Absence of Breast Cancer Cells in a Single-Day Peripheral Blood Progenitor Cell Collection After Priming With Cyclophosphamide and Granulocyte-Macrophage Colony-Stimulating Factor

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The effect of priming on occult tumor cell involvement of peripheral blood (PB) and PB progenitor cell (PBPC) collections is poorly characterized. Using sensitive immunocytochemistry (ICC) and tumor clonogenic assays (TCA) specific for epithelial-derived tumor cells, hematopoietic specimens were analyzed for PBPC and occult tumor cell involvement in 28 patients with chemotherapy-sensitive stage IIIB or IV breast cancer. Before PBPC priming, tumor was detected by ICC in PB of 1 of 23 (4%) patients and in bone marrow (BM) harvests of 4 of 27 (15%) patients. Fifteen days after cyclophosphamide and granulocyte-macrophage colony-stimulating factor (GM-CSF) priming, 2 of 27 (8%) patients had ICC-positive PBPC collections. The median amplification of PBPC during this time was over 19-fold (range, <1 to 199). One patient had pretreatment tumor involvement of both PB and BM. One patient grew tumor colonies in TCA; the PB and BM were ICC- and TCA-negative, but the PBPC collection was ICC-positive but TCA-negative. After cytoreduction with conventional-dose chemotherapy, patients with advanced breast cancer and histologically negative BM biopsy specimens have rare tumor cell involvement of PB and BM. Despite effective PBPC priming with cyclophosphamide and GM-CSF, occult breast cancer cells were not found in the PBPC collection performed on day 15.

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PERIPHERAL BLOOD progenitor cells (PBPCs) are used increasingly for hematopoietic rescue after high-dose chemotherapy. In patients with overt tumor involvement of bone marrow (BM) or prior irradiation of harvest sites, PBPC grafts are preferred over BM grafts. However, at the steady-state level, the PB concentration of hematopoietic progenitors is very low, requiring multiple leukapheresis to collect an adequate number of PBPCs for hematopoietic rescue. Furthermore, the kinetics of engraftment after reinfusion of PBPCs collected at the steady-state level are similar to those observed after BM reinfusion. In contrast, reinfusion of PBPCs mobilized by myelosuppressive chemotherapy and/or hematopoietic growth factors is associated with faster hematopoietic recovery.

In most cases, patients eligible for high-dose chemotherapy with BM rescue must not have evidence, by routine pathologic examination of tumor involvement of BM. However, by using more sensitive diagnostic techniques, some of these patients are found to have occult tumor contamination. In some series, occult tumor contamination of hematopoietic grafts is associated with shortened disease remission and survival. Preliminary data suggest that occult tumor contamination of PBPC collections is less frequent than contamination of BM harvest products. Recently, however, a mobilization effect on tumor cells of chemotherapy and hematopoietic growth factor administration was reported in a small number of patients with recently diagnosed metastatic breast cancer and small cell lung cancer. In an elegant study, Brugger et al analyzed serial PB specimens for tumor cell contamination after administration of etoposide, ifosfamide, and cisplatin (VIP) followed by granulocyte colony-stimulating factor (G-CSF). Of a total of seven patients with stage IV breast cancer, two had evidence of tumor cell involvement of PB at the steady-state level, but all seven had evidence of tumor cell involvement of PB after VIP and G-CSF administration. This reported mobilization of tumor cells raises the possibility that PBPC priming with chemotherapy and hematopoietic growth factor might result in increased tumor cell contamination of PBPC collections.

To clarify the impact of PBPC priming on occult tumor cell contamination of PBPC collections, we analyzed PB and BM samples pretreatment and PBPC collection samples performed 15 days after cyclophosphamide and granulocyte-macrophage CSF (GM-CSF) priming from women with chemotherapy-sensitive advanced breast cancer, using sensitive immunocytochemical (ICC) and tumor clonogenic assays (TCA) specific for epithelial-derived tumor cells. We found a low percentage of occult tumor cell contamination of hematopoietic products at the steady-state level and no evidence of increased tumor cell involvement of PBPC collections harvested 15 days after priming with cyclophosphamide and GM-CSF.

