High-Dose Chemotherapy and Autologous Stem Cell Support followed by Posttransplantation Doxorubicin as Initial Therapy for Metastatic Breast Cancer

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ABSTRACT
High-dose chemotherapy is associated with a high complete response rate and possibly some survival advantage in patients with metastatic breast cancer. We designed a clinical trial consisting of a two-step high-dose chemotherapy regimen followed by posttransplantation doxorubicin as the first chemotherapy treatment for metastatic disease. Twenty-one patients with metastatic breast cancer and no previous chemotherapy for metastatic disease were treated with high-dose cyclophosphamide (Cyt; 5000 mg/m²), followed by granulocyte colony-stimulating factor. Peripheral blood stem cells were collected. Subsequently, patients received Cyt (6000 mg/m²), thiotepa (500 mg/m²), and carboplatin (800 mg/m²) (CTCb) with hematopoietic rescue. Upon recovery of hematopoietic and gastrointestinal toxicity, three cycles of doxorubicin (Dox; 60 mg/m²) were delivered. After Cyt, nine patients (45%) developed neutropenic fevers. There were no episodes of bacteremia. Patients received CTCb 37 days after starting Cyt and had a hospital stay of 19 days. After CTCb, the median number of days to an absolute neutrophil count >5 x 10⁹/liter was 8, and the median number of days to a platelet count >20 x 10⁹/liter was 9. Neutropenic fevers occurred in 12 patients. There were no hemorrhagic complications. Fifty-five of the 63 planned courses of Dox were delivered. The median time from peripheral blood stem cell infusion to the first Dox cycle was 38 days. The median time to the second Dox cycle was 28 days, and to the last cycle was 30 days. Three episodes of neutropenic fevers were observed. Two patients developed herpes zoster. This regimen is feasible, with acceptable toxicity.

INTRODUCTION
MBC² remains an incurable disease. Despite a better understanding of the natural history of breast cancer, the overall median survival of patients with metastatic disease has not been improved by current standard-dose chemotherapy. Dose-intensive chemotherapy followed by stem cell transplantation has been associated with increased response rates. HDC programs have consisted mainly of standard-dose induction therapy followed by one or occasionally two doses of HDC for patients who first achieve an objective response with induction treatment. The rationale has been that the initial induction produces a degree of cytoreduction prior to the administration of HDC. Multiple regimens comprising a variety of numbers and schedules of drugs have been used in the MBC patient population. At least 42 studies utilize a single HDC cycle with complete response rates averaging 50% and 10–20% disease-free survival at 4–5 years (1). This strategy can be challenged on several aspects. It can be argued that the induction phase may allow expansion of populations that are only partial sensitive to HDC treatment, compromising the ability to be cured. If this assumption is correct, HDC should be used as initial therapy and not after induction therapy. The cytoreductive component may, in fact, be a relatively minor player in many cases. Partial remission, which is usually accepted as a criterion for proceeding with HDC likely represents no more than several logarithms of cell kill that constitute a relatively small step toward eradication of tumor. The use of HDC as initial therapy in MBC may, in theory, limit the emergence of drug resistance clones. At least 87 patients with MBC have received HDC as initial therapy. Response rates of 55% were obtained, with 33% of patients remaining in complete continuous remission from 11 to 120 months (1). The leading cause of treatment failure following HDC is relapse. If we assume that this reflects the survival of resistant cancer cells among the heterogeneous clones in the original tumor, clinical advances in this area would require additional therapy posttransplantation. On the basis of the above concept, we hypothesize that up-front HDC would be able to achieve a state of minimal residual disease in a substantial percentage of patients with MBC. We believe that posttransplantation chemotherapy with non-cross-resistant agents may be able to improve on the current results. We believe that the initial HDC will translate clinically into an effective induction of response. Moreover, the initial cytoreduction may act as a...
powerful kinetic stimulus for the nondividing cells to begin cycling. Previously dormant cells surviving the initial phase will then be kinetically more active and more sensitive (2). At this point, the administration of multiple cycles of chemotherapy may improve on the response rate and its duration.

