A Feasibility Study of Multiple Cycle Therapy with Melphalan, Thiotepa, and Paclitaxel followed by Mitoxantrone, Thiotepa, and Paclitaxel with Autologous Hematopoietic Cell Support for Metastatic Breast Cancer


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ABSTRACT

Dose-intensive chemotherapy appears to be important in the treatment of patients with recurrent solid tumors. Expanding upon our prior experience, we report the results of our most recent approach to administering dose-intensive therapy using four cycles of moderately high-dose chemotherapy with hematopoietic cell support for patients with metastatic breast cancer. This outpatient therapy includes high-dose melphalan, thiotepa, and paclitaxel for two cycles followed by mitoxantrone, thiotepa, and paclitaxel for two cycles, each cycle supported with autologous peripheral blood progenitor cells (PBPCs).

Between December 1994 and June 1996, 16 patients with recurrent or refractory breast cancer were enrolled in this prospective study. They had received a median of two previous chemotherapy regimens, with a median of nine prior cycles of chemotherapy for mobilization of autologous PBPCs, patients received cyclophosphamide, 4 g/m2, followed by granulocyte colony-stimulating factor (G-CSF). PBPCs were collected by apheresis. Each day’s collection was divided into four equal fractions, and each fraction was infused after each cycle of combination therapy. Cycles 1 and 2 consisted of melphalan, 80 mg/m2, thiotepa, 300 mg/m2, and paclitaxel, 200 mg/m2. Cycles 3 and 4 were comprised of mitoxantrone, 30 mg/m2, and thiotepa and paclitaxel at the same doses as in the first two cycles. The cyclophosphamide infusion was administered in the hospital, whereas all subsequent infusions of chemotherapy and PBPCs were performed on an outpatient basis. The first seven patients were randomized to receive alternate cycle G-CSF or placebo on day +1 of each cycle.

Including the initial pulse of cyclophosphamide, 67 (84%) of a planned 80 total courses of chemotherapy were delivered. Of the planned 64 cycles of high-dose combination chemotherapy, 52 cycles (81%) were delivered. Treatment was discontinued for progressive disease (one patient) or morbidity (five patients). Twelve of 16 patients completed at least three cycles of therapy. Nine patients completed all four cycles. One death resulted from fungal sepsis. In 20 cycles delivered to the first seven patients, day +1 G-CSF versus placebo was administered, with a median WBC recovery of 10 versus 13 days, respectively (P = 0.048 in cycle 1). The median duration of response was almost 9 months, and the median survival was 18 months after therapy. With a median follow-up of 1.5 years and longest follow-up of 4.2 years, two patients continue to be without evidence of disease. The 3-year event-free survival, freedom from progression, and overall survival are 19%, 20%, and 31%, respectively.

This four-cycle regimen of high-dose combination therapy supported with hematopoietic progenitor cells is feasible, but it is associated with a range of posttransplant complications. The efficacy of such a treatment would have to be substantially superior to that of other currently available therapies, including single autologous transplant procedures, to justify the prolonged period of treatment, multiple episodes of pancytopenia, and associated toxicities, including infectious risks. G-CSF administration after each PBPC infusion appears to accelerate time to neutrophil recovery but does not affect red cell or platelet engraftment.

INTRODUCTION

Much experience has been gleaned throughout decades of research into tumor growth kinetics and cytotoxic therapy. The observation of a specific log-kill curve for an individual chemotherapeutic drug led to the development of treatment schemes using combinations of agents, with each one potentially adding to the overall cell kill. Subsequently, the timing and sequence of chemotherapy, the method of drug delivery, dose escalation, and dose intensity have become important concepts in the treatment of cancer.

Substantial data exist to support the importance of dose intensity (the amount of drug delivered per unit of time) in the treatment of solid tumors (1–3). However, increasing dose intensity to levels that require cytokine support has not been unequivocally established as more efficacious than standard dose therapy in prolonging overall survival in the treatment of
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(Alkeran) was substituted for mitoxantrone (ATT) in the first cycle, eliminating from the repetitive cycle regimen, and melphalan (Alkeran), thiotepa, paclitaxel (Taxol). MTT, mitoxantrone, and cyclophosphamide. Each patient was assessed for toxicity and response after each cycle of high-dose therapy. A patient could be taken off the study if she did not demonstrate continued improvement of disease status with each cycle of therapy based upon investigator discretion and assessment of clinical status.