PATIENTS AND METHODS

Patient population. Between April 1993 and March 1994, 28 consecutive patients with breast cancer participated in this study of high-dose chemotherapy with reinfusion of mobilized PBPC and 4-hydroperoxycyclophosphamide (4HC)-purged BM. Study eligibility criteria included histologically documented stage IIIB or IV breast cancer responsive to conventional-dose systemic chemotherapy, a leukocyte count ≥ 3 × 10⁹/µL, a platelet count ≥ 100 × 10⁹/µL, and a normocellular BM biopsy specimen without pathologic evidence of tumor involvement by breast cancer. The study was approved by the Joint Committee for Clinical Investigation (the Institutional Review Board of the University of California, Los Angeles, and Cedars-Sinai Medical Center).

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Review Board) of the Johns Hopkins Hospital (Baltimore, MD), and written informed consent was obtained from all patients.

The median age was 43 years (range, 31 to 61 years). A total of 5 patients had stage IIIIB breast cancer, and 23 patients had stage IV. Of the patients with stage IV breast cancer, 4 were in complete remission and 19 were in partial remission at study entry after 2 or more courses of conventional-dose cytotoxic chemotherapy, and 6 of 23 had radiologic evidence of BM metastases.

The day after bone harvest, cyclophosphamide (4 g/m² administered only once) and GM-CSF (5 μg/kg/d, administered subcutaneously, for 15 days; Sargramostim; Immunex CO, Seattle, WA) were administered for PBPC priming. A single large-volume leukapheresis was performed 15 days after cyclophosphamide administration. One week later, patients received BM ablative chemotherapy with cyclophosphamide and thiotepa (6 g/m² and 800 mg/m², respectively, by continuous infusion over 96 hours), followed by reinfusion of PBPCs and 4HC-purged BM.

Samples of pretreatment PB obtained before BM harvest, samples of harvested BM, and samples of PBPCs collected 15 days after priming with cyclophosphamide and GM-CSF were analyzed for occult breast cancer involvement. The efficacy of PBPC priming was evaluated by measuring PB concentration of GM colony-forming units (CFU-GM) and of CD34+ cells before priming and on days 13, 14, and 15 after initiation of cyclophosphamide and GM-CSF, on recovery from myelosuppression.

**Tumor cell detection by ICC.** A total of 3 to 5 mL of aseptically collected pretreatment PB and BM or mobilized PBPC specimens, containing a minimum of 1 × 10⁶ cells/mL, were collected in sterile sodium heparin tubes and shipped overnight at room temperature to BHS Laboratories (Reseda, CA). The ICC staining procedure for the presence of tumor micrometastases in PB, BM, and PBPC collections has been described previously.12 Briefly, mononuclear cells were incubated with a cocktail of IgG murine monoclonal antibodies (MoAbs) directed against breast or glandular epithelial antigens (MAS-385 and SB-6, from Accurate Chemical, Westbury NY; SB-3, from CalTag, San Francisco, CA; and TFS-2, from Biodesign, Kennebunkport, ME). Cytospin preparations were then immunostained using the Zymed streptavidin immunoperoxidase kit (Zymed, San Francisco, CA). Tumor cells stained bright red, whereas hematopoietic cells stained blue with the hematoxylin counterstain. To facilitate tumor detection, an ICC technique was used in a blinded fashion in the laboratory of one of the investigators (T.J.M.) at Cedars-Sinai Medical Center (Los Angeles, CA). ICC immunostaining has been reported previously.

In previous direct tumor cell seeding experiments of breast cancer cell lines into normal BM and PBPC samples to assess assay sensitivity, the ICC assay was able to detect as few as 1 tumor cell/5 × 10⁵ hematopoietic cells.12,13 Immunostaining of 48 normal BM samples and 27 BM samples from patients with hematologic malignancies to assess assay specificity showed no nonspecific or cross-reactive immunostaining.13 In 7 PBPC collections from patients with malignancies other than breast cancer, none have shown positivity with the antibodies used by ICC (A.A.R., unpublished data). The criteria for tumor cell positivity by the ICC assay have been previously described.12

For tumor cell quantification, up to 5 × 10⁶ cells per specimen were directly examined. The total number of fields required to analyze a total of 10⁶ cells was calculated from the average number of cells present per high-power field (40× objective). If 5 or greater ICC-positive tumor cells were detected per 10⁶ BM cells, the number of tumor cells per 10⁵ was mathematically extrapolated. If fewer than 5 tumor cells were detected per 10⁶ BM cells, then up to 5 × 10⁶ cells were directly examined to eliminate sampling error. Virtually all patients had 5 × 10⁶ cells directly examined.

