Paclitaxel-containing High-dose Chemotherapy in High-risk Breast Cancer Patients

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Despite standard-dose adjuvant chemotherapy, the prognosis for patients with breast cancer and extensive axillary lymph node involvement at diagnosis is poor. The efficacy of a paclitaxel-containing, high-dose chemotherapy protocol in 21 high-risk breast cancer patients is assessed. After standard-dose chemotherapy followed by peripheral blood stem cell (PBSC) mobilization, high-dose therapy with paclitaxel, carboplatin, and cyclophosphamide and CD34-selected PBSC rescue was given. Hematologic reconstitution after high-dose therapy was rapid. Main toxicity included diarrhea grade I or II in about half of the patients and infections were observed in 19%. Five-year probabilities for relapse and failure-free survival were 32% and 62%, respectively. High-dose consolidation with paclitaxel, carboplatin, and cyclophosphamide achieves a high failure-free survival in patients with high-risk breast cancer with acceptable toxicities and stable, long-term hematopoietic reconstitution. Evaluation of the benefit of high-dose therapy in these patients in larger prospective, randomized trials is warranted and currently under way.

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Despite standard-dose adjuvant chemotherapy, the prognosis for patients with breast cancer and extensive axillary lymph node involvement at diagnosis is poor. Ten-year survival is 35% to 65% for those with one to three involved axillary lymph nodes, 30% to 40% for those with four to nine involved axillary nodes, and 15% to 30% in those with more than nine involved axillary nodes (1–3). Because of the poor long-term results obtained with standard-dose chemotherapy in high-risk primary breast cancer, the use of high-dose chemotherapy with hematopoietic stem cell rescue has been widely explored in selected patients in recent years (4–8). Retrospective analyses showed superior outcomes in high-risk breast cancer patients. In addition to a recently published study, various large, randomized phase III trials are currently under way (9–11).

So far, combinations of cyclophosphamide (CY), cisplatin, and carmustine or CY, thiotepa, and carboplatin were mainly used in high-dose regimens (3–5, 8, 11). Paclitaxel, a drug with known activity in breast cancer, acts in part by enhancing microtubule assembly and inhibiting the depolymerization of tubulin (12, 13). Selected in vitro and animal studies have shown that paclitaxel-induced apoptosis exhibits a steep dose-response curve similar to the cell kill seen with alkylating agents (14, 15). When this study was initiated, paclitaxel at doses ranging from 200 to 250 mg/m^2 had achieved promising results in phase II trials in patients with metastatic breast cancer (13, 16). However, myelosuppression was the most frequent and severe adverse effect of paclitaxel therapy. Since carboplatin and CY represent antineoplastic agents widely used in breast cancer patients with well-known toxicity profiles, they were combined with paclitaxel and used as novel high-dose chemotherapy in our study. To reduce risk of relapse due to tumor cell contamination of peripheral blood stem cell (PBSC) products, a positive selection for CD34-progenitors using a biotin-avidin immunoadsorption method was performed as previously reported (17, 18).

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MATERIAL AND METHODS

Patients

Women younger than 60 years with histologically proven non-inflammatory breast cancer who had undergone primary tumor excision and axillary node dissection showing 7 or more lymph nodes involved were eligible for the study. The patients were required to have a performance status $\geq 80\%$ (Karnofsky), no evidence of cardiac, pulmonary, liver, or renal impairment as determined by echocardiographic left ventricular ejection fraction $\geq 50\%$, forced expiratory volume in one second greater than 70%, liver function tests of less than 2 times the normal, and serum creatinine level < 1.5 mg/dl. Pretreatment baseline evaluation included physical examination, chest x-ray, bone scan, liver echography and serology including appropriate tumor markers. Patients were excluded if they had a history of previous antineoplastic therapy or metastatic disease including bone marrow involvement. The latter was assessed by unilateral bone marrow biopsy for conventional histology and immunohistochemical staining with mouse anticytokeratin monoclonal antibodies, as described (19). The study was approved by the Ethics Committee of our institution and all patients gave written, informed consent.

The patients' pre-transplant characteristics are presented in Table 1. Most of the patients had undergone modified radical mastectomy (76%), while only 5 had breast-conserving surgery.

