**Purpose:** Multipotential mesenchymal stem cells (MSCs) are found in human bone marrow and are shown to secrete hematopoietic cytokines and support hematopoietic progenitors in vitro. We hypothesized that infusion of autologous MSCs after myelosuppressive therapy would facilitate engraftment by hematopoietic stem cells, and we investigated the feasibility, safety, and hematopoietic effects of culture-expanded MSCs in breast cancer patients receiving autologous peripheral-blood progenitor-cell (PBPC) infusion.

**Patients and Methods:** We developed an efficient method of isolating and culture-expanding a homogenous population of MSCs from a small marrow-aspirate sample obtained from 32 breast cancer patients. Twenty-eight patients were given high-dose chemotherapy and autologous PBPCs plus culture-expanded MSC infusion and daily granulocyte colony-stimulating factor.

**Results:** Human MSCs were successfully isolated from a mean ± SD of 23.4 ± 5.9 mL of bone marrow aspirate from all patients. Expansion cultures generated greater than 1 × 10^6 MSCs/kg for all patients over 20 to 50 days with a mean potential of 5.6 to 36.3 × 10^6 MSCs/kg after two to six passages, respectively. Twenty-eight patients were infused with 1 to 2.2 × 10^6 expanded autologous MSCs/kg intravenously over 15 minutes. There were no toxicities related to the infusion of MSCs. Clonogenic MSCs were detected in venous blood up to 1 hour after infusion in 13 of 21 patients (62%). Median time to achieve a neutrophil count greater than 500/μL and platelet count ≥ 20,000/μL untransfused was 8 days (range, 6 to 11 days) and 8.5 days (range, 4 to 19 days), respectively.

**Conclusion:** This report is the first describing infusion of autologous MSCs with therapeutic intent. We found that autologous MSC infusion at the time of PBPC transplantation is feasible and safe. The observed rapid hematopoietic recovery suggests that MSC infusion after myelosuppressive therapy may have a positive impact on hematopoiesis and should be tested in randomized trials.


Human bone marrow contains mesenchymal progenitors (mesenchymal stem cells [MSCs]) that produce adventitial cells in the marrow microenvironment; these cells provide support to hematopoiesis by producing membrane-bound and soluble signals and cytokines. These stromal progenitors can be readily isolated from bone marrow and demonstrate extensive proliferative capacity in vitro. Purified and culture-expanded human MSCs differentiate along the osteogenic, chondrogenic, and adipogenic lineages both in vitro and in vivo. In unstimulated cultures, MSCs appear as fusiform fibroblasts with expression of unique surface proteins (SH2, SH3, SH4) that are not found on hematopoietic precursors. MSCs lack expression of hematopoietic markers such as CD45, CD14, and CD34. MSCs constitutively secrete interleukin (IL)-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, macrophage colony-stimulating factor, Flt-3 ligand, and stem-cell factor, and they are inducible with IL-1α to produce IL-1α, leukemia-inhibiting factor, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor. Similar to Dexter-type stromal cultures, MSCs can support human long-term culture-initiating cells (LTC-1Cs). Therefore, we postulated that MSCs may enhance hematopoietic engraftment rate and quality after myelosuppressive and stroma-damaging treatments.

In a pilot study, our group demonstrated the safety of ex vivo expansion and subsequent infusion of autologous MSCs in 15 patient volunteers. These individuals had hematologic malignancies that were in remission at the time of MSC collection and infusion and were not given preparative chemotherapy. Only 1 to 50 × 10^6 total autologous MSCs were administered via intravenous (IV) infusion without any toxicity. However, human bone marrow--de-
rived, culture-expanded MSCs have never been adminis-
tered via IV infusion into patients at the time of peripheral-
nerve outgrowth and bone marrow–derived culture-
extended autologous MSCs infused into patients in the
course of high-dose chemotherapy and hematopoietic stem-
cell rescue. Our results show that autologous MSCs can be
successfully culture-expanded and infused along with PB-
PCs after high-dose chemotherapy in advanced breast can-
cer patients, are free of toxicity, and are associated with
rapid hematopoietic recovery.