**TCA technique.** The TCA technique for the in vitro growth of tumor colonies in PB, BM, and PBPC specimens has been described previously.12 Briefly, 5.0 × 10⁵ mononuclear cells/ml were plated in triplicate in 35-mm² grid-bottom petri dishes (Nunc, Inc, Naperville, IL) in a soft agar-based medium with supplemental growth factors as previously described.12,15 Negative control plates consisted of medium without supplemental growth factors. Tumor colonies (>40 cells) were counted after 14 days of incubation using an inverted-phase contrast microscope.

The TCA system was tested on nearly 200 BM17 and 63 PBPC specimens from patients with breast cancer. These studies have shown that the combination of growth factors used supports the in vitro clonogenic growth of tumor colonies in both BM and PBPCs. We have observed no tumor colony growth in normal BM specimens with this combination of growth factors.17 In addition, we have previously reported that in vitro tumor colony growth in the TCA system is highly correlated (P < .0001, χ² test) with the ICC detection of tumor cells both in BM and in PB.15 In that study, tumor colonies grew in 4 of 5 PBPC specimens where tumor cells were observed by ICC and failed to grow in all 22 PBPC specimens where the ICC was negative.

**Immunostaining of tumor colonies in TCA assay.** TCA immunostaining was performed in a blinded fashion in the laboratory of one of the investigators (T.J.M.) at Cedars-Sinai Medical Center (Los Angeles, CA). TCA immunostaining has been reported previously.12 Briefly, immunofluorescence staining with fluorescein isothiocyanate-labeled anticytokeratin MoAb SB-3 or breast cancer-associated MoAbs 520C9, 260F9, and 317G5 (kindly provided by Baxter Healthcare, Immunotherapy Division, Santa Ana, CA) were used to identify tumor colonies. The specificity of these antibodies in this assay has been previously validated.12,17,18

**Quantification of hematopoietic progenitors.** Quantification of hematopoietic progenitors was performed by measurement of CFU-GM in short-term in vitro culture assay and by measurement of CD34+ cells by flow cytometry using techniques previously described.19,20 PBPC concentration was measured at the steady-state level (before BM harvest), on days 13, 14, and 15 after initiation of priming with cyclophosphamide and GM-CSF and on the leukapheresis product. Calculation of PBPC content per milliliter of PB was performed according to the following equations:

\[
\text{CD34}^+ \text{cell/mL} = \frac{PB \text{ leukocytes/mL} \times \text{PB sample Ficoll recovery} \times \text{PB sample CD34}^+ \times \text{(PB sample Ficoll recovery) \times (no. CFU-GM colonies/seeding density)}}{\text{Ficoll separation}}
\]

These calculations assume that all PBPCs are recovered by Ficoll gradient separation.

**Statistical analysis.** Confidence intervals (CIs) for the proportion of tumor-positive PB, BM, and PBPC collection specimens were calculated using exact binomial CIs. The binomial distribution was used to compare tumor contamination of paired BM and PBPC specimens, testing the probability (P) that discordant pairs of either type was .5. Fisher's exact test was used for comparison of categorical data.

**RESULTS**

Hematopoietic specimens were analyzed for breast cancer cell contamination in 28 consecutive patients with breast cancer. ICC and TCA assays were performed in 23 patients on PB and BM harvest samples pretreatment and on PBPC collection samples after cyclophosphamide and GM-CSF priming. In 5 patients, only BM harvest and PBPC collection
samples were analyzed by ICC and TCA assays because of failure to collect the PB sample before BM harvest.

The results of the ICC assay are shown in Table 1. Evidence by ICC assay of tumor contamination of pretreatment PB specimens was observed in only 1 patient (patient no. 21) who had 2 circulating tumor cells/10^5 PB mononuclear cells. This patient's BM harvest, performed the same day the PB was drawn, was also positive (4 tumor cells/10^5 BM mononuclear cells), as was the PBPC collection 2 weeks later (1 tumor cells/10^5 collected PBPC). The remaining 22 PB samples (96%; 95% CI, 78 to 100) had no evidence of tumor cell contamination by ICC on day 0.

Four BM harvest samples were positive by ICC assay, with 2 to 18 tumor cells/10^5 BM mononuclear cells. In 1 other case (patient no. 9), ICC results were inconclusive; for this reason, this patient was removed from further analysis. Thus, 4 of 27 (15%; 95% CI, 4 to 34) BM harvest specimens were positive by ICC.