We devised a treatment consisting of three steps. During the first step, patients receive high-dose cyclophosphamide. The goal of this step is dual. High-dose cyclophosphamide is used as a mobilizing agent for the collection of PBSCs and also for its antitumor effect in breast cancer. The second step includes the use of high-dose chemotherapy followed by PBSCs rescue. As a conditioning regimen, we chose CTXb due to its proven efficacy and low morbidity and mortality in patients with breast cancer. After the initial two steps, a large percentage of patients should have achieved a state of minimal residual disease. At this point, we planned to deliver three cycles of doxorubicin, the most active single agent against breast cancer.

The present trial studied the feasibility and safety of a HDC program consisting of an initial cycle of high-dose cyclophosphamide followed by CTXb and PBSC rescue and posttransplantation doxorubicin.

PATIENTS AND METHODS

The protocol was approved by the Institutional Review Board for the Biomedical Research at the University of Pittsburgh. Informed written consent was obtained from all the patients prior to entry into the study.

Patient Eligibility. Eligibility criteria included histologically proven metastatic breast carcinoma; age less than 65 years old; Eastern Cooperative Oncology Group performance status of 0 or 1; or brain metastases; less than 250 mg/m² of cumulative doxorubicin; cardiac ejection fraction greater than 50%; adequate pulmonary function, including a carbon monoxide diffusion capacity of more than 60%; serum creatinine of less than 1.5 mg/dl; no prior chemotherapy for metastatic disease; and failure of one or more hormonal manipulations in patients with ER-positive receptors with bone and/or soft tissue disease alone.

Treatment Plan. The study design is depicted in Table 1. The study consisted of three steps. In step I, the patients were admitted to the hospital, and a double-lumen leukapheresis vascular catheter was inserted into the subclavian vein. Patients were hydrated and treated with cyclophosphamide at 5000 mg/m² given as a continuous i.v. infusion over 24 h. All patients were given 2-mercaptoethane sulfonate as uroprotection. Imme-

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<table>
<thead>
<tr>
<th>Table 1 Study design</th>
<th>Table 2 History of prior therapies</th>
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<tr>
<td><strong>Agent</strong></td>
<td><strong>Dose</strong></td>
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<tr>
<td>Cyclophosphamide</td>
<td>5000 mg/m²</td>
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<tr>
<td>G-CSF</td>
<td>5 μg/kg/day</td>
</tr>
<tr>
<td>Leukapheresis</td>
<td>7 × 10⁶ mononuclear cells/kg</td>
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<tr>
<td><strong>Step I</strong></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>6000 mg/m²</td>
</tr>
<tr>
<td>Thiotepa</td>
<td>500 mg/m²</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>800 mg/m²</td>
</tr>
<tr>
<td>Hematopoietic rescue</td>
<td>PBSCs</td>
</tr>
<tr>
<td><strong>Step II</strong></td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>60 mg/m² × 3 cycles</td>
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</table>

Step I. The study consisted of three steps. In step I, the patients were admitted to the hospital, and a double-lumen leukapheresis vascular catheter was inserted into the subclavian vein. Patients were hydrated and treated with cyclophosphamide at 5000 mg/m² given as a continuous i.v. infusion over 24 h. All patients were given 2-mercaptoethane sulfonate as uroprotection. Imme-

Step II. Patients received cyclophosphamide (1500 mg/m² daily) on days −6, −5, −4, and −3; thiotepa (125 mg/m²) on days −6, −5, −4, and −3; and carboplatin 200 mg/m² on days −6, −5, −4, and −3. These drugs were delivered i.v. via continuous infusion. 2-Mercaptoethane sulfonate uroprotection at 150 mg/m² was administered daily from day −6 to day −2. PBSCs were infused on day 0, and daily G-CSF was reinstated at 5 μg/kg/day on day +1 and continued until the patient achieved a sustained neutrophil count of greater than 1 × 10⁹/liter for 3 days. Step III was initiated after patients recovered from any GI toxicity to grade I or better and after patients achieved an absolute neutrophil count of equal or more than 1.5 × 10⁹/liter and a platelet count of more than 100 × 10⁹/liter. It consisted of three cycles of doxorubicin at 60 mg/m² delivered i.v. every 3 weeks if possible. Each cycle of doxorubicin was postponed until the above-listed hematological parameters were achieved.

