the complete blood count was repeated weekly and Sequential Multiple Analysis-14 (SMA-14) monthly. The study was conducted after approval was obtained from the local Ethics Committee and each patient provided written informed consent. During immunotherapy and hormonal therapy, laboratory studies, repeated every four months, included plasma estradiol, progesterone, FSH, LH, CEA, CA15-3, $CD4^+/CD8^+$ ratio and natural killer (NK) cell number. Mammograms and computed tomography of the chest and liver were repeated yearly or more frequently as required.

Statistical analyses. Between May 1995 and September 2009, a total of 42 patients were entered into the study. The accrual period of this study was particularly long, because breast cancer in young women is a relatively rare disease (1). The median follow-up was 10 years (range=6-20 years). The primary endpoint of the study was to assess

Table I. *Characteristics of patients*.

Characteristic	No.	%
No of patients	42	100
Age, years		
Median	41	
Range	34-44	
Histology		
Ductal infiltrating carcinoma	33	78
Lobular infiltrating carcinoma	9	22
PS (ECOG)		
0	36	86
1	6	14
Estrogen receptors		
Positive	23	55
Negative	19	45
Progesterone receptors		
Positive	12	29
Negative	30	71
No. of involved axillary nodes	636	
Median	14	
10-12	16	38
13-15	11	26
16-19	5	12
Over 20	10	24
Grading		
G2	19	45
G3	23	55
Surgery type		
Mastectomy	23	55
Lumpectomy	19	45

ECOG PS: Eastern Cooperative Oncology Group Performance Status.

whether the present therapeutic regimen produced an improvement in the expected DFS and OS of these high-risk premenopausal patients. All patients were recruited at the two centers consecutively. The DFS was defined as the time between the start of high-dose chemotherapy with PBPC transplantation to any relapse and the appearance of a second primary cancer or death, whichever occurred first. The OS was measured from study entry to death, or May 31, 2015 for censored patients. Statistical analysis of DFS and OS was performed using the Kaplan–Meier method (22). All comparisons were performed using Pearson's χ^2 contingency table analysis. Patients were evaluated for toxicity at each study visit according to the National Cancer Institute Common Toxicity Criteria Version 2.0 (23). Statistical analysis was performed with SAS statistical software (version 8.12, 2000; SAS Institute Inc, Cary, NC, USA).

Results

Patients' characteristics. Patients' characteristics are listed in Table I. The median patient age was 41 years (range=34-44 years). Twenty patients had both ER^+ and PGR^+ tumor, while five patients had ER^+ and PGR^-; five patients had HER2-overexpressing disease and tumors in six patients were triple negative. The majority of patients (33/42) had ductal-infiltrating carcinoma. The median number of involved axillary nodes was 14 (range=10-25). The mean Ki-67 index was 39%, while the median duration of treatment from initiation of LH-RH analog and anastrazole was five years.

Table II. *Immune parameters*.

	NK (n/mm^3±sd)	$CD4^+/CD8^+$ (ratio±sd)
Time point		
Baseline (42 patients)	304±15	1.1±0.8
2 Years (39 patients)	509±22	1.87±0.06
	$p<0.0001$	$p<0.05$
5 Years (35 patients)	519±24	1.96±0.1
	$p<0.0001$	$p<0.05$

$CD4^+/CD8^+$ ratio; NK: natural killer cells; sd: standard deviation.

Efficacy. Estradiol levels were evaluated at various time points to assess the hormonal status. The dose of 11.25 mg leuprorelin administered every 84 days was not sufficient in four patients to keep castration levels of estrogen. In two of these patients, the dose of leuprorelin had to be administered every 56 days and every 28 days in the other two patients. As we had previously shown (20, 21), the immunological intervention with IL2 and RA was able to restore normal immune function. In fact after the immunotherapy, our patients exibited improved $CD4^+/CD8^+$ ratios, and an increased number of NK cells (Table II). After a median follow-up of 120 months, DFS rates (Figure 1) were as follows: 5-year: 73%, 8-year: 73%, 10-year: 69.7%, 15- and 20-year: 63.8%. Overall survival rates (Figure 2) were as follows: 5-year: 85%, 8-year: 85%; 10-, 15- and 20-year: 81.6%. Fourteen patients (33.3%) suffered with locoregional and systemic recurrences, after a median time of 24 (range=9-134) months. Six of them were salvaged with surgery and second-line chemotherapy and disease never recurred, while eight of them (19%) died of metastatic disease after a median of 29 (range=13-95) months. Late recurrences, after 90 and 134 months, were observed in two women with ER^+ tumors. Thirteen patients, entered into the study before the age of 40 years, resumed normal menses and three of them had four full-term pregnancies one to two years after completion of hormonal therapy. The compliance rate with the therapy was good: in fact more than 90% of patients adhered to the therapeutic regimen.