Table 1

Patient characteristics

No.	21
Median age in years	43
Range	24–58
Tumor size	
<5 cm	14
>5 cm	7
Histology	
Lobular	4
Ductal	14
Lobular and ductal	3
No. of involved lymph nodes	
Median	14 (7–32)
Less than 10	4
10–15	11
16–19	3
More than 20	3
Hormone receptor status	
ER or PR or both positive	15
ER and PR negative	5
Unknown	1
Menopausal status	
Premenopausal	15
Postmenopausal	6
Median follow-up from surgery for patients in CCR, in months	34 (12–61)

Abbreviations: No. = number; ER = estrogen receptor; PR = progesteron receptor; CT = chemotherapy; PBSCT = peripheral blood stem-cell transplantation; CCR = continuous complete remission.

Standard-dose adjuvant therapy

The median time from surgery to first cycle of outpatient standard-dose adjuvant therapy was 23 days (range, 5–40). All patients received 2 cycles of FAC (5-fluorouracil 500 mg/m^2 i.v. days 1 and 8, doxorubicin 50 mg/m^2 i.v. day 1, and cyclophosphamide 500 mg/m^2 i.v. day 1) chemotherapy at 28-day intervals.

Peripheral blood stem cell (PBSC) harvest and CD34 selection

For PBSC mobilization, cyclophosphamide 4 g/m^2 was administered by 1-h infusion with urine alkalization, intensive intravenous hydration, and intravenous 2-mercaptoethane sulfonate (mesna) for uroprotection. To accelerate hematopoietic recovery and to improve PBSC harvesting, recombinant human granulocyte colony-stimulating factor (G-CSF; Filgrastim, Amgen, Thousand Oaks, CA, USA) was given at 10 μg/kg/day subcutaneously, starting 6 days after CY. When CD34-positive cells in peripheral blood (PB) increased above 20/μl, consecutive leukaphereses using a Fenwall CS 3000 (Baxter Healthcare, Chicago, IL, USA) or COBE Spectra (COBE, Denver, CO, USA) cell separator were performed.

PB CD34-positive cells were identified by immunofluorescence staining and analysis as described (8). CD34-positive cells were isolated from one to two leukapheresis products with the Stem Cell Concentrator Ceprate SC (CellPro, Inc, Bothell, WA, USA) and cryopreserved as described (8).

Granulocyte-macrophage colony-forming units (CFU-GM) were assayed and scored after 14 days of incubation in a humidified atmosphere at 37°C in 5% CO_2, as previously described (17).

High-dose therapy

After PBSC harvest, patients received high-dose therapy after a median of 33 days (range, 22–62). The therapy consisted of paclitaxel 250 mg/m^2 infused over 3 h on day -8, carboplatin 300 mg/m^2/day infused over 1 h on 5 days (days -7 to -3), and cyclophosphamide 60 mg/kg patient's body weight (b.w.) infused over 1 h on 2 days (days -3 and -2), respectively. Before paclitaxel infusion, all patients were premedicated with dexamethasone, cimetidine, and diphenhydramine as previously described (16). All doses of chemotherapy were calculated on the actual patient body weight unless the actual weight was $>$ 20% over the ideal body weight, in which case the average of the actual and ideal weight was used to calculate the body surface area. CD34-selected PBSCs were injected intravenously 48 h after completion of chemotherapy (day 0). G-CSF at 10 μg/kg/day was infused continuously starting on day $+1$ until an absolute neutrophil count

(ANC) $> 1 \times 10^9$/l was achieved and maintained for at least three consecutive days.

Patients were hospitalized in single rooms with reverse isolation. They received antimicrobial prophylaxis with non-absorbable antibiotics, and low-bacterial, low-fungal content diet with standard mouth care. Antibiotics were given i.v. for febrile episodes associated with aplasia. Packed red blood cells (RBC) were given to maintain hemoglobin concentration above 8.0 g/dl and platelet transfusions to keep the platelet count above 20×10^9/l.

Postintensification therapy

Approximately 6 to 8 weeks after high-dose therapy, patients with multicentric carcinoma or marked lymphangiosis carcinomatosa received 50 Gy of irradiation to the chest wall and regional lymph nodes using standard radiation techniques.

All patients with hormone-positive receptor status were given tamoxifen 10 mg orally twice daily for 5 years.

Evaluation of toxicity and hematologic reconstitution

All cases of non-hematologic dysfunction were considered regimen-related toxicities and graded according to the WHO scale as described (20).

All patients had peripheral blood and reticulocyte counts on a daily basis until hematologic reconstitution. Hemograms were obtained with the Sysmex NE-1000 and white blood cell (WBC) differential counting was carried out from smears after modified Wright staining. ANCs were calculated from leukocyte and differential counts. Engraftment was defined as ANC of 0.5×10^9/l, platelets of 20×10^9/l and independence of RBC and platelet transfusions.