Supportive Care. During the second phase of the study, all patients were cared for in private rooms with a positive-pressure, high-efficiency particle-filtration air system. Antimicrobial prophylaxis with ciprofloxacin and fluconazole was used routinely as well as acyclovir in patients with elevated herpes simplex antibody titers. All blood products were filtered and irradiated. Parental nutrition and broad spectrum antibiotic coverage was provided as needed. Patients were transfused with leukocyte-free packed RBCs and platelets to maintain hemoglobin values greater than 8 g/dl and a platelet count greater than 10 × 10⁹/liter.

Hematopoietic Progenitor Cells. PBSCs were collected at the time of hematopoietic recovery after cyclophosphamide and G-CSF starting when leukocyte and platelet counts reached 1 × 10⁹/liter and 50 × 10⁹/liter, respectively. Harvesting was performed using a Cobe Spectra cell separator (Cobe BCT, Inc.) until the total collected mononuclear cell count was 7 × 10⁹/kg. The PBSC product was cryopreserved in the usual manner. The total number of CD34+ and lineage-negative (CD34+lin−)
cells were determined in daily PBSC products by flow cytometry by staining cells with two anti-CD34, FITC-conjugated monoclonal antibodies, 8G12 (Becton-Dickinson, San Jose, CA) and Qbend-10 (Gen Track, Plymouth, PA) and four lineage-specific phycoerythrin-conjugated monoclonal antibodies with specificities for CD3, CD11b, CD14, and CD19 (Becton Dickinson). Granulocyte macrophage colony-forming units were assayed using the Pike’s method and scored after 14 days of incubation.

**Response Criteria, Toxicity, and Statistical Analysis.**

All patients were considered evaluable for toxicity. Toxicity was graded according to the National Cancer Institute common criteria. CR was defined as the complete disappearance of all evidence of tumor, including normalization of laboratory parameters. PR was defined as 50% or greater reduction in the sum of the perpendicular diameters of measurable lesions without evidence of progression. The persistence of uptake in bone scan despite sclerosis of previous lytic lesions was defined as PR.

**PBSC Collection**

Leukapheresis was performed following the first phase of the study (high-dose cyclophosphamide) and was devoid of any significant morbidity. A median of 38.2 \( \times 10^6\) cells CD34+lin− cells (range, 0.97–114) and of 9.11 \( \times 10^6\) granulocyte macrophage colony-forming units/kg (range, 0.73–38.7) were collected and infused. The median number of leukapheresis was 6 (range, 3–11).

**Toxicity**

**Step I.** All 21 patients completed step I of the study. Grade I-II GI toxicity was observed in 11 of the 20 patients. The median days of a neutrophil count less than 0.5 \( \times 10^9\) and a platelet count less than 20 \( \times 10^9\) were 4 (range, 3–8) and 3 (range, 0–10). Ten patients (48%) developed neutropenic fevers and were treated with broad-spectrum antibiotics. These patients required readmission to the hospital. There were no episodes of documented bacteremia. Eight of the nine patients who developed neutropenic fevers had not received prophylactic antibiotics.

**Step II.** All 21 patients completed step II of the study. The median number of days elapsed from the start of cyclophosphamide until admission for CTCb was 37 (range, 25–60). Grade I-II GI toxicity was observed in 16 of 20 patients. Neutropenic fevers developed in 13 patients. There was only one episode of documented bacteremia. Two patients experienced elevation in both serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase to levels twice and three times the upper limits of normal. These patients had no evidence of prior liver disease, and liver functions returned to baseline levels within 1 month after finishing CTCb. One patient developed pancreatitis, which was documented by an increase in amylase and lipase. Evaluation included abdominal ultrasound, a computed tomography scan of the pancreas and endoscopic retrograde cannulation of the pancreatic duct, all of which failed to reveal an etiology. The levels of the amylase and lipase returned to normal within 6 weeks. One patient developed herpes simplex localized to the oral region. One patient developed *Clostridium difficile* diarrhea. The median number of days to recovery of a neutrophil count to greater than 0.5 \( \times 10^9\) was 8 (range, 7–10), and to a platelet count greater than 20 \( \times 10^9\) was 9 (range, 8–11). The median number of RBC transfusions was two (range, 1–4). Nineteen patients received platelet transfusions during this phase. The median number of platelet transfusions was four (range, 1–5). No hemorrhagic complications occurred. The median duration between the first day of hospitalization until discharge from the hospital during CTCb was 19 (range, 8–30). No cases of hemorrhagic cystitis or veno-occlusive disease were observed. There were no deaths due to toxicity during this phase of the study.

