Mobilization, Collection, and Characterization of Peripheral Blood Hemopoietic Progenitors after Chemotherapy with Epirubicin, Paclitaxel, and Granulocyte-Colony Stimulating Factor Administered to Patients with Metastatic Breast Carcinoma

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BACKGROUND. As single agents, both paclitaxel and epirubicin in combination with cytokines can mobilize peripheral blood progenitor cells (PBPCs). The authors have demonstrated previously that the combination of epirubicin and paclitaxel is very active against metastatic breast carcinoma and tolerated by patients.

METHODS. Twenty-one patients with metastatic breast carcinoma received epirubicin 90 mg/m^2 in combination with paclitaxel 200 mg/m^2 given as a 3-hour infusion, and granulocyte-colony stimulating factor (G-CSF) starting 24 hours after chemotherapy to mobilize PBPCs. An immunophenotypic analysis for CD3, CD4, CD8, CD19, CD33, CD34, and CD38 antigen expression was performed on apheresis products. Eighteen patients underwent high dose chemotherapy and were engrafted with PBPCs primed with paclitaxel, epirubicin, and G-CSF.

RESULTS. The median number of circulating CD34+ cells at peak was 70/μL; in the patients less heavily pretreated, it was 106.7/μL. The mean number of CD34+, CD34+/CD33−, and CD34+/CD38− cells/kg collected per apheresis was 6.3×10^6, 2.0×10^6, and 0.18×10^6, respectively. The mean number of CD34+ cells/kg per apheresis was 7.8×10^6 when the preleukapheresis CD34+ cell count was more than 50/μL and 0.9×10^6 when the CD34+ cell count was less than 50/μL. The mean number of CD3+, CD4+, and CD8+ cells/kg collected per apheresis was 90×10^6, 50×10^6, and 30×10^6, respectively.

CONCLUSIONS. Epirubicin plus paclitaxel in combination with G-CSF mobilizes PBPCs, including more primitive progenitors capable of supporting myeloablative treatment. Moreover, the mononuclear cells collected in this study contained high levels of cytotoxic effector cells suitable for ex vivo manipulation to augment the antitumor effect. ***Cancer* 1998;82:867–73.** © *1998 American Cancer Society.*

KEYWORDS: epirubicin, paclitaxel, peripheral blood progenitors, high dose chemotherapy.

High dose chemotherapy with autologous hemopoietic support induces high response rates in patients with metastatic breast carcinoma; however, long term disease free survival is rare.[1–2] Early consolidation/intensification treatment, after a good response to standard dose therapy, allows an increase in the complete response rate; moreover, 15–30% of the patients are progression free after 5 years.[3] Therefore, a good response to conventional chemotherapy is considered one of the most important predictors of prolonged progression free survival after high dose consolidation chemotherapy.[4]

Peripheral blood progenitor cell (PBPC) autografts provide accelerated hematopoietic recovery after myeloablative and myelosuppressive chemotherapy, decreasing the morbidity and the cost of the procedure.[5] Several cytotoxic agents plus cytokines have been utilized to mobilize PBPCs to be harvested by apheresis procedure. Paclitaxel and antracycline compounds (doxorubicin and epirubicin) are the most active agents against metastatic breast carcinoma.[6] Moreover, both paclitaxel and epirubicin as single agents plus cytokines mobilize PBPCs,[7,8] and it has been demonstrated that paclitaxel in combination with cyclophosphamide can improve the mobilization of PBPCs versus cyclophosphamide alone.[9,10]

The combination of paclitaxel and doxorubicin, although very active against metastatic breast carcinoma, has been demonstrated to be quite cardiotoxic. We have recently shown that the combination of paclitaxel and epirubicin as first-line treatment of metastatic breast carcinoma yelds a response rate of 84% without relevant cardiotoxicity.[11] Of concern is the possibility of using this tolerable and very active combination as a PBPC mobilizing regimen.