Safety. Toxicity frequencies and grades are shown in Table III. Grade 4 hematological toxicity was observed in all patients after high-dose chemotherapy. The median time from transplantation to engraftment of leukocytes at a value greater than 1,000/dl was 10 days (range=5-15 days). The median time to engraftment of platelets at a value greater than 20,000/dl was 10 days (range=5-15 days). One episode

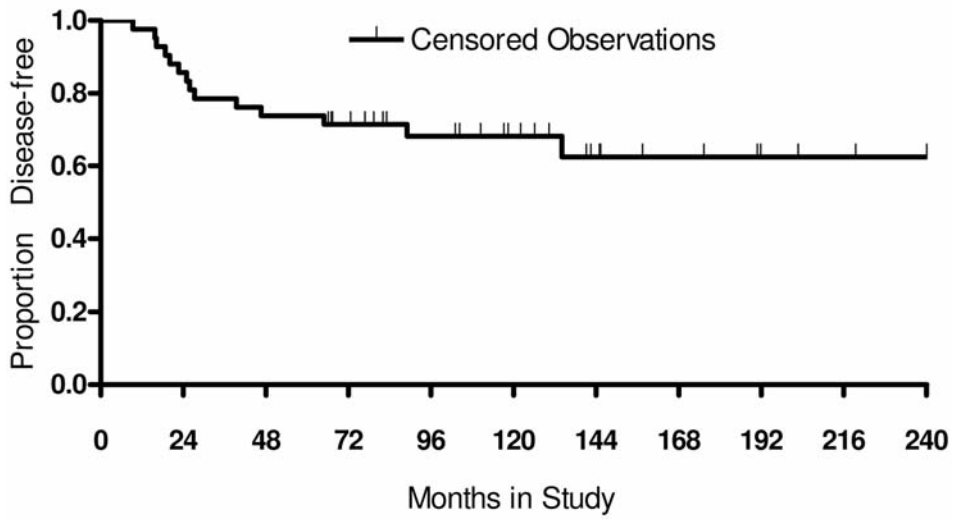

Figure 1. *Disease-free survival. Events n=14 (33.3%). Censored n=28 (66.7%). DFS rate: 5-year: 73%.8-year: 73%, 10-year: 69.7%, 15- and 20-year: 63.8%. Median follow-up=120 months.*

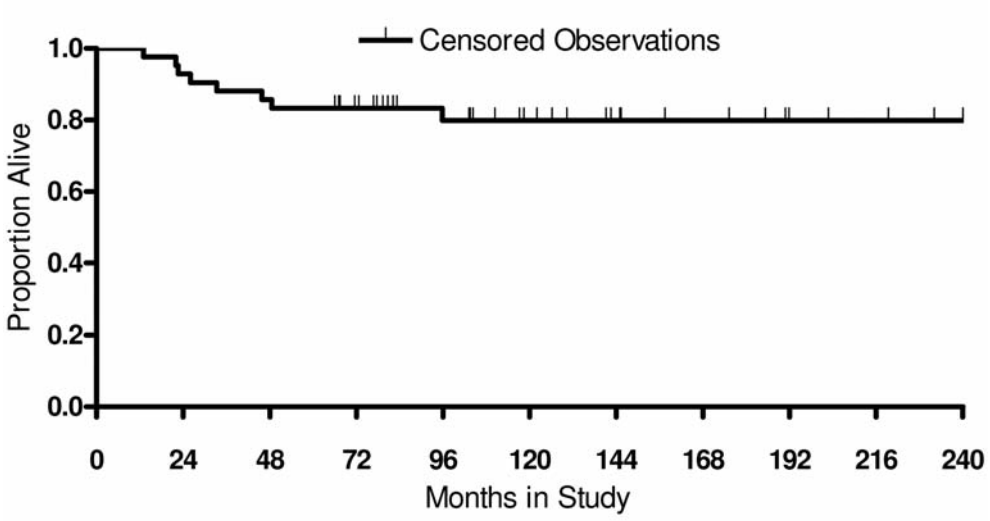

Figure 2. *Overall survival. Events 8: 19%. Censored 34: 81%. OS rate: 5-year 85%; 8-year: 85; 10-, 15- and 20-year: 81.6%. Median follow-up 120 months.*