**Step III.** All but one patient proceeded to step III. This patient failed to achieve a platelet count of more than 100 \( \times 10^9\) as stipulated in the study design as a criterion to proceed to step III. Four patients failed to complete all three cycles of doxorubicin. One patient progressed after the first cycle of doxorubicin. Three other patients received two cycles of doxorubicin each but refused the last cycle. A total of 55 cycles of doxorubicin were delivered. Three episodes of neutropenic fevers were observed. Blood cultures were positive in
two of these episodes. Two patients developed herpes zoster. One patient developed chest pain after two cycles of doxorubicin. Evaluation by cardiac radionuclide-gated blood pool scanning showed a normal left ventricular ejection fraction. Cardiac enzymes and electrocardiogram results were normal during the chest pain episode. The median time from the day of PBSC infusion to the first doxorubicin dose was 38 days (range, 22–65). Although hematopoietic recovery was prompt after step II, prolonged GI toxicity precluded earlier initiation of the first dose of doxorubicin. The median time from the first doxorubicin dose to the second was 28 days (range, 21–46). Thirty % of patients were able to receive the second dose of doxorubicin precisely 3 weeks after the first cycle. The median time from the second doxorubicin dose to the third was 30 days (range, 21–48). Three cycles of doxorubicin were delivered with G-CSF. Grafts are stable at 6 months posttransplantation, with patients having a median leukocyte count of 4.6 \times 10^9/liter (range, 2.4–4.9) and a median platelet count of 181 \times 10^9/liter (range, 41–312).

**Response**

Median duration of follow-up for all patients is 28 months (range, 12–39). Restaging, whenever possible, was performed after each phase of the study. Eighteen patients wererestaged after step I. Five patients achieved a PR, 10 patients showed StD and 3 PD. After step II, all patients were restaged, with 12 achieving a PR, 1 CR, and 8 StD. Due to the short interval preceding subsequent steps, tumor response to cyclophosphamide and CTCb is difficult to interpret. At completion of step III, there were nine PRs and eight CRs, two patients had StD, and two had PD. The overall response rate was 81%, with 43% PRs and 38% CRs (Table 4). Four of the eight patients in CR after the completion of therapy remain in continuous CR. The predominant sites of disease were visceral in three of these patients and bone in one patient. Three were ER and progesterone receptor negative, and one was ER and progesterone receptor positive. Two patients received adjuvant cyclophosphamide and doxorubicin and another cyclophosphamide, methotrexate, and fluorouracil. Four of the eight patients in CR progressed at 9, 16, 17, and 31 months. Of the nine patients who achieved a PR, eight have disease progression. One patient remained progression-free in PR at 25 months. The recurrence pattern has been predominantly in sites of previous bulk disease. The estimated median progression-free survival is 13.4 months. Overall median survival is projected to be 26.6 months. The estimated 2-year survival is 68%. Progression-free survival at 2 years is estimated to be 30%. For the patients achieving a CR, the estimated progression-free survival at 2 years is 58%.

**DISCUSSION**

The majority of recent studies using HDC for MBC are used in women responding to conventional chemotherapy. Standard-dose chemotherapy is used as induction therapy to reduce tumor bulk and allow for selection of chemotherapy-sensitive tumors for intensification with HDC. Using such an approach, multiple studies show that approximately 15–30% of patients are alive and disease free at 3–5 years (4–7). Despite these encouraging results, such a strategy may not be optimal, because, after induction of standard chemotherapy, the patient may be left with clones of cell that may be resistant to HDC (8). A more rational approach would be to apply HDC as initial therapy in previously untreated patients with metastatic disease. Several groups have treated a series of patients with a single HDC regimen at the time of relapse from primary disease. These studies show a CR rate of 30–50%, of which 10–20% of patients are still in complete remission at 2–3 years. In data from Peters et al. (9) and colleagues updated with follow-up now to 8 years, 3 of 22 (14%) poor-prognosis patients who were premenopausal, ER negative, and had residual disease have remained continuously disease free. In most of the published studies, the HDC treatment consists of a single cycle (4–7). Although this approach is associated with an increased response rate, rapid repopulation with potentially sensitive clones may require additional therapy. In this study, we have attempted to improve upon previous results by administering posttransplantation doxorubicin after a two-step HDC regimen in 21 patients with MBC. None of the patients treated with this approach had received prior chemotherapy for the treatment of metastatic disease. All patients completed the first two phases of the study. One patient did not receive any doxorubicin due to persistent thrombocytopenia. Another patient received only one cycle of doxorubicin, having progressed after that. Three patients elected not to pursue therapy with doxorubicin after receiving two cycles each.

