Chemotherapy Immediately following Autologous Stem-Cell Transplantation in Patients with Advanced Breast Cancer


ABSTRACT

Most patients relapse after high-dose chemotherapy (HDCT) with autologous stem-cell transplantation (ASCT) for metastatic breast cancer. Further chemotherapy immediately after hematopoietic recovery from ASCT is not given for fear of irreversibly damaging the newly engrafted cells in a pilot chemoprotection trial, autologous CD34+ cells from patients with metastatic breast cancer were exposed to a replication-incompetent retroviral vector carrying MDR-1 cDNA and then reinused after HDCT. Immediately on recovery, patients received multiple courses of escalating dose paclitaxel. All of the 10 patients tolerated reininfusion of modified cells without any toxicity and had myeloid engraftment within 12 days (range, 11–14). The bone marrow cells of three patients contained vector MDR-1-positive cells only at the time of the first course of post-transplant paclitaxel, indicating that the MDR-1 vector-modified cells had only short-term engrafting potential. A total of 83 courses of paclitaxel were administered starting at a median of 30 (range, 21–32) days from ASCT. The median dose of paclitaxel was 225 mg/m² and the median interval between paclitaxel cycles of therapy was 21 (range, 20–41) days. Five of the six CR patients were able to receive all of the 12 courses of paclitaxel. Three patients who had achieved less than a complete response to the HDCT (2 patients) and partial response (1 patient) were converted to complete clinical responses during the 12 cycles of paclitaxel. Three patients who had achieved less than a complete response to the HDCT (2 patients) and partial response (1 patient) were converted to complete clinical responses during the 12 cycles of paclitaxel. No delayed toxicity or bone marrow failure was noted in these patients with a median follow-up of 2 years from ASCT. This is the first study of chemotherapy immediately after transplantation with autologous CD34+ cells. These data indicate that paclitaxel can be safely administered immediately after ASCT without any delayed toxicities. Paclitaxel given immediately after ASCT can further improve the response to pretransplant chemotherapy in patients with advanced breast cancer.

INTRODUCTION

Breast cancer is moderately chemosensitive and several preclinical studies (1, 2), retrospective reviews (3, 4), and randomized studies (5, 6) have shown a relationship between dose and response. HDCT with ASCT has consistently resulted in CR rates of 50–60%, and responses have been reported even in patients with tumors that are resistant to standard-dose chemotherapy. However, most of the patients eventually relapse after HDCT and ASCT, and only 15–20% of patients with overtly metastatic breast cancer are reported to survive 5 years after HDCT. (7, 8)

Although the alkylating agent containing HDCT may damage the DNA of all of the cancer cells, some of the cells may escape genotoxic stress-induced apoptosis by DNA repair mechanisms. Patients who have undergone HDCT with ASCT are usually not given additional posttransplant chemotherapy immediately posthematopoietic recovery after ASCT because of concern that the newly engrafted hematopoietic stem cells would be resistant to a single agent. The development of multidrug resistance (MDR) is an important mechanism that contributes to the clinical failure of chemotherapy. The ability of tumor cells to express MDR proteins, such as Pglycoprotein (P-gp) encoded by MDR-1 gene, results in the development of drug resistance (1, 7, 8). The development of chemoresistance in breast cancer could result in partial or no responses to conventional chemotherapy, limited duration of response, and a high rate of relapse after ASCT (9, 10).

The abbreviations used are: HDCT, high-dose chemotherapy; ASCT, autologous stem-cell transplantation; CR, complete response; MDR, multiple drug resistant (or multidrug resistance).
very sensitive to myelosuppression and might be irreversibly damaged. Although the MDR-1 gene is expressed in early hematopoietic precursor cells at high enough levels to confer resistance to conventional dose chemotherapy, the expression levels of the MDR-1 gene in all but very immature normal bone marrow cells are so low that most hematopoietic cells are particularly sensitive to the cytotoxic effects of many chemotherapy drugs, such as anthracyclines, taxanes, and Vinca alkaloids, that require the MDR-1 gene product, P-glycoprotein, for their removal from the cell.