We therefore designed a study to evaluate the combination of epirubicin and paclitaxel with granulocyte-colony stimulating factor (G-CSF) as a PBPC priming regimen administered to metastatic breast carcinoma patients and optimize the collection to support a course of high dose chemotherapy. Moreover, we performed a characterization of PBPCs for CD3, CD4, CD8, CD19, CD33, CD34, and CD38 antigen expression on apheresis products to evaluate the presence of more primitive hemopoietic progenitors and T-lymphocyte populations. The CD34+ cell population is very heterogeneous, and the lack of CD33 or CD38 antigen expression can identify more immature hemopoietic progenitor cells (CD34+/CD33− and CD34+/CD38−), putative stem cells responsible for long term bone marrow reconstitution after myeloablative treatment. The number of CD34+/CD33− cells may help predict the short term repopulation capacity of PBPCs.[12] Moreover, the PBPCs are an ideal cell source of T cells suitable for ex vivo manipulation to augment the antitumor properties of these effector cells.[13]

PATIENTS AND METHODS

Patients

Twenty-one women with a median age of 48 years (range, 28–62 years) entered the study. The main eligibility criteria were Stage IV breast carcinoma, performance status 0–1 (Eastern Cooperative Oncology Group scale), no prior treatment for metastatic disease, bone marrow morphologically not involved, chemoresponsive disease, and written informed consent. The protocol was approved by the Institutional Ethical Committee. The relevant characteristics of these patients are summarized in Table 1.

TABLE 1
Patient Characteristics

Characteristic	No. of patients
No. of patients/no. of courses	21/26
Median age, yrs (range)	49 (28–62)
Median PS (range)	0 (0–1)
Site of metastatic disease (no. of patients)	
Liver	8
Lung	5
Bone	11
Lymph nodes	7
Bone marrow	0
Soft tissue	10
Previous adjuvant chemotherapy (no. of patients)	
Yes	10
No	11
Previous radiation therapy (no. of patients)	
Yes	10
No	11
No. of courses before mobilization (no. of patients)	
<6 courses	14
≥6 courses	7

PS: Eastern Cooperative Oncology Group performance status.

Treatment Plan

Induction/mobilization phase

All patients were given epirubicin 90 mg/m^2 as a bolus injection immediately followed by paclitaxel 200 mg/m^2 administered as a 3-hour infusion on the same day every 3 weeks as treatment for metastatic breast carcinoma. The patients were premedicated with prednisone, orphenadrine, and cimetidine. An evaluation of response was performed after 2, 4, 6, and 8 courses of cytoreductive chemotherapy. After a minimum of 2 and a maximum of 8 courses, depending on the time of the evidence of response, the patients received a course of the same regimen followed by G-CSF (filgrastim) 5 μg/kg/day administered subcutaneously starting 24 hours after chemotherapy (day +1) to mobilize PBPCs. The growth factor was discontinued after the last leukapheresis or when the white blood cell count (WBC) was $>50 \times 10^9$/L.

Consolidation phase

The high dose regimen was administered immediately to patients in complete remission (CR) or after two additional cycles to patients in partial remission (PR).

The patients received a median of 6 cycles (range, 4–9) before the consolidation phase. The high dose chemotherapy regimen included thiothepa 600 mg/m^2 (Day −3) and melphalan 120 mg/m^2 (Day −1) 1 day apart. Thiothepa was given in three equal doses as a 1-hour infusion, each 1 hour apart. Melphalan was administered in three equal doses as an intravenous bolus, each two hours apart. Autologous PBPCs were reinfused 24 hours after the last dose of melphalan. At the time of reinfusion, frozen PBPCs were thawed rapidly in a 37 °C warm bath and reinfused through a venous central catheter (Certofix, Braun). After PBPC reinfusion, rhG-CSF 5 μg/kg/day was administered until the WBC was >1000/mL for 3 consecutive days.

Hemopoietic Progenitor Analysis

The analysis of hemopoietic progenitors was performed on peripheral blood and on leukapheresis products.