The administration of high-dose cyclophosphamide followed by G-CSF was free of any significant morbidity and allowed collection of PBSCs at the beginning of hematological recovery. This is in accordance with results reported previously by several investigators (10, 11). Neutropenic fevers were a common complication of high-dose cyclophosphamide treatment. Forty-five % of our patients experienced neutropenic fevers after having received high-dose cyclophosphamide. However, only one patient demonstrated positive blood cultures. Recovery from myelosuppression and nonhematological toxicities was prompt to allow the administration of CTCb to begin within a median of 37 days of starting cyclophosphamide.

The second step of our study included the use of high-dose chemotherapy with peripheral stem cell rescue. As a conditioning regimen, we used CTCb, a regimen described previously by Antman et al. (4). The median number of days to a neutrophil count greater than 0.5 \times 10^9/liter was 8 days, and to a platelet count greater than 20 \times 10^9/liter was 9 days. These results compare favorably to the ones reported by Antman et al. (4). In her series, the median time to recovery to granulocyte to greater

### Table 4 Response rates

<table>
<thead>
<tr>
<th>Response to step I</th>
<th>CR</th>
<th>PR</th>
<th>STD</th>
<th>PD</th>
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<tbody>
<tr>
<td>18 Assessable</td>
<td>5 (30%)</td>
<td>10 (58%)</td>
<td>3 (12%)</td>
<td></td>
</tr>
<tr>
<td>Response to Step II</td>
<td>1 (5%)</td>
<td>12 (57%)</td>
<td>8 (35%)</td>
<td></td>
</tr>
<tr>
<td>21 Assessable</td>
<td>8 (38%)</td>
<td>9 (43%)</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
</tr>
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</table>
than $0.5 \times 10^{9}$/liter was 21 days, and to a platelet independence greater than $20 \times 10^{9}$/liter was 23 days. This may be explained by the fact that we used PBSCs as hematopoietic rescue as well as G-CSF posttransplantation. Our results are similar to the ones reported by Elias et al. (12) using the same regimen (CTCb) and PBSC rescue. Of note, we did not experience any cardiac toxicity during this phase as described previously also by Antman et al. (4) who, in a series of 29 patients, reported a 21% incidence of transient congestive heart failure.

Twenty patients in our series progressed to the third phase, which consisted of three cycles of doxorubicin. The first course of doxorubicin could be delivered on average by 38 days after CTCb. Although hematopoietic recovery was prompt after CTCb, GI toxicity precluded an earlier initiation of the first dose of doxorubicin. Of note, only 3 of the 55 cycles of doxorubicin were supported by G-CSF. Only three episodes of neutropenic fevers were observed during the 55 cycles of doxorubicin delivered. The toxicities observed throughout the studies were acceptable. Progressive decline in bone marrow reserve is seen in patients treated with multiple cycles of chemotherapy. Chemotherapy soon after transplantation has rarely been considered for concerns of toxicity to engraftment. Using growth factors and mobilized PBSC, this trial demonstrates the feasibility of safely administering a two-step HDC regimen followed by standard-dose doxorubicin. Our trial differs from the majority of previously reported trials in that no induction standard-dose chemotherapy was delivered up front, in an attempt to select patients to proceed with HDC. Our study population consisted of chemotherapy-naive patients with metastatic disease. All patients enrolled in this trial, irrespective of tumor sensitivity, received HDC followed by posttransplantation doxorubicin rather than the commonly used approach of limiting the use of HDC to patients responding to standard-dose therapy. The median progression-free survival for our patient population is 13.4 months, which is similar to the best published results for HDC in patients who are sensitive to induction chemotherapy.

In conclusion, standard-dose doxorubicin can safely be administered posttransplantation. However, longer follow-up is necessary to assess whether this approach compares favorably with the use of standard-dose chemotherapy alone.

ACKNOWLEDGMENTS

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REFERENCES


High-dose chemotherapy and autologous stem cell support followed by posttransplantation doxorubicin as initial therapy for metastatic breast cancer.

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