**PATIENTS AND METHODS**

**Patients**

Between October 1996 and July 1998, 32 patients with locally advanced or metastatic breast cancer who were eligible for high-dose chemotherapy and PBPC transplantation were enrolled onto this phase I-II trial at the Ireland Cancer Center, University Hospitals of Cleveland, Case Western Reserve University in Cleveland, OH, after obtaining written informed consent. The clinical trial protocol and the consent form were approved by the Institutional Review Board for Human Investigation of the University Hospitals of Cleveland. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 or 1 and were required to have adequate visceral organ function, including a left ventricular ejection fraction of at least 50%, forced expiratory volume in 1 second and diffusion capacity of carbon monoxide greater than 50% predicted, serum direct bilirubin less than 2.0 mg/dL, and an actual or calculated creatinine clearance greater than 60 mL/min. At the start of therapy, a neutrophil count greater than 1.2 × 10⁹/L and a platelet count greater than 100 × 10⁹/L were required. Patients were excluded if they had cumulative doxorubicin exposure in excess of 500 mg/m², major CNS dysfunction, visceral organ function, including a left ventricular ejection fraction of performance status of 0 or 1 and were required to have adequate bone marrow biopsy specimens.

**Ex Vivo MSC Culture**

On enrollment, approximately 35 days before scheduled PBPC infusion (see High-Dose Chemotherapy and PBPC Infusion), 20 to 25 mL of bone marrow aspirate was obtained under sterile conditions by puncture of bilateral posterior iliac crests of patients under local anesthesia. Aspirates were obtained 2 to 48 hours before high-dose cyclophosphamide mobilization chemotherapy. The aspirate was taken to the class 10,000 quality clean production suite of the Cell and Gene Therapy Core Facility at the Case Western Reserve University. Aspirate was mixed with two volumes of Dulbecco’s phosphate-buffered saline (DPBS; Gibco, Grand Island, NY) in a sterile class II biologic safety cabinet and centrifuged at 900 × g for 10 minutes at 20°C in a Beckman GS-6R centrifuge (Palo Alto, CA). Pellets were layered onto 25 mL of Percoll (density, 1.073 g/mL) (Sigma, St Louis, MO) at a density of 1 to 2 × 10⁶ cells/mL. Gradients were centrifuged at 900 × g for 30 minutes at 20°C, and recovered mononuclear cells were resuspended in DPBS and centrifuged at 460 × g for 10 minutes at 20°C. Cells were resuspended at 1 × 10⁶ cells/mL in Dulbecco’s modified Eagle medium, low glucose (DMEM-LG) (GibcoBBL, Grand Island, NY) with 10% fetal bovine serum (Hyclone, Logan, UT,) and 30 mL of cell suspension was plated in a 175 cm² flask (Falcon, Franklin Lakes, NJ). The serum lot used was selected on the basis of optimal MSC growth with maximal retention of osteogenic differentiation as assessed with in vitro and in vivo assays.⁷ MSCs were cultured in humidified incubators with 5% CO₂ and initially allowed to adhere for 72 hours, followed by media change every 3 to 4 days. When cultures reached more than 90% confluence, adherent cells were detached with 0.05% trypsin-EDTA (Gibco) and replated (passaged) at a density of 1 × 10⁶ per 175 cm² flask until processing for infusion. Cell cultures were tested for sterility weekly (University Hospitals Microbiology, Cleveland, OH) and for the presence of breast cancer cells by immunocytochemical method (BIS Labs, Reseda, CA)⁸ and endotoxin by limulus amebocyte lysate test (Associates of Cape Cod, Falmouth, MA), and *Mycoplasma* by DNA-fluorochrome stain (Bionique, Saranac Lake, NY) before infusion.

**Flow Cytometry**

Surface expression of SH-2, SH-3, SH-4, CD14, and CD45 was determined on culture-expanded MSCs. Cells were detached with 0.05% trypsin-EDTA (Gibco), washed with DPBS plus 2% bovine albumin, fixed in 1% paraformaldehyde, blocked in 10% normal goat serum, and incubated separately with primary SH-2, SH-3, and SH-4 antibodies (Osiris Therapeutics, Baltimore, MD) followed by phycoerythrin-conjugated antimouse IgG(H+L) antibody (CalTag, Burlingame, CA) or with fluorescein isothiocyanate conjugated CD45 and phycoerythrin-labeled CD14 with appropriate isotype controls (Becton Dickinson, San Jose, CA). Flow cytometry was performed on a FACScan (Becton Dickinson, Parsippany, NJ) equipped with an argon laser, and data were analyzed with CellQuest software (Becton Dickinson).