From Day +6 to Day +16, a sample of peripheral blood was collected daily from each patient. A sample of each apheresis product was also collected. Total and differential WBC and an analysis for CD34 and CD33 antigen expression was performed on each peripheral blood sample. Cell surface immunophenotyping of PBPCs was performed on the product of leukapheresis for the expression of the following antigens: CD3, CD4, CD8, CD19, CD34, CD33, and CD38. Cell immunophenotypic characterization was performed by flow cytometry using direct immunofluorescence analysis. A small aliquot (100 μL) of sample was incubated with a mixture of CD34 fluoresceine isothiocyanate (FITC)-conjugated anti-HPCA-2 antibody (Becton-Dickinson, San José, CA) and CD33 phicoerytrin-conjugated anti-WM-54-RPE antibodies (Dako Glastrup, Denmark). The CD38 antigen expression was evaluated using a phicoerytrin-conjugated antibody (clone HB-7, Becton-Dickinson, San José, CA). The expression of CD3, CD4, CD8, and CD19 antigens was investigated with the commercially available products Simultest CD4 CD8 and Simultest CD3 CD19 (Becton-Dickinson, San José, CA). The CD34+/CD33−, CD34+/CD33+, CD34+/CD38+ and CD34+/CD38− populations were determined as a percentage of all analyzed white blood cells (10,000 events). The absolute counts of circulating CD34+/CD33−, CD34+/CD33+, CD34+/CD38+, and CD34+/CD38− populations were determined by multiplying the percentage of positive cells by the total white blood cell number.

Progenitor Cell Collection

A double-lumen vascular access catheter (Certofix Duo, Braun) was placed before mobilizing chemotherapy. Provided a WBC of >1,500/μL, a platelet count of >50,000/μL, and circulating CD34+ cells >10/μL, daily leukapheresis was performed using a Fenwall CS 3000 cell separator (Baxter, Chicago, IL). Eight to 10 liters of whole blood were processed per procedure, using continuous flow centrifugation at a flow rate of 50 mL/minute. The procedure usually lasted between 150 and 210 minutes and was not associated with significant complications. A target level of 2.0×10^6 CD34+ cells/kg was considered adequate to support a single course of high dose chemotherapy.

Statistical Analysis

The statistical significance of the data obtained were analyzed by the Student's unpaired t test using a commercially available software program (Statworks, Cricket Software, Philadelphia, PA). A P value of <0.05 was considered significant.

RESULTS

Activity of the Induction Phase

Seven patients achieved a CR after a median of 5 cycles (range, 4–8); 14 patients achieved a PR after a median of 6 cycles (range, 2–9). All the induction phase was given on an outpatient basis. When G-CSF was administered to mobilize PBPCs, 11 patients (52.3%) experienced Grade 4 neutropenia; however, no episode of febrile neutropenia was reported.

Peripheral Blood Progenitor Mobilization

The mobilization of CD34+ cells was assessed during 26 courses: in 5 patients during the third course, in 3 patients during the fourth course, in 2 patients during the fifth course, in 6 patients during the sixth course, in 3 patients during the seventh course, in 2 patients during the eighth course, and in 2 patients during the ninth course. Five patients were assessed in two consecutive courses.

Taking in account the entire group of patients, the peak of circulating CD34+ cells occurred as a median on Day +11 (range, 9–16), and the median number of CD34+ cells/μL at peak was 69.5 (range, 1.4–251). The peak of circulating CD34+/CD33− cells occurred as a median on Day +11 (range, 8–16), and their median number was 14.3/μL (range, 1.74–195). In the group of patients who had received fewer than 6 courses of induction chemotherapy before mobilization, the peak of CD34+ cells in peripheral blood occurred as a median on Day +11 (range, 9–13), and the median number of CD34+ cells at peak was 106.7/μL (range, 2.26–251). Patients more heavely pretreated before mobilization (6 or more courses) showed a lower number of CD34+ cells at peak: median 7.3/μL (range, 1.41–64.7), and the median day of peak was Day +14 (range, 11–16). The difference of circulating CD34+

TABLE 2
Peak of Circulating Hemopoietic Progenitors and Day of Peak after Mobilization in Patients Pretreated with <6 or ≥6 Courses of Chemotherapy

	All patients	<6 courses	≥6 courses	*P* value
Median no. of CD34+ cells/μL (range)	69.5 (1.4–251)	107 (2.26–251)	7.3 (1.41–64.7)	0.02
Day of peak (range)	+11 (9–16)	+11 (9–13)	+14 (11–16)	
Median no. of CD34+/CD33− cells/μL (range)	14.3 (1.74–195)	24.4 (1.74–195)	3.92 (1.78–15.1)	0.18
Day of peak (range)	+11 (8–16)	+11 (10–14)	+13 (8–16)	