Recently, several studies (9–12) involving animal models have demonstrated that retroviruses carrying the MDR-1 gene can be used to modify cells used to engraft lethally irradiated mice. These transplants have been associated with increased levels of resistance to chemotherapy in the engrafted cells compared with transplants with hematopoietic cells that have not been genetically modified (9–12). In addition, the level of chemotherapy resistance in genetically modified cells increases with each succeeding exposure to chemotherapy after transplantation (11, 12). In a pilot clinical trial, we tested the hypothesis that autologous hematopoietic stem cells exposed to retroviral vectors carrying MDR-1 cDNA would be less sensitive to paclitaxel. By administering paclitaxel immediately posttransplant, we also tested whether tumor cells recently damaged by intensive pretransplant HDCT that contained alkylating agents would be more sensitive to paclitaxel in the immediate posttransplant period. The MDR-1 vector-modified hematopoietic cells persisted in the patients only a short time posttransplant, possibly because of the modification of short-term but not long-term engrafting cells. Thus, no conclusion can be reached about the utility of MDR-1 modification and chemotherapy resistance of the normal hematopoietic cells posttransplant from this trial. However, we did observe that three patients who achieved only minor (2) or partial (1) responses to the pretransplant HDCT were converted to complete clinical responses by the posttransplant conventional dose paclitaxel. These results suggest that conventional dose chemotherapy administered immediately posttransplant may increase the percentage of patients achieving a CR post-ASCT.

PATIENTS AND METHODS

This trial involved 10 patients with metastatic breast cancer, who were partially responsive to standard-dose chemotherapy. All of the patients signed an informed consent form approved by the institutional review board of The University of Texas M. D. Anderson Cancer Center, the Yale Human Investigations Committee, the NIH Recombinant DNA Advisory Committee, the Chemotherapy Evaluation Program of the National Cancer Institute, NIH, and the Food and Drug Administration.

Two engrafting doses (4 × 10^8 nucleated cells/kg; or 20,000 colony-forming units, granulocyte-macrophage/kg; or 2 × 10^6/kg of CD34+ positive cells) of peripheral blood cells were collected during granulocyte colony-stimulating factor stimulation after mobilization with chemotherapy (4 gms/m^2 of cyclophosphamide IVPB × one). One-half of the progenitor cells were frozen unmodified and CD34+ cells were selected from the remaining half using a Ceprate SC Selector (CellPro, Inc., Bothell, WA) and incubated with replication-incompetent retrovirus containing MDR-1 cDNA as described previously (13). Two methods were used to measure the transduction frequency of the vector MDR-1-modified cells at the DNA level: (a) in situ DNA PCR assay, which can only detect MDR-1 vector-modified cells when they are present at the 1% level or greater; and (b) solution DNA PCR assay, which is sufficiently sensitive to detect one MDR-1 vector-modified cell in an excess of between 5,000 and 20,000 unmodified cells, as described previously (9, 10, 13). The modified cells were also frozen using a programmed freezer.

The patients then received HDCT consisting of thiotepa 200 mg/m^2 IVPB daily × 3; 1,3-bis(2-chloroethyl)-1-nitrosourea 150 mg/m^2 IVPB daily × 3; and cyclophosphamide 1.5 g/m^2 IVPB daily × 3. Six days after the last dose of chemotherapy, a mixture of the unmodified and vector-exposed peripheral blood stem cells (designed to add up to 2 engrafting doses) were reinfused. After transplantation, and depending on patients’ willingness before every or every other course of paclitaxel, bone-marrow aspirations were performed. These bone marrow cells were then analyzed for vector MDR-1 cDNA by the solution DNA-PCR assay as previously described in detail (9, 13–15).

When the patients had fully recovered from the effects of HDCT and platelets had increased to 100,000/μl and WBC count to 4,000/μl, paclitaxel was given as a single 24-h infusion. The starting dose of paclitaxel was 60 mg/m^2 and the next course was given in 3 or more weeks (as soon as patients recovered from the previous course). Subsequent doses were increased to 120, 180, 225, and 275 mg/m^2 if the patient experienced no grade III toxicity; otherwise, the dose was kept the same. If the patient experienced grade IV toxicity, the dose was decreased to the previous level. Patients with recurring grade IV toxicity after dose reduction or progressive disease were taken off the study. The protocol called for 12 courses of posttransplant paclitaxel.