Collection of PBPCs. All patients underwent placement of a double-lumen tunnelled catheter (12 French Cook, Bloomington, IN) before the administration of high-dose cyclophosphamide. Patients received cyclophosphamide at 4 g/m² i.v. over 2 h. They were discharged the following day and treated with G-CSF (Amgen, Thousand Oaks, CA) at ~10 µg/kg beginning 24 h after the completion of cyclophosphamide infusion. G-CSF was continued daily until the target number of CD34+ cells or alternate target of mononuclear cells was collected by apheresis. Prophylactic oral rifampin and ciprofloxacin were begun at the time of discharge and continued until recovery from neutropenia. The collection of autologous PBPCs by apheresis was initiated after recovery from cyclophosphamide the day after the leukocyte count was >1,000/µl and was continued daily until the target goal of 10 × 10⁶ CD34+ cells/kg or an alternate goal of 1 × 10⁹ mononuclear cells/kg was collected. Cells expressing the surface marker CD34 were identified by indirect immunofluorescence with the monoclonal antibody HPCA-2 (Becton Dickinson, Mountain View, CA) conjugated to phycoerythrin using a FAC-STAR cell sorter (Becton Dickinson). Bone marrow was not collected from any patient. Platelet counts were maintained >10,000/µl with irradiated (2500 cGy) platelet concentrates. The PBPCs were processed and cryopreserved as described previously (20).

MATERIALS AND METHODS

Patient Eligibility. Sixteen patients with recurrent breast cancer were enrolled in this study between January 1995 and June 1996. Patient eligibility criteria included the following: age <65 years, Karnofsky performance status ≥80%, the absence of central nervous system metastasis, and demonstration of adequate organ function and HIV seronegativity. Chemotherapy-responsive disease or minimal tumor volume was not criteria for eligibility. The risks and benefits of the therapy were explained to patients at the initial clinic visit and again at enrollment when written consent was obtained. The clinical protocol was approved by the Administrative Panel on Human Subjects in Medical Research at Stanford University. The study schema is depicted in Fig. 1. Patient characteristics are listed in Table 1. Patients were assessed for toxicity and response after each cycle of high-dose therapy. A patient could be taken off the study if she did not demonstrate continued improvement of disease status with each cycle of therapy based upon investigator discretion and assessment of clinical status.

Fig. 1 Schematic of chemotherapy administration. Days between administration of cycles were scheduled every 21 days.
Chemotherapy Regimen and PBPC Infusion. This schema called for administration of four cycles of high-dose therapy every 21 days on an outpatient basis. After the adequate collection of PBPCs, melphalan at 80 mg/m² was infused i.v. over 20 min on day 2. Thiotepa, 200 mg/m², was then delivered by continuous infusion pump over 48 h from day 2 to day 2. After completion of thiotepa, patients received paclitaxel, 30 mg/m², and thiotepa were delivered by continuous infusion over 48 h from day 2 to day 2. Paclitaxel and PBPCs were administered as in the first two cycles.

Supportive Care. Patients were evaluated twice daily on an outpatient basis. Each morning, patients presented to the Day Hospital for physician evaluation and infusions of medications or blood products, if necessary, as determined by the previous evening’s laboratories. In the evening, patients received home care nursing assessment, blood sampling for laboratory analysis, and infusion of medications. Continuous infusion of i.v. fluid containing low-dose heparin (10 units/kg/day) was administered by Cadd pump (Sims Deltech, St. Paul, MN) at the start of each chemotherapy and continued until recovery from neutropenia. With the first cycle of therapy, all patients received prophylactic trimethoprim-sulfamethoxazole (160 mg/800 mg IV bid) for 3 days on day 2. Inhaled pentamidine (300 mg) was substituted if patients were allergic to sulfa antibiotics. Except for the initial patient, all patients received either prophylactic trimethoprim-sulfamethoxazole or pentamidine at the beginning of each cycle. At the time of neutropenia, patients received prophylactic i.v. antibiotics starting with vancomycin (1 g bid). Ceftriaxone (2 g daily) was added for low-grade febrile episodes. Patients were admitted to the hospital when fever was >38.5°C. Antibiotics were continued until the ANC was >500/μl. All patients received transfusions of irradiated packed RBCs or platelet concentrates to maintain a hematocrit >30% and platelet count >10,000/μl. The first seven patients enrolled were also randomized to start day +1 G-CSF in cycles 1 and 3 and placebo in cycles 2 and 4 versus placebo in cycles 1 and 3 and G-CSF in cycles 2 and 4. All subsequent patients received G-CSF after each cycle starting day +4. G-CSF was discontinued the day after the ANC was >1500/μl.