of fever was observed in eight patients during the aplastic period following transplantation. Infection was diagnosed in three patients (12%). No patient died due to treatment-related causes. No statistically significant decrease of the baseline values of left ventricular ejection fraction was observed after the end of treatment. Grade 2 skin toxicity was observed in three patients after radiation therapy. Toxicity of immunotherapy (Table IV) was mild with grade 1 and 2 fever and cutaneous autoimmune symptoms. The most frequent toxicities of hormonal therapy were hot flashes (45%), arthralgia (20%), and fatigue (15%). All these symptoms decreased in younger patients after the interruption of LH-RH analog.

Discussion

The present study analyzed, after a median follow-up of 10 years, the results of a complex therapeutic regimen

Table III. *Toxicity of chemotherapy.*

	Toxicity grade of chemotherapy							
	1		2		3		4	
Toxicity	N	%	N	%	N	%	N	%
Hematological								
Leucopenia	0	0	0	0	0	0	42	100
Neutropenia	0	0	0	0	2	5	40	96
Thrombocytopenia	0	0	0	0	4	10	38	90
Anemia	8	20	11	27	12	40	5	13
Infection	0	0	8	20	8	20	0	0
Gastrointestinal								
Oral	27	64	10	23	5	13	0	0
Nausea and vomiting	3	7	7	17	5	13	0	0
Diarrhea	3	7	4	10	0	0	0	0
Hepatic	1	2	2	6	0	0	0	0
Neurotoxicity	2	6	0	0	0	0	0	0
Cutaneous								
Alopecia	0	0	42	100	0	0	0	0

Table IV. *Toxicity of immunotherapy.*

	Toxicity of immunotherapy							
	1		2		3		4	
	N	%	N	%	N	%	N	%
Gastrointestinal								
Oral	17	40	0	0	0	0	0	0
Triglycerides	0	0	8	20	0	0	0	0
Diarrhea	8	20	0	0	0	0	0	0
Hepatic	11	27	0	0	0	0	0	0
Fever	14	33	4	10	0	0	0	0
Cutaneous	11	27	8	20	0	0	0	0
Autoimmune disease	13	30	22	53	0	0	0	0

including, high-dose chemotherapy with PBPC transplantation, immunotherapy and hormonal therapy, in a group of premenopausal patients with very high risk of relapse. Indeed, it has been observed that premenopausal patients with early breast cancer and some unfavorable prognostic features have particularly high risk of locoregional and systemic recurrence (24). The reasons for such high risk may be identified in the biology of premenopausal breast cancer for the presence of high serum estradiol levels (6).

The initial enthusiasm for high-dose chemotherapy and PBPC transplantation for the treatment of high-risk breast cancer declined after the publication of the fraudulent results of a study (25). An early study, by Gianni *et al*. reported a 5-year DFS rate of 42% and a 5-year OS rate of 61% in patients with more than 10 involved axillary nodes treated with sequential adriamycin–CMF (2). A large randomized trial of standard-dose chemotherapy has shown the severity of prognosis of premenopausal patients with high-risk breast cancer treated with conventional chemotherapy (26). Indeed, the Danish Breast Cancer Cooperative Group randomized 1,146 premenopausal patients who had positive axillary lymph nodes to four groups: No systemic therapy, levamisole, oral cyclophosphamide (CTX), or CMF for 12 cycles (26). The 10-year DFS rate was 38.6% in the control arm compared to 55.5% in the CTX arm, 48.8% in the CMF arm, and 35.2% in the levamisole arm. The 10-year OS rate was 48% in the control group, 59.7% in the CTX group and 62.2% in the CMF group. In this study, the survival benefit occurred mostly from 5 to 10 years after randomization, and persisted up to 25 years.