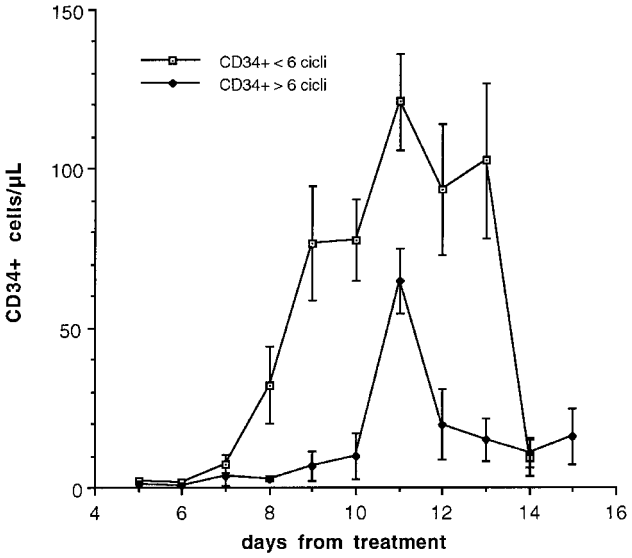

FIGURE 1. Kinetic of circulating CD34+ cell mobilization is shown for the patients who had received more than 6 courses of chemotherapy before mobilization versus patients who had received less than 6 courses before mobilization. Error bars show the range of CD34+ cell counts.

cell numbers at peak between the two groups was statistically significant ($P = 0.02$) (Table 2). The kinetic of CD34+ cell mobilization in the peripheral blood in the two groups of patients is shown in Figure 1. The median number at peak of more primitive CD34+/CD33− cells was 24.4/μL (range, 1.74–195.4) versus 3.92/μL (range, 1.78–15.1), respectively, in patients who had received <6 or ≥6 courses of chemotherapy before mobilization. The difference was not statistically significant ($P = 0.12$).

Collection of Peripheral Hemopoietic Progenitors and Characterization of Lymphocyte Subpopulations

Eighteen of 21 patients underwent leukapheresis procedure to harvest circulating progenitors after priming with epirubicin, paclitaxel, and G-CSF to support a course of high dose chemotherapy as consolidation treatment. Three patients refused the treatment procedure.

TABLE 3
Collection of Circulating Hemopoietic Progenitors

	Mean % (range)	Mean total no. of cells/lk (range)	Mean no. of cells/kg/lk (range)
MNC	100	15.5×10^9 (0.7–94.5)	26.8×10^7 (1.5–135)
CD34+	3.0 (0.8–5.1)	37.7×10^7 (2.34–75.6)	6.25×10^6 (0.52–20.4)
CD34+/33−	0.8 (0–4.3)	12.2×10^7 (0.5–69.1)	2×10^6 (0.08–11.0)
CD34+/38−	0.11 (0–0.22)	0.86×10^7 (0–2.13)	0.18×10^6 (0–44)

The median day for starting apheresis was Day +11 (range, 9–14) after chemotherapy plus G-CSF; 70% of the patients underwent the apheresis procedure on Day +11. The median WBC, platelet count, and CD34+ cell count on the first day of collection were 14.1×10^9/L (range, 1.9–26.3), 106×10^9/L, and 113.8/μL, respectively.

The mean number of MNC, CD34+, CD34+/33−, and CD34+/CD38− cells/kg collected per apheresis procedure were 2.7×10^8 (range, 0.15–13.5), 6.3×10^6 (range, 0.52–20.4), 2.0×10^6 (range 0.8–11), and 0.18×10^6 (range, 0–44), respectively. A target level of 2.0×10^6 CD34+ cells/kg was achieved with two apheresis procedures in 90% of the patients and with a single apheresis procedure in 70% of the patients (Table 3). As shown in Figure 2, the number of preleukapheresis circulating CD34+ cells correlated significantly with the harvested CD34+ cell count per kilogram (r: 0.7, $P = 0.01$); in the patients with an absolute CD34+ cell number in the peripheral blood higher than 50/μL, the mean of CD34+ cells/kg collected per apheresis procedure was 7.8×10^6 (range, 1.25–20.4) versus 0.9×10^6 (range, 0.52–2.0) in patients who had a lower peripheral CD34+ cell count ($P = 0.006$).

To determine the proportion of the various lymphocyte subset populations, an immunophenotypic characterization of the mononuclear cell population of the PBPC harvests was performed.