Engraftment Evaluation. The number of days to WBC and platelet engraftment was defined as the number of days after hematopoietic cell reinfusion (day 0) to ANC >500/μl and platelets >20,000/μl. The number of transfusion products (packed red cells or platelets) transfused was monitored after each cycle of chemotherapy.

Toxicity Monitoring. Toxicity monitoring was graded using the Southwest Oncology Group common toxicity criteria. Patients were removed from the trial for any grade III-IV nonhematological toxicity or for PD.

Assessment of Response. Breast cancer lesions were assessed by physical examination and imaging studies at the start of high-dose therapy. Patients were evaluated before each cycle of therapy with physical examination and chest X-ray. Computed tomographic reassessment, if applicable, was performed after two cycles of therapy, CR, VGPR, and PR were defined by standard criteria: disappearance of all measurable...
RESULTS

Patient Data. Patient characteristics and outcome data are listed in Table 1. The median age was 43 years (range, 33–58 years). All but one patient had premenopausal primary breast cancer. Prior primary treatment included the following: all but two patients had received primary adjuvant chemotherapy; seven patients had been initially treated with a doxorubicin-containing regimen; seven patients had previously undergone primary treatment with radiotherapy; and four patients had received hormonal therapy. In the development of recurrent disease, eight patients demonstrated visceral metastases, and eight patients manifested bony disease, with none of them associated with bone-only disease. Salvage chemotherapy was administered to 13 patients for a median of one regimen (range, 0–3). Four patients received radiotherapy, and five patients had hormonal manipulation for relapsed disease. Before enrollment, all patients had received a doxorubicin-containing regimen. Seven patients also had treatment with a paclitaxel-containing salvage regimen. The median number of prior chemotherapy regimens at the time of high-dose therapy was two (range, 1–4), with the number of prior cycles of therapy being nine (range, 4–15).

Mobilization Data. Fifteen of sixteen patients received cyclophosphamide and G-CSF for the mobilization of PBPCs. The median time to WBC recovery using G-CSF was 10 days (range, 9–13). All patients achieved the apheresis goal of 10 × 10^6 CD34+ cells/kg patient weight or the alternate goal of 100 × 10^3 mononuclear cells/kg. The median number of aphereses was 3 (range, 1–7). A median of 2 units of packed red cells (range, 0–3) and zero platelet products (range, 0–3) were transfused around the time of cell collection. The median number of days from high-dose cyclophosphamide to commencement of the multicycle therapy was 27 (range, 24–56).

One patient did not receive cyclophosphamide because her PBPCs had been collected with G-CSF, as specified in another protocol from which she became ineligible when her disease progressed through standard dose chemotherapy.

Hematological Recovery (with or without G-CSF). To infuse equivalent numbers of PBPCs at each cycle, each day’s apheresis collection was split into four equal fractions. Each day’s quarter fraction was subsequently thawed and reinforced on day 0 of each cycle. The median number of mononuclear cells infused per cycle was 2.8 × 10^9/kg (range, 0.64–11.47 × 10^9). Of 10 patients for whom numbers of CD34+ cells are available, the median number of CD34+ cells infused per cycle was 3.15 × 10^9/kg (2.2–5.0 × 10^9).

In our previous report on multiple cycle therapy supported with PBPCs, time to neutrophil and platelet recovery remained constant throughout the four courses of therapy when G-CSF was initiated on day +1 of each cycle (20). At the time this present study was proposed, the impact of G-CSF after autologous PBPC transplant was unclear. Therefore, a secondary aim was to determine whether G-CSF was necessary to accelerate time to neutrophil or platelet recovery following infusion of “primed” PBPCs collected after recovery from chemotherapy and G-CSF. Patients received either G-CSF or placebo in alternate cycles starting day +1. To avoid an effect from initial cycle or prior cycle G-CSF, patients were randomized to G-CSF in cycles 1 and 3 and placebo in cycles 2 and 4 versus G-CSF in cycles 2 and 4 and placebo in cycles 1 and 3. The first seven patients agreed to randomization. However, because the sixth and seventh patients both refused postinfusion blinded treatment after cycle 2, they received G-CSF in cycles 3 and 4. Because of patient preference and reports of the utility of posttransplant G-CSF (21), this aspect of the protocol was suspended, and the nine subsequent patients enrolled received G-CSF in each cycle.