RESULTS

The ages of the patients and their sites of metastatic disease are shown in Table 1. All of the patients had two engrafting doses of peripheral blood progenitor cells collected without any toxicity. After transduction of CD34+ cells, the marrow cells of all of the patients were positive for the vector MDR-1 transgene by the solution DNA PCR assay. The in situ PCR hybridization assay indicated that an average of 5.7% (range, 2.5–9.0%) were positive pretransplant for the MDR-1 vector-modified cells. The average percentage increment, pretransplant, of colony-forming units, granulocyte-macrophage, which were more resistant to paclitaxel after the transduction was 4.9% (range, −0.22–18.1%) by a methylcellulose plating assay. These pretransplant transduction data have been reported previously (13, 16).

No unusual or excessive toxicity was noted during the HDCT or reinfusion of vector-modified and unmodified progenitor cells. The median time to recovery of absolute neutrophil count to 500/μl was 12 days (range, 11–14) and platelet count to 20,000/μl without transfusion was 14 days (range, 10–19+). One patient (patient 10) developed respiratory syncytial virus pneumonia, diagnosed 9 days after ASCT, which was complicated by pulmonary hemorrhages. At the time of death on day
Evidence of vector MDR-1

then received 83 courses of posttransplant paclitaxel. Only five indicating that only short-term reconstituting cells had been detectable at the time of the first cycle of posttransplant paclitaxel. Despite repeated testing, there was no subsequent evidence of exogenous MDR-1 by PCR assay (Table 2). These cells were transfusion dependent.

The neutrophil count was 9,000/µl, but the patient was still platelet-dependent.

Three of the eight evaluable patients (patients 4–6) had evidence of vector MDR-1 cDNA sequences in bone marrow before and during posttransplant paclitaxel therapy.

Results of DNA PCR analysis of bone marrow cells during the first four courses of posttransplant paclitaxel are shown here. All of the remaining DNA PCR analyses performed up to the twelfth course of paclitaxel were negative. This PCR assay was sufficiently sensitive to detect one MDR-1 vector-modified cell in an excess of between 5,000 and 20,000 unmodified cells. The blank spaces represent the courses of paclitaxel when bone marrow samples were not analyzed.

Table 1 Sites of metastatic disease, maximum responses achieved, survival durations, and final status of all of the 10 patients undergoing HDCT with ASCT immediately followed by paclitaxel for advanced breast cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sites of metastasis</th>
<th>Pre-ASCT HDCT</th>
<th>Post-ASCT paclitaxel</th>
<th>No. of courses</th>
<th>Dose intensity (mg/m²/wk)</th>
<th>Prog free surv (wk)</th>
<th>Overall surv (wk)</th>
<th>Final status from ASCT</th>
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<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>SC nodes</td>
<td>CR</td>
<td>CR</td>
<td>12</td>
<td>72</td>
<td>NR</td>
<td>NR</td>
<td>Alive in CR (136)</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>Lungs</td>
<td>MR</td>
<td>CR</td>
<td>12</td>
<td>63</td>
<td>NR</td>
<td>NR</td>
<td>Alive in CR (131)</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>Lungs</td>
<td>PR</td>
<td>PD after 7 courses</td>
<td>7</td>
<td>50</td>
<td>33</td>
<td>90</td>
<td>Died</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>SC LN, bones, skin</td>
<td>PR</td>
<td>CR</td>
<td>12</td>
<td>68</td>
<td>100</td>
<td>NR</td>
<td>Alive with PD (100)</td>
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<tr>
<td>5</td>
<td>35</td>
<td>Lungs</td>
<td>MR</td>
<td>CR</td>
<td>12</td>
<td>75</td>
<td>NR</td>
<td>NR</td>
<td>Alive in CR (111)</td>
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<tr>
<td>6</td>
<td>44</td>
<td>Chest wall, SC and Ax LN</td>
<td>SD</td>
<td>PD after 7 courses</td>
<td>7</td>
<td>49</td>
<td>26</td>
<td>82</td>
<td>Died</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>Chest wall, lungs</td>
<td>IE</td>
<td>CR then OP due to neuropathy</td>
<td>8</td>
<td>42</td>
<td>37</td>
<td>NR</td>
<td>Alive with PD (106)</td>
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<tr>
<td>8</td>
<td>32</td>
<td>SC LN</td>
<td>CR</td>
<td>CR</td>
<td>12</td>
<td>64</td>
<td>79</td>
<td>NR</td>
<td>Alive with PD (104)</td>
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<tr>
<td>9</td>
<td>52</td>
<td>SC LN, lungs</td>
<td>IE</td>
<td>CNS metastasis after first cycle (PD)</td>
<td>1</td>
<td>NA</td>
<td>12</td>
<td>63</td>
<td>Died</td>
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<tr>
<td>10</td>
<td>37</td>
<td>Chest wall</td>
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<td>OP</td>
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Max* response after Post-ASCT paclitaxel