PBPC were collected in a single 6-hour leukapheresis 15 days after beginning priming with cyclophosphamide and GM-CSF. PBPC concentration was measured at the steady-state level and on the 3 days preceding leukapheresis. Data on CD34+ cell mobilization are also presented in Table 1. CD34+ numbers in the PB increased from a median 470 (range, 110 to 2,130) cells/mL on day 0 to a median 11,200 (range, 110 to 103,200) cells/mL on day 15, confirming the effectiveness of the mobilization strategy. The median fold increase in CD34+ cells in the PB was 19.5. This resulted in a median PBPC collection of 4 × 10^7 CD34+ cells/kg (range, 0 to 27 × 10^7). In 10 of 26 patients (38%) the peak concentration of PB CFU-GM/L and of CD34+ cells/L was measured on the day of leukapheresis. In 2 other patients, the day of peak concentration of PB CFU-GM and CD34+ cells did not coincide, one peak occurring on the day of leukapheresis and the other peak the day before leukapheresis. In 8 patients, the peak concentration of PB CFU-GM and CD34+ cells occurred the day before, and in 3 patients 2 days before leukapheresis. The remaining 3 patients had their peak concentration of PB CFU-GM and CD34+ cells noted before administration of cyclophosphamide and GM-CSF and had very low numbers of PBPCs collected during leukapheresis.

PBPC collected on day 15 after priming with cyclophosphamide and GM-CSF were also analyzed for tumor cell contamination. Only 2 of 28 (7%; 95% CI, 1 to 24) collections had evidence of tumor contamination by ICC assay. Patient no. 21, who had 1 tumor cell/10^5 cells in the PBPC collection, also had evidence of tumor involvement of PB and BM before PBPC priming. This patient had metastatic disease at multiple sites including bone and was in partial remission at study entry. Another patient (patient no. 16) had tumor contamination of the PBPC collection (1 tumor cell/10^5 collected PBPC) despite tumor-negative PB and BM before mobilization. This patient had lung and lymph node metastases and was in partial remission at study entry. Of 23 patients without tumor involvement of PB or BM before PBPC priming, 22 had no evidence of tumor involvement of PBPC collections 15 days after cyclophosphamide and GM-CSF administration, whereas 3 of 4 patients with breast cancer contamination of PB or BM at study entry did not have tumor involvement of PBPC collections 2 weeks later, after cyclophosphamide and GM-CSF priming.

TCA was positive in only 1 patient (patient no. 21). This patient had ICC-positive PB, BM, and PBPC collection specimens and TCA-positive pretreatment PB and BM specimens but a TCA-negative PBPC collection specimen. In 3 other patients (patients no. 5, 7, and 8), the TCA results of ICC-positive BM specimens were inconclusive; tumor colonies were detected by inverted-phase microscopic evaluation, but the immunostaining of these TCA specimens was inconclusive. No TCA positivity was observed in ICC-negative specimens.

In the 5 patients with tumor involvement of hematopoietic products detected by ICC, the peak concentration of PB CFU-GM and CD34+ cells occurred on the day of leukapheresis in 3 patients (patients no. 7, 8, and 16), 2 days before leukapheresis in 1 patient (patient no. 9), and before PBPC
mobilization in 1 patient (patient no. 5). In patient no. 21, it was not possible to estimate the day of peak PBPC concentration because of incomplete data collection.

There was no relationship between tumor involvement of hematopoietic products and disease stage, presence of bone metastases, or clinical evidence of breast cancer at study entry. Of 22 patients with stage IV disease, 4 had at least one ICC-positive hematopoietic specimen, as compared with 3 of 21 without bone metastases (P = .3). Of 8 patients without clinical evidence of breast cancer at study entry (ie, patients with stage IIIB after neoadjuvant chemotherapy and locoregional treatment, and patients with stage IV in complete remission), 2 had at least one ICC-positive specimen, as compared with 3 of 19 patients with stage IV breast cancer in partial remission at study entry (P = .6). With a median follow-up since study entry of 212 days (range, 27 to 390), 2 patients have progressed after high-dose chemotherapy (patients nos. 3 and 10). Neither patient had evidence of tumor involvement of any hematopoietic specimen.


discussion

PBPCs are used with increasing frequency for hematopoietic rescue after high-dose chemotherapy. This is because of the early hematopoietic recovery associated with reinfection of mobilized PBPCs and the belief that PBPC collections are less likely than BM harvest products to be contaminated with circulating tumor cells. In breast cancer patients without evidence of tumor involvement of BM by routine pathologic examination, sensitive ICC, TCA, or polymerase chain reaction techniques can detect occult BM involvement with tumor cells. Although the clinical relevance of occult tumor contamination of hematopoietic rescue products is unclear, an association between occult BM tumor involvement, detected by cell culture assay, and increased tumor relapse has been reported in patients with breast cancer. A similar association was reported for patients with non-Hodgkin’s lymphoma who underwent high-dose chemotherapy.