The mean percentage (range) of cells positive for T-cell markers CD3, CD4, and CD8 were 52.1% (34.7–60%), 32% (21.8–43.4%), and 20.2% (15–24.5%), respectively. The mean percentage (range) of cells positive for B-cell marker CD19 was 2.0% (0.1–7.2). The

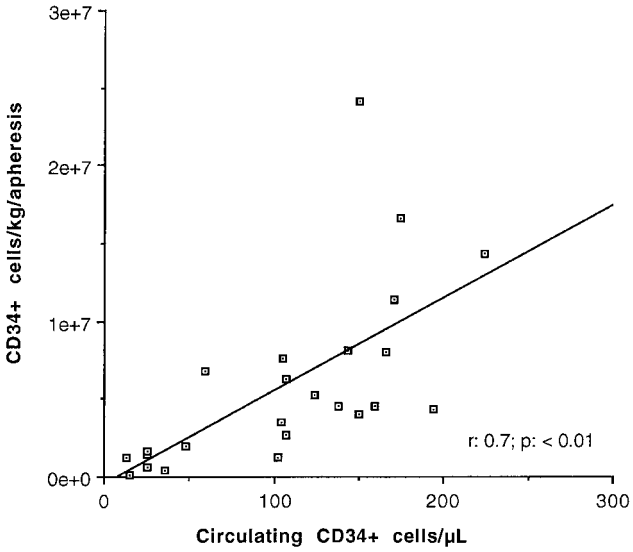

FIGURE 2. The correlation between the number of CD34+ cells/kg collected per apheresis and the preleukapheresis circulating CD34+ cell count is shown (r: 0.7; $P < 0.01$).

TABLE 4
Lymphocyte Subset Populations in the Peripheral Blood Progenitors Harvest

	Mean % (range)	Mean total no. of cells/lk × 10^9 (range)	Mean no. of cells/kg/lk × 10^7 (range)
MNC	100	15.5 (0.7–94.5)	26.8 (1.5–135)
CD3	52.1 (34.7–60)	4.3 (1.1–7.4)	9.0 (2.2–15.4)
CD4	32 (21.8–43.4)	2.62 (0.5–4.9)	5.0 (1.13–10.3)
CD8	20.2 (15–24.5)	1.6 (0.5–3)	3.0 (1.0–6.0)
CD19	2.0 (0.1–7.2)	0.2 (0.002–0.95)	0.33 (0.01–1.0)

mean number (range) of CD3, CD4, and CD8 positive cells collected per kg per apheresis were 90×10^6 (22–154), 50×10^6 (11.3–103), and 30×10^6 (10–60) (Table 4).

Engraftment Kinetics after Blood Cell Transplantation

Eighteen of the 21 patients received a single course of high dose chemotherapy, including thiotepa 600 mg/m^2 plus melphalan 120 mg/m^2, and were engrafted with PBPCs collected after priming with paclitaxel, epirubicin, and G-CSF. One patient who had not reached the target level of CD34+ cells because of a very poor circulating progenitor cell collection (0.53×10^6/kg CD34+ cells) underwent bone marrow harvest and was rescued with both the stem cell sources.

The median MNC, CD34+, and CD34+/33− cells reinfused were 2.6×10^8/kg (range, 0.3–10.1), 5.33×10^6/kg (range, 0.53–17), and 3.87×10^5 (range, 0.85×10^5–3.7×10^6), respectively. The median days to reach an absolute neutrophil count of 0.5×10^9/L and a platelet count of 20×10^9/L were 9.5 (range, 9–22) and 10 (range, 9–35), respectively.

Activity of the Consolidation Therapy

After high dose regimen, 6 patients in PR were converted into CR (conversion rate, 43%). Therefore, the CR rate after induction plus consolidation phase was 13/18 (72%).