A, axillary; CNS, central nervous system; IE, inevaluable; LN, lymph nodes; MR, minor response; NA, not applicable; NR, not reached; PD, progressive disease; PR, partial response; Prog, progression; Surv, survival; SC, supraclavicular.

Table 2 Evaluation of vector MDR-1-modified cells after transplantation by DNA PCR in bone marrow of patients before and during posttransplant paclitaxel therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Posttransplantation paclitaxel course</th>
<th>Pre-ASCT</th>
<th>Post-ASCT</th>
<th>No. of courses</th>
<th>Dose intensity (mg/m²/wk)</th>
<th>Prog free surv (wk)</th>
<th>Overall surv (wk)</th>
<th>Final status from ASCT</th>
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</table>

All of the five patients who received the total program of 12 courses of paclitaxel were in CR after completion of the therapy. Three of these five patients (patients 2, 4, and 5) had residual disease after pretransplant HDCT before the start of paclitaxel and were converted to CR during posttransplant paclitaxel (Table 1). Three patients (patients 1, 2, and 5) are still alive in CR after 111, 131, and 136 weeks from ASCT. Two of the patients (2 and 5) who are still alive in CR had residual disease after pretransplant HDCT and were converted to CR during posttransplant paclitaxel. Both of these patients had multiple pulmonary metastases proven by biopsies, which persisted post transplant after the HDCT. The serial computerized tomographic images of representative pulmonary metastases in these two patients, before the start of protocol, after HDCT but before paclitaxel, and after 12 courses of paclitaxel, are shown in Fig. 1. The remaining two patients who received all of the 12 courses of paclitaxel had a median dose of 275 mg/m².

All of the five patients who received the total program of 12 courses of paclitaxel were in CR after completion of the therapy. Three of these five patients (patients 2, 4, and 5) had residual disease after pretransplant HDCT before the start of paclitaxel and were converted to CR during posttransplant paclitaxel (Table 1). Three patients (patients 1, 2, and 5) are still alive in CR after 111, 131, and 136 weeks from ASCT. Two of the patients (2 and 5) who are still alive in CR had residual disease after pretransplant HDCT and were converted to CR during posttransplant paclitaxel. Both of these patients had multiple pulmonary metastases proven by biopsies, which persisted post transplant after the HDCT. The serial computerized tomographic images of representative pulmonary metastases in these two patients, before the start of protocol, after HDCT but before paclitaxel, and after 12 courses of paclitaxel, are shown in Fig. 1. The remaining two patients who received all of the 12 courses of posttransplant paclitaxel are still alive but developed progressive disease 100 and 104 weeks after ASCT.

Three patients (3, 6, and 9) were taken off the protocol because of progressive disease (Table 1). One patient (patient 9) received a diagnosis of central nervous system metastasis just as she was finishing the first cycle of paclitaxel, and each of the remaining two patients developed progressive disease after seven courses of paclitaxel (Table 1). Only one patient (patient 7) stopped receiving paclitaxel after eight courses because of side effects of posttransplant paclitaxel therapy. This patient had a combination of severe numbness and painful paresthesia. We have not noticed any delayed toxicity of posttransplant pacli-
2720 Posttransplant Paclitaxel