Whether tumor contamination of hematopoietic grafts is a source of tumor relapse after high-dose chemotherapy or simply a marker of tumor bulk or resistance to chemotherapy is unknown. However, using gene-marking techniques in patients with acute leukemia, Brenner et al have shown that tumor cells reinjected in the hematopoietic rescue product do contribute to disease relapse.

In patients with breast cancer, the prevalence of occult tumor contamination of PBPC collections is less frequent than that of BM harvests. In patients undergoing high-dose chemotherapy, analysis of paired PBPC collection and BM harvest samples documented not only that PBPC collections were significantly less contaminated with tumor cells than were BM, but also that the concentration of tumor cells in tumor-involved specimens was 30-fold lower in PBPC collections than in BM.

The concentration of tumor cells in hematopoietic grafts is important because PBPC products usually contain a larger cell number than BM products. For instance, in the series reported here, a median 8 x 10^8 nucleated cells/kg were collected in the PBPC product compared with a median 4 x 10^8 nucleated cells/kg collected during BM harvest.

The impact of PBPC mobilization strategies on occult tumor contamination of hematopoietic products is poorly characterized. Recently, Brugger et al using an ICC assay with MoAbs against cytokeratin and epithelial antigens, reported a mobilization effect of tumor cells, after treatment with VIP and G-CSF, in seven newly diagnosed patients with stage IV breast cancer. Two patients without tumor involvement of PB and BM before chemotherapy had circulating tumor cells detected by ICC assay between days 1 and 7 after VIP treatment but not between days 9 and 16. Of five patients with tumor-involved BM, two patients had tumor contamination of PB before chemotherapy, and all five patients had tumor contamination of PB between days 9 and 16 after VIP therapy. It was not clear whether the BM contamination was clinically apparent (ie, documented by routine pathologic examination) or occult (ie, detected by ICC assay alone) or whether these patients had received any systemic chemotherapy for metastatic breast cancer or were in remission before treatment with VIP and G-CSF. The reported mobilization of tumor cells into the PB by chemotherapy and hematopoietic growth factor obviously raises the question of the impact of such treatment strategies on tumor cell contamination of PBPC collections.

We analyzed hematopoietic specimens for occult tumor contamination by ICC and TCA assays, performed before and 15 days after PBPC priming, in 28 patients with stage IIIB or IV breast cancer and histologically negative BM biopsy specimens, who had previously received cytoreductive chemotherapy to assess drug responsiveness. This analysis was performed to see if the mobilization of tumor cells into PB reported by Brugger et al would translate into increased numbers of tumor cells in PBPC collections harvested around the time of maximal mobilization of CD34+ cells. PB and BM specimens were analyzed the day before beginning PBPC priming with cyclophosphamide and GM-CSF, and PBPC collection specimens were analyzed 15 days after cyclophosphamide administration. A low prevalence of occult tumor contamination was found in patients with BM (1 of 23; 4%) and BM (4 of 27; 15%) specimens by ICC assay, as was a low absolute number of tumor cells in ICC-positive specimens (2 to 18 tumor cells/10^5 mononuclear cells). There was no evidence of increased numbers of tumor cells in PBPC collections performed 15 days after cyclophosphamide and GM-CSF priming. Of 23 patients without tumor involvement of PB or BM before PBPC priming, 22 had no evidence of tumor involvement of PBPC collections after cyclophosphamide and GM-CSF administration. Of 4 patients with evidence of breast cancer contamination of PB or BM at study entry, 3 did not have tumor involvement of PBPC collections 2 weeks later, after PBPC priming. Furthermore, in patient no. 21, whose PB, BM, and PBPC collection specimens were all ICC-positive, the tumor cells obtained from PB and BM samples before cyclophosphamide and GM-CSF administration had in vitro clonogenic capacity, but the tumor cells obtained from the PBPC collection did not. The change in TCA results observed in this
patient suggests that the tumor cells in the PBPC collection were either nonviable cells or cells incapable of in vitro proliferation, possibly as a result of priming with cyclophosphamide. Only 1 of 28 (4%) patients had evidence of tumor contamination of mobilized PBPC collection without occult tumor involvement of PB or BM harvest before PBPC mobilization. One limitation of this study is that tumor contamination of PB was not examined serially after PBPC priming. Therefore, increased tumor contamination of PB occurring after cyclophosphamide administration, but before the day of leukapheresis, would have been missed. The data from Brugger et al suggest this is possible. In seven patients with stage IV breast cancer, these investigators detected tumor cells in the PB of only two patients at the the steady-state level but in all seven after PBPC recruitment with chemotherapy and growth factors. In the five patients who had tumor in their BM, Brugger et al were able to detect cancer cells in the PB between 9 and 16 days after priming. In the two patients with negative BM, tumor cells appeared in the PB only between days 1 and 7. Even in those patients with positive BM, in whom tumor cells were detectable at days 9 through 16, the concentration of tumor cells in the PB was decreasing by day 16. Because no patient in the current study had evidence of tumor contamination in BM on routine histology and because only 4 were positive by a sensitive immunohistochemical technique, it is reasonable to assume that, if mobilization of tumor cells did occur into the PB in a manner similar to that observed by Brugger et al, it would have happened around day 7.