DISCUSSION

In metastatic breast carcinoma, high dose chemotherapy with hemopoietic support is one of the most promising strategies for overcoming drug resistance. The achievement of a major clinical response (CR or good PR) with conventional chemotherapy has been shown to be an important prognostic factor for long term disease free interval after high dose chemotherapy with autologous hemopoietic support.[14] The combination of anthracyclines and paclitaxel is very active, and its use in an adjuvant setting is under investigation.[15,16] When bolus doxorubicin is combined with paclitaxel given over 3 hours, the CR rate is high (30–40%), with an objective response rate ranging from 70% to 80%; however, a significant proportion of patients experience cardiac toxicity (decline of left ventricular ejection fraction and signs of congestive heart failure).[17,18] In our experience, the combination of epirubicin and paclitaxel was devoid of cardiotoxicity and resulted in an objective response rate of 84%.[11] In the current study, we have explored the possibility of using this very active combination as a PBPC mobilizing regimen.

Our data show that epirubicin in combination with paclitaxel and G-CSF mobilizes PBPCs, with a median peak of 70/μL CD34+ cells in the peripheral blood. The number of CD34+ cells was significantly higher in patients who had received fewer than 6 courses of chemotherapy before the mobilization course: 107/μL vs. 7.3/μL ($P = 0.02$). The median peak of more primitive progenitors (CD34+/CD33−), although not statistically significant, was also higher in the patients not heavely pretreated: 24.4/μL versus 3.92/μL. Moreover, the median day of peripheral blood CD34+ cell peak was delayed in the patients who had received 6 or more courses: 11 versus 14 ($P = 0.01$).

The minimum number of CD34+ cells necessary for rapid and durable engraftment is still being debated. However, recent studies have reported a significant correlation between the number of CD34+ cells reinfused and hemopoietic engraftment, and a

number greater than 2.5×10^6/kg is considered to be sufficient for a safe hemopoietic reconstitution.[19–21] In our study, the CD34+ cell target level was 2×10^6/kg. In 90% of the patients it was achieved by 2 apheresis procedures, and in 70% of the patients by a single apheresis procedure. All but 1 patient were transplanted with a number of CD34+ cell greater than the cell target level (median, 5.33×10^6/kg), and the engraftment was complete and rapid, with a median neutrophil ($>0.5 \times 10^9$/L) and median platelet ($>20 \times 10^9$/L) recovery, on Days +9.5 and +10, respectively. The patient who received less than 2×10^6 CD34+ cells/kg in combination with autologous bone marrow engrafted over a significantly longer period of time.

In agreement with other authors,[22] we have found a good correlation between the absolute number of circulating CD34+ cells/μL and the number of CD34+ cells/kg collected per apheresis procedure (r: 0.7, $P = 0.01$); when the preleukapheresis CD34+ cell count exceeded a threshold of 50/μL, a single apheresis procedure was sufficient to yield a mean of 8.9×10^6 CD34+ cells/kg.

The CD34+ cells are a heterogeneous population, with the CD34 antigen present on both progenitor cells and more immature, putative stem cells, the latter responsible for long term bone marrow reconstitution. Few data are reported about the quality of circulating CD34+ cells mobilized using different chemotherapy regimens plus growth factors. The number of CD34+/CD33− cells may help predict the short term repopulation capacity of PBPCs.[12] We characterized peripheral blood CD34+ cells for CD33 and CD38 antigen expression to identify more primitive hemopoietic progenitor cell subsets in the PBPC harvest. The mean number of CD34+/CD33− and CD34+/CD38− cells per leukapheresis was 2×10^6/kg and 0.18×10^6/kg, respectively. Our data suggest that epirubicin and paclitaxel are not toxic for very primitive hemopoietic progenitors. Moreover, in agreement with others authors,[23] our study shows that PBPC harvests contain a significant number of cells positive for T-cell markers, an ideal cell source for ex vivo manipulation to augment antitumor properties of these effector cells. Recently, Neubauer et al. demonstrated that the lymphocytes present in the stem cell harvest show lympholine-activated killer cell activity before and after autologous peripheral blood stem cell transplantation.[24]

In conclusion, our data suggest that epirubicin plus paclitaxel in combination with G-CSF are able to mobilize hemopoietic progenitors, including more primitive progenitors, capable of sustaining a rapid and complete hemopoietic recovery after myeloablative treatment. The best mobilization is achieved when the procedure is performed earlier during the induction phase. The yield of CD34+ cell collection is predictable on the basis of the circulating CD34+ cell count. Finally, the considerable number of T cells in the PBPC harvest supports the hypothesis that PBPCs contain antitumor effector cells that might mediate an antitumor effect.

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