Fig. 1 Serial computerized tomographic images of representative pulmonary metastases from patients 2 and 5. Both patients had biopsy-proven pulmonary metastases from breast cancer. After pretransplant HDCT, both patients were in partial response, but after 12 courses of posttransplant paclitaxel, both achieved CR. Both patients are alive without evidence of disease 2 years from the HDCT with ASCT. Images A-C are from patient 2 and D-F are from patient 5. A and D show pulmonary metastases before pretransplant HDCT; B and E show pulmonary metastases after HDCT but before posttransplant paclitaxel; and C and F show pulmonary metastases after 12 courses of posttransplant paclitaxel.

taxel after a median follow-up of 2 years from transplantation. All of the patients are maintaining normal blood counts, and there was no delayed engraftment failure, failure to engraft, or myelodysplasia associated with posttransplant paclitaxel or the genetic modification procedure.

DISCUSSION
This is the first study of multiple courses of cytotoxic chemotherapy given immediately after pretransplant intensive chemotherapy and autologous transplantation. The cells used for the transplant were exposed to a MDR-1 retroviral vector. The patients participating in the trial had metastatic breast cancer that was only partially responsive to conventional dose chemotherapy.

The in situ and solution DNA PCR assays were able to detect vector MDR-1 cDNA in the autologous CD34+ cells of all of the patients after transduction but before transplantation. However, only three of eight evaluable patients had evidence of vector MDR-1 cDNA posttransplant in their bone marrow cells. These modified cells were detectable at the time of the first course of posttransplant paclitaxel in three patients, and most of the courses of paclitaxel were delivered in the absence of such detectable cells. These data suggested that the transduction procedures used succeeded in modifying only cells of short-term engrafting potential. Factors in this trial that possibly contributed to the failure to modify long-term engrafting cells include: (a) a low ratio (0.5) of infectious vector particles/nucleated cell; (b) a low number of receptors of the long-term engrafting potential cells for the envelope proteins of the retroviral vector necessary for infection; and (c) the use of serum-containing medium supplemented with interleukin 3 for 4 days in vitro during the transduction procedure. (Interleukin 3 and serum-containing medium have been shown to be toxic for the long-term engrafting cells.) Efforts are under way in many laboratories to improve the gene delivery vectors in serum-free medium for the purpose of increasing the frequency of modification of the long-term engrafting cells with MDR-1 and other chemotherapy resistance genes.

All of the patients except one tolerated this posttransplant cytotoxic therapy well without any excessive toxicity. We have
not noticed any delayed toxicity after a median follow-up of 2 years from transplantation. All of the patients are maintaining normal blood counts, and there was no delayed engraftment or loss of engraftment caused by 12 cycles of relatively high-dose posttransplant paclitaxel given after engraftment. We have also not noticed any myelodysplasia at the time of this analysis (median follow-up, 2 years from ASCT).

This pilot trial was conducted in a small number of patients with advanced breast cancer, and its results need to be viewed with caution and need to be verified in a larger trial. However, the results show that it is safe to administer cytotoxic chemotherapy immediately following ASCT in selected individuals. In addition, the administration of posttransplant paclitaxel therapy converted three patients who had received pretransplant intensive chemotherapy, from a minor response (2) and a partial response (1) to a CR. Two of these patients who were converted to a CR by posttransplant paclitaxel are still alive in CR more than 2 years from ASCT.

When a decision has been made to administer cytotoxic chemotherapy to patients with metastatic breast cancer, the present practice is to administer therapy with the highest activity first. It has also been shown in a randomized trial that disease-free and overall survival of patients with breast cancer is significantly better when more active therapy is given first as compared with alternating therapies (17). Presently, patients with breast cancer are given multiple courses (usually 4–6 courses) of standard-dose chemotherapy and then HDCT. With this approach, a large number of patients still have experienced relapse of the tumor caused by resistant disease (18). Despite higher response rates reported with HDCT, a modification of the sequence of therapy to include posttransplant as well as pretransplant chemotherapy has not been attempted because of concerns about irreversibly damaging the newly engrafted hematopoietic stem cells. This trial has dispelled some of these fears and opened the door for trials of alternative sequencing of therapy for patients with metastatic breast cancer.

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