Nevertheless several studies, with conflicting results, have explored the utility of high-dose chemotherapy with PBPC transplantation in the treatment of high-risk breast cancer. The study of International Breast Study Group randomized 344 patients to epirubicin–cyclophosphamide chemotherapy followed or not by high-dose chemotherapy. There was a trend for a better 5-year DFS for patients randomized to high-dose chemotherapy (70%) *versus* patients treated with standard-dose chemotherapy (61%) (27). The same trend for a better DFS of those treated with high-dose chemotherapy compared to standard-dose chemotherapy was shown by Nitz *et al*. (28): Four-year event-free survival (intention-to-treat analysis) was 60% (95% confidence interval=53-67%) in the high-dose chemotherapy group and 44% (95% confidence interval=37-52%) in the control group (p=0.00069). The corresponding OS was 75% (95% confidence interval=69-82%) *versus* 70% (95% confidence interval=64-77%; p=0.02). Another randomized study of high-dose adjuvant chemotherapy with autologous hematopoietic stem-cell support *versus* standard-dose chemotherapy in patients with breast cancer with 10 or more positive lymph nodes also showed a trend for a superiority of high-dose chemotherapy. In fact, after a median follow-up of 6.1 years, with respect to DFS, 91 events have been observed in the standard therapy arm and 75 events in the high-dose chemotherapy arm, with hazard ratios of high-dose *versus* standard-dose arm estimated as 0.80 (95% confidence interval 0.59-1.08; p=0.15). The trend for a superiority of high-dose as compared with standard-dose with respect to DFS seems to be more pronounced in premenopausal patients as compared with postmenopausal patients, and in patients with tumor grade 3 as compared with patients with tumor grade 1 or 2 (29). In another randomized study, an identical 5-year DFS was achieved by Peters *et al*.: In this study, the 5-year DFS rate of patients with high-risk breast cancer was 71% both in the high-dose and standard-dose chemotherapy arms (30). Indeed, a meta-analysis exploring the role of high-dose chemotherapy with PBPC transplantation, with respect to standard-dose chemotherapy,

showed that there was a significant benefit in DFS for the high-dose group, which at 5 years approached statistical significance (relative risk=1.06, 95% confidence interval=1.00-1.13). However, OS rates were not significantly different at any stage of follow-up and there was insufficient evidence supporting routine use of high-dose chemotherapy with autologous graft for treating early breast cancer with poor prognosis (31).

As shown previously, ovarian suppression given concurrently with chemotherapy gives an advantage with respect to chemotherapy alone. Additionally, we chose a chemotherapy with docetaxel and epirubicin, followed by radiation therapy over approximately 18 weeks in order to avoid the possible development of resistance (32). Afterward, a course of high-dose chemotherapy with carboplatin, ifosfamide and etoposide was administered when the response to chemotherapy was at its maximum, and we would maximize the probability of eradicating micrometastatic disease. After high-dose chemotherapy, a depressed immune function, with low $CD4^+/CD8^+$ ratios, and low levels of NK cells was observed. Cancer immunotherapy, initiated with high-dose IL2 (15) has made considerable progress (33). It is important to stress the significant improvement of immunological parameters ($CD4^+/CD8^+$ ratio, and the number of NK cells) after IL2 and RA immunotherapy.

The long-term efficacy of the aromatase inhibitor anastrozole as post-surgical adjuvant treatment for postmenopausal women with hormone-sensitive tumors has been clearly established (34). In another study, premenopausal women with node-positive disease received chemotherapy followed by randomization to tamoxifen or placebo for 5 years. Patients with ER^+ tumors who achieved chemotherapy-induced amenorrhea had a significantly improved outcome (hazard ratio for amenorrhea *vs*. no amenorrhea=0.61; 95% confidence interval=0.44 to 0.86; p=0.004) (35). After chemotherapy, we treated our patients for 5 years with concurrent anastrazole and LH-RH analog, producing a total estrogen blockade. In our study, OS achieved at 5 years was 85%: this figure persisted roughly up to 18 years (81.6%).

In conclusion, sequential treatment with LH-RH analog, high-dose CT and immunotherapy for patients with BC>10 is feasible, has moderate toxicity, and while preserving ovarian function, seems to improve the expected DFS and OS for these high-risk patients.

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