However, the potential clinical concern about mobilization of tumor cells by PBPC-priming strategies relates to the risk of contamination of PBPC collection products. Therefore, it is critical to examine PBPC specimens collected at the clinically relevant time. After PBPC priming with chemotherapy and hematopoietic growth factors, leukaphereses are performed on recovery from myelosuppression, corresponding to a peak of PBPC concentration in the circulation. Thus, this is the clinically relevant time to examine for a tumor cell mobilization effect, and this was the approach used in this study. Table 1 shows that substantial increments in CD34+ cell numbers were observed in the PB on day 15 in the majority of patients; at which time, leukapheresis was performed. This mobilization of CD34+ cells was not associated with large numbers of tumor cells in the progenitor cell collections. Serial assessment of CD34+ cells and CFU-GM showed that in 20 of 26 (77%) patients PBPC collection was performed the day of, or 1 day after, the peak of PB CFU-GM and CD34+ cell concentrations.

The low frequency of occult tumor cell involvement of PB and BM before PBPC priming is probably because of prior systemic chemotherapy administered to reduce disease before high-dose chemotherapy. However, no specimens to support this interpretation were collected at the time of presentation with stage IIIB or IV breast cancer. The absence of in vitro clonogenic capacity by TCA of the PBPC collection specimen from the only patient with premobilization TCA-positive PB and BM specimens further suggests that administration of chemotherapy before collection of PBPCs may decrease the frequency of tumor contamination of the hematopoietic graft. A similar prevalence of occult tumor involvement of BM harvest products was observed in a previous cohort of patients with breast cancer who were treated with high-dose chemotherapy after systemic cyto reduction (5 of 20; 25%). Furthermore, in that study, tumor contamination of PBPC collections was reduced by further courses of chemotherapy, and the in vitro clonogenic activity of tumor cells assessed by TCA was suppressed by in vitro treatment with 4HC. We believe that most studies of high-dose chemotherapy for advanced breast cancer enroll patients similar to the ones included in this series. However, it is possible that tumor mobilization into pheresis collections caused by PBPC-priming strategies may occur in patients with larger tumor bulk or untreated disease, such as patients with BM metastases that is evidenced by routine pathologic evaluation or before administration of cytoreductive chemotherapy. It is also possible that PBPC priming with hematopoietic growth factors alone has a different impact on mobilization of tumor cells into PBPC collections. This is currently under investigation.

In summary, sensitive and specific ICC and TCA assays were used to analyze hematopoietic specimens for occult breast cancer cell involvement and in vitro growth potential, before and 15 days after PBPC priming, in 28 patients with advanced breast cancer. There was a low prevalence and a low concentration of occult tumor cell contamination of PB and BM harvest products before PBPC priming. More importantly, whereas effective recruitment of CD34+ cells by cyclophosphamide and GM-CSF was documented around the time of PBPC collection, there was no evidence of increased tumor contamination of the PBPC collection. These findings suggest that patients may benefit from the early hematopoietic recovery associated with reinfusion of primed PBPCs without the potential risk of increased disease relapse associated with reinfusion of tumor cells in the hematopoietic graft.

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REFERENCES


6. To LB, Roberts MM, Haylock DN, Dyson PG, Branford AL, Thorp D, Ho JQK, Dart GW, Horvath N, Davy MLJ, Otweny CLM, Abdi E, Juttner CA: Comparison of hematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. Bone Marrow Transplant 9:277, 1992


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