Statistical analysis

Descriptive statistics are reported as frequencies and medians. Differences in time to engraftment between patients given more or less than 3×10^6 CD34-positive cells/kg b.w. were assessed by the Mann-Whitney test. Transplant-related mortality, relapse and failure-free survival were calculated according to the Kaplan–Meier method. Data were analyzed as of November 30, 1998.

Treatment failure was defined as disease recurrence or toxicities related to antineoplastic disease and affecting survival.

RESULTS

PBSC collection and CD34 selection

PBSC harvest was initiated after a median of 12 days (range, 10–16) following the administration of CY. To obtain at least 3×10^6 CD34-positive cells/kg b.w., a median of 1.5 leukaphereses (range, 1–3) were performed for CD34 selection without any significant complications, with only one patient needing three sessions. Following CD34 selection the mean total nucleated cell recovery was 1.6%, while the mean CD34-positive cell recovery was 57%. Patients received a mean of 6.6×10^6 CD34-positive cells/kg b.w. with a mean purity of 79%. Seven out of 21 patients were given less than 3×10^6 CD34-positive cells/kg b.w., respectively.

Table 2

Non-hematologic toxicities

	No. of patients				
Toxicity	0	I	II	III	IV
Mucositis	17	3	1	0	0
Nausea/vomiting	18	2	1	0	0
Diarrhea	9	3	8	1	0
Neuropathy	15	6	0	0	0
Arthralgia/myalgia	20	0	1	0	0
Rise of transaminases	13	5	1	2	0
Hemorrhagic cystitis	20	0	0	1	0
Infection	17	0	2	1	1
Bleeding	21	0	0	0	0

Hematologic reconstitution after high-dose therapy

One patient died on day 10 and thus could not be assessed for hematopoietic regeneration. In all other patients a rise in ANC $> 0.5 \times 10^9$/l and platelet counts $> 20 \times 10^9$/l was seen after a median of 11 days. RBC and platelet transfusion independence was achieved after a median of 8 and 10 days, respectively. In patients given less than 3×10^6 CD34-positive cells/kg b.w., hematologic reconstitution was significantly prolonged compared to patients receiving higher CD34-cell numbers ($p = 0.007$).

Non-hematologic toxicity

Data obtained on non-hematologic toxicity of high-dose chemotherapy are presented in Table 2. Infections were observed in 19% of all patients and included *Candida albicans* enteritis (n = 1) or stomatitis (n = 1), staphylococcus bacteremia (n = 1), and *E. coli* sepsis in one patient. The *E. coli*-infected patient died on day 10 after PBSC infusion, despite appropriate antimicrobial therapy. The probability of transplant-related mortality 100 days after high-dose therapy was 5%.

Analysis of relapse and failure-free survival

As of November 30, 1998, 5 patients (24%) experienced relapse involving the bones (n = 3) or contralateral breast (n = 2). Response to salvage chemotherapy or local irradiation was poor. All patients progressed with treatment. With a median follow-up of 34 (range, 12–61) months since initial surgery the actuarial probability of relapse at 5 years is 32% (Fig. 1). Probability of failure-free survival at 5 years is 62% (Fig. 2). Mean time to treatment failure was 13.5 (range, 5–22) months (95% confidence intervals).

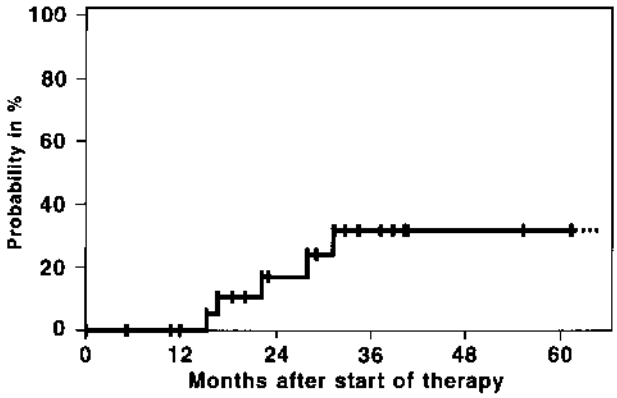

Fig. 1. Probability of relapse in patients with high-risk breast cancer given high-dose chemotherapy.