The median number of days to WBC recovery (with and without G-CSF) and platelet recovery after each cycle of therapy are listed in Table 2. As listed in Table 3, 20 cycles of G-CSF or placebo were administered to seven patients on day +1. The median time to WBC recovery using G-CSF was 10 days compared to placebo at a median of 13 days. Because there were so few patients evaluable for this part of the study, only cycle 1 demonstrated a statistically significant WBC recovery time with the use of G-CSF after autograft infusion (P = 0.048). The median time to platelet recovery and the median number of transfusions of packed RBCs and platelets were not statistically significant between the groups who received G-CSF versus placebo. The median number of transfusions administered at each cycle are listed in Table 4.

Toxicities. The toxicities associated with this treatment (Table 5) were primarily related to infections rather than organ toxicity. There were no cases of hemorrhagic cystitis, veno-occlusive disease of the liver, nor documented cardiac dysfunction. There was one mortality secondary to fungemia.

Infections. Despite prophylaxis with i.v. sulfamethoxazole/trimethoprim, the first study patient developed PCP and diffuse alveolar hemorrhage on day +13 of cycle 3. She recovered fully; nonetheless, a fourth cycle of therapy was not administered. Patient SPN 1254 developed Candida tropicalis sepsis in cycle 2. She experienced multiorgan failure and ex-

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Table 2  Time to recovery of absolute neutrophil count >500/µl and platelet count >20,000/µl following each cycle of therapy

<table>
<thead>
<tr>
<th>Cycle #</th>
<th>Days to ANC &gt; 500 (range)</th>
<th>Days to PLTs &gt; 20 K (range)</th>
<th>Interval to next cycle (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>10 (9–15)</td>
<td>11 (8–23)</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>10 (8–13)</td>
<td>10 (6–19)</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>11 (9–17)</td>
<td>10 (9–17)</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>10 (8–17)</td>
<td>11 (8–16)</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* #, number of patients; PLT, platelet.
Table 3  The effect of G-CSF versus placebo on recovery of ANC > 500 by cycle on the seven patients randomized to posttransplant therapy

<table>
<thead>
<tr>
<th>Cycle</th>
<th>No. pts&lt;sup&gt;a&lt;/sup&gt;</th>
<th>+ G-CSF</th>
<th>Placebo</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>10</td>
<td>13</td>
<td>0.048</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>8.5</td>
<td>12</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>14</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>11.5</td>
<td>17</td>
<td>0.54</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

<sup>a</sup> No. pts, number of patients who received G-CSF or placebo by cycle.

<sup>b</sup> Analysis performed by rank sum test.

was 27% and at 3 years was 20%. Overall survival at 3 years was 31%.

DISCUSSION

Tumor response in the setting of recurrent disease may differ from that seen in the treatment of primary disease. Although the strategy of “high-dose sequential” or “dose-dense” serial monotherapy over short intervals appears promising in the treatment of patients with newly diagnosed high-risk lymphoma (22) and breast cancer (23, 24), its role in the treatment of recurrent disease is unclear. Given the greater degree of tumor heterogeneity, administration of a single drug, even at a maximal dose, may kill only a small population of susceptible cells while allowing the remaining population of resistant cells to proliferate. For patients with recurrent breast cancer, reports of single-agent high-dose cyclophosphamide (60 mg/kg × 2; Ref. 25) or melphalan (140–180 mg/m²; Ref. 26) used for cytoreduction before myeloablative chemotherapy and autografting have not demonstrated improvement in survival outcomes. Because dose intensity and total dose appear to be important factors in achieving CR and in the duration of response (27), multiple-cycle high-dose combination therapy administered in relatively short intervals and supported with hematopoietic cells may be the optimal treatment strategy for recurrent disease.

In our previous report on four-cycle therapy supported with PBPCs (20), we described the feasibility of repetitive cycles of high-dose mitoxantrone, 18 mg/m², thiopeta, 150–200 mg/m², and cyclophosphamide, 4500–5000 mg/m². We subsequently modified this regimen by adding paclitaxel at 150 mg/m² and escalating the thiopeta dose from 200 mg/m² to 300 mg/m² per cycle. However, 10 of 18 patients experienced one or more toxicities related to the cumulative dose of cyclophosphamide at 18 g/m². These included: hemorrhagic cystitis (four episodes), interstitial pneumonitis (five episodes), and cardiac dysfunction (four episodes). Our present report resulted from the last modification of multicyle treatment, which eliminated cyclophosphamide from the repetitive cycle regimen, increased the mitoxantrone dose, increased the paclitaxel dose, and substituted melphalan (Alkeran) into the first two cycles (ATT).