DISCUSSION

High-dose therapy is increasingly used for treatment of chemotherapy-sensitive hematologic malignancies and solid tumors. In this study we analyzed the toxicity and efficacy of high-dose therapy in patients with high-risk breast cancer and well-defined pretreatment characteristics using a new regimen consisting of paclitaxel, carboplatin, and cyclophosphamide after standard-dose adjuvant therapy. Paclitaxel has shown excellent activity in breast cancer with response rates of up to 62% in metastatic disease (13, 16, 21). Hypersensitivity reactions were among the major toxicities observed in some patients treated with paclitaxel (12, 22, 23). With proper premedication, as performed in our study, these reactions can be prevented. Gastrointestinal- and neurotoxicities were reported to be dose-limiting in previous studies (22, 24). Twelve patients developed diarrhea, which in our series was the main non-hematological toxicity, and resolved in all patients. Neuropathies were mild and reversible after 2 to 3 weeks, respectively. Whereas up to 28% of patients given conditioning with CY, cisplatin, and carmustine or CY, thiotepa, and carboplatin for high-dose therapy had moderate renal, cardiac, and hepatic toxicity and about a third of patients had pulmonary toxicity (3, 4), these side effects were not observed in our study using paclitaxel, carboplatin, and CY. Documented infections occurred in 4 patients (19%) and responded rapidly to broad-spectrum antibiotics. However, there was one death due to *E. coli* sepsis. Further analysis revealed that all documented infections were observed in patients given less than 3×10^6 CD34-positive PBSC/kg b.w. compared to patients transplanted with higher cell numbers.

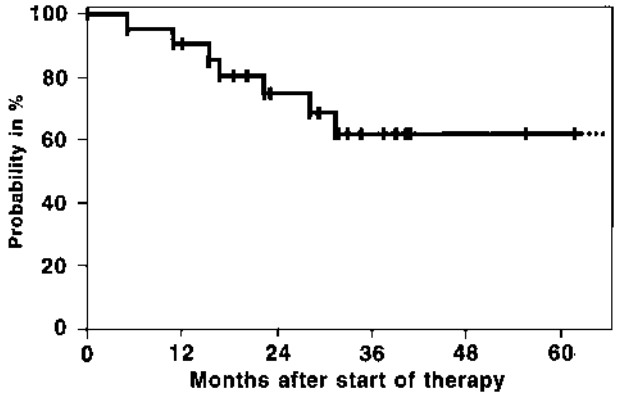

Fig. 2. Probability of failure-free survival in patients with high-risk breast cancer after adjuvant high-dose chemotherapy.

Various centers have reported treatment-related mortality rates of between 4% and 30% in pilot series (3–6, 8). Recently, improvements in inpatient supportive care management resulted in a decrease in treatment-associated deaths (25).

Many trials with high-dose therapy used an average CD34-positive cell number between 0.5 and 5×10^6/kg b.w. Our engraftment data with PB CD34-positive cells compare favorably with previous reports on breast cancer patients receiving positively selected CD34 cells with G-CSF mobilized blood (8, 18). In accordance with previous findings, a significant prolongation of both neutrophil and platelet recovery was seen in our patients given PBSC with a lower CD34 content (26, 27). Thus, more than 3×10^6 CD34-positive cells/kg b.w. should be considered as appropriate PBSC rescue in future studies.

Immunohistochemical or long-term culture techniques can usually detect occult tumor cells in marrow and peripheral blood of breast cancer patients, even when routine histologic evaluation is normal (19, 28, 29). For autologous marrow transplants, it has been shown that reinfusion of tumor cells might be an important prognostic indicator in predicting relapse (30, 31). Recently, a risk of co-mobilization of tumor cells and hematopoietic progenitor cells during PBSC recruitment has been observed (28). To reduce tumor cell contamination of PBSC a positive selection of CD34 progenitor cells with a biotin-avidin immunoadsorption method can be applied resulting in an up to 4-log tumor cell depletion (8, 17, 18). In our patients immunohistochemical staining of all marrow samples prior to PBSC mobilization did not reveal any tumor cell infiltration. Whether the positive selection of hematopoietic progenitor cells from PBSC harvests had an impact on relapse-free survival in our patients cannot be assessed with certainty. Considering the costs and effort involved and the lack of prospective randomized trials comparing unselected and CD34-positive PBSC transplants in breast cancer patients, in vitro tumor cell depletion techniques should not be recommended for clinical routine.

In our study the Kaplan–Meier estimates of relapse and failure-free survival at 5 years were 32% and 62%, respectively, and this compares favorably with results previously reported in high-risk breast cancer patients (3–5). However, only a small and selective number of patients were included in our study and data should be confirmed in

more patients. This novel chemotherapy regimen is highly effective, with acceptable toxicities and rapid and stable long-term trilinear hematopoietic engraftment. So far, the question of whether high-dose chemotherapy is beneficial in high-risk breast cancer patients remains unanswered since larger prospective randomized trials are still in progress (9, 10).

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