The drugs included in our protocol have been used alone or in various combinations in the setting of high-dose therapy for stage IV or metastatic breast cancer. Melphalan and thiopeta are alkylators with steep dose-response curves in breast cancer cell lines. In Antman’s initial review of intensive therapy with stem cell support for breast cancer (28), a survey of clinical trials for advanced or refractory breast cancer using single agents yielded
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In a single cycle of myeloablative therapy, thiotepa (900 mg/m²), busulfan at 12 mg/kg dose therapy, and paclitaxel as escalated to 825 mg/m² yielded promising results in patients treated to CR before high-dose therapy. Delays in therapy were usually due to slow WBC recovery (median, 13 days) but not in red cell or platelet transfusion resulting in a delay in WBC recovery (median, 13 days). Cycles of therapy of 24-day intervals. Otherwise, grade 1–2 diarrhea and enteritis were the most frequently noted side effects. However, two patients developed infections (PCP and cytomegalovirus pneumonitis) characteristic of T-cell immunodeficiency. Although serious pulmonary infections may be found in T-cell-depleted autograft recipients (35), children undergoing autografting (36, 37), and those treated with immunosuppressive pretransplant therapies (38), we have not observed this morbidity with single-cycle autotransplants for breast cancer nor with our prior multicyle regimens. Other centers have reported this rare complication in standard or high-dose chemotherapy, however (39, 40). Review of both patients' prior history and treatment did not yield information that would predict the development of these complications. Lymphocyte analysis was not performed at the time of these diagnoses; however, subsequent management included prolonged antimicrobial prophylaxis of PCP. Because our prior regimens had included cyclophosphamide, thiotepa, mitoxantrone, and later paclitaxel, perhaps the addition of melphalan to this high-dose combination regimen may have contributed to this type of immunocompromise.

The patients enrolled in this study generally had poor risk features of disease. In our multivariate analysis of prognostic variables associated with outcome after high-dose therapy for patients with metastatic breast cancer (41), factors associated with poor outcome were identified and were similar to those for women with breast cancer treated with standard therapies. These included hormone receptor-negative disease, the presence of visceral metastases, two or more prior regimens, refractory disease, and less than VGPR to high-dose therapy. Other groups have identified prior history of anthracycline exposure (42) as an additional adverse risk factor. In contrast, the achievement of CR to induction therapy and presence of metastasis to a single site (43) are predictors of better outcome. In our study population, all patients had received prior anthracycline therapy, with a median of two prior regimens. Eight patients demonstrated visceral metastases, and 13 patients had disease metastatic to two or more sites. Only three patients had achieved minimal disease status before the time of high-dose therapy, all of whom had metastasis to the chest wall that was resected. One of these patients remains without evidence of disease, one patient died from regimen-related toxicity, and the third progressed at 839 days after high-dose therapy. Two other patients have done well; one continues without evidence of disease progression, and the other developed PD at 1358 days. Both patients had limited cycles of prior chemotherapy (one regimen each.)

Another possible adverse determinant of outcome may be prior paclitaxel therapy. The development of drug resistance mediated through the multidrug resistance model has been well-documented for several drugs, including anthracyclines and taxanes, which are substrates for the P-glycoprotein receptor. In this study, seven patients had also received paclitaxel treatment before high-dose therapy. Of these patients, only one with chest wall recurrence remains free of disease. All six other patients have since relapsed, with five having developed PD between 147 and 270 days after high-dose therapy. The sixth patient developed PD at 489 days after therapy.

In conclusion, we have reported the results of a novel four-cycle regimen of high-dose melphalan, thiotepa, and paclitaxel for two cycles followed by mitoxantrone, thiotepa, and paclitaxel for two cycles, with each cycle supported with hema-
topoietic progenitor cells. This therapy is feasible, but it is associated with a range of toxicities, including infectious complications. G-CSF administration after each PBPC infusion accelerates time to neutrophil recovery but appears to have no effect on red cell or platelet engraftment. The outcomes of this approach appear comparable to those reported to the North American Bone Marrow Transplant Registry for single courses of high-dose therapy in the treatment of recurrent breast cancer (44); however, they may represent treatment efficacy given that this study population has multiple poor prognostic features. The survival results of autotransplantation are best for patients who achieve CR with standard-dose salvage therapy. Another strategy to improve outcome would be to apply novel chemotherapy regimens such as this at standard doses to achieve minimal disease before single-course high-dose therapy. Administration of dose-intensive multiple-cycle therapy with hematopoietic cell support is possible; however, the efficacy of such a treatment would have to be substantially superior to that of currently available therapies, including single autotransplants, to justify the prolonged period of treatment, multiple episodes of pancytopenia, and associated toxicities, including infectious risks.

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