**High-Dose Chemotherapy and PBPC Infusion**

The PBPC mobilization regimen consisted of cyclophosphamide 4.0 g/m² IV infusion over 6 hours on day 1, along with mesna (first 3.0 g/m² IV, then 500 mg every 3 hours orally/IV for eight doses) and prednisone 2.0 mg/kg orally on days 1 through 4.⁷ At 36 to 48 hours after completion of the cyclophosphamide, patients began subcutane-
ously injections of recombinant human G-CSF (Amgen, Thousand Oaks, CA) 10 µg/kg/d. On recovery of neutrophils to greater than 1 × 10⁹/L (usually 12 to 15 days after cyclophosphamide treatment) patients underwent a leukopheresis procedure using Cobe Spectra (COBE, Lakewood, CO) pheresis equipment. Apheresis with a mean volume of 20 L (4 × total blood volume) was performed in each session, which was repeated until ≥ 2.0 × 10⁹ CD34⁺ cells/kg or ≥ 12 × 10⁶ mononuclear cells/kg of patient weight was obtained. Cells were cryopreserved using a controlled-rate liquid nitrogen freezer using previously published methods.¹⁰ After PBPC procurement, high-dose chemotherapy with cyclophosphamide 6,000 mg/m², thiotepa 500 mg/m², and carboplatin 800 mg/m² were administered as continuous IV infusion over 96 hours through a central venous catheter from days T–7 through T–3.¹² PBPCs were thawed and infused 72 hours after the completion of high-dose chemotherapy (day T–0). All patients received recombinant human G-CSF 10 µg/kg subcutaneously (Amgen) daily starting 4 hours after the PBPC infusion until neutrophil engraftment (absolute neutrophil count > 0.5 × 10⁹/L for 3 days). Platelet engraftment was defined as the first of 7 consecutive days on which platelet count was greater than 20 × 10⁹/L without transfusion.
Support. Bone marrow aspirates were obtained on days T+7, T+14, T+42, and T+72 to determine hematopoietic colony-forming unit (CFU) recovery. Patients underwent restaging evaluation with computed tomography and bone scans 42 days after transplantation and every 3 months thereafter.

Supportive Care

All patients had multilumen, indwelling central venous pheresis catheters and were cared for in single hospital rooms. Antibiotics were given empirically for fever and neutropenia, and all patients were supported with irradiated blood components. Irradiated, packed RBC transfusions were given in an attempt to keep the hematocrit greater than 25%, and irradiated platelet transfusions were given for platelet counts less than $10 \times 10^9/L$ or bleeding complications. Cytomegalovirus-negative blood products were given to cytomegalovirus-seronegative patients. Toxicity grading was accomplished using the National Cancer Institute common toxicity criteria.

MSC Infusion

Confluent MSCs in 175 cm$^2$ flasks (15 to 85 per patient) were washed with Tyrode's salt solution (Sigma), incubated with Medium 199 (M199 [Gibco]) for 60 minutes, and detached with 0.05% trypsin-EDTA (Gibco). Cells from 10 flasks were detached at a time and MSCs were resuspended in 40 mL of M199 plus 1% human serum albumin (HSA [American Red Cross, Washington, DC]). MSCs harvested from each 10-flask set were stored for up to 4 hours at 4°C and combined at the end of the harvest. A total of 1 to $2 \times 10^6$ MSCs/kg were combined and resuspended in M199 + 1% HSA and centrifuged at $460 \times g$ for 10 minutes at 20°C. Cell pellets were resuspended in fresh M199 + 1% HSA media and centrifuged at $460 \times g$ for 10 minutes at 20°C for three additional times. MSCs were then resuspended in M199 + 1% HSA at $1 \times 10^6$ cells/mL and transferred into 20-mL syringes. Total harvest time was 3 to 5 hours on the basis of each MSC yield per flask and the target dose. Freshly harvested autologous MSCs were infused into patients 1 or 24 hours after PBPC infusion (on day T−0 or T+1) through a side port of a running 0.9% saline IV infusion into a central catheter over 15 to 20 minutes. Patients were premedicated with acetaminophen 650 mg and diphenhydramine 50 mg. The protocol was amended in March 1998 to allow cryopreservation of the harvested MSCs and to increase the cell dose to $2 \times 10^6$ MSCs/kg. In eight patients, harvested MSCs were cryopreserved with a rate-controlled freezer in a final concentration of 10% dimethyl sulfoxide (Research Industries, Salt Lake City, UT) and 5% autologous plasma in Cryoocyte (Baxter, Deerfield, IL) freezing bags. Bags were overfilled with $\approx 10$% MSCs to account for cell loss during freeze and thaw. On the day of infusion cryopreserved units were thawed at the bedside in a 37°C water bath and transfused into 60-mL syringes within 10 minutes and infused into patients as described above. Vital and clinical signs and symptoms were monitored at the time of infusion and every 15 minutes thereafter for 3 hours, followed by every 2 hours for 6 hours and every 8 hours for 3 days.

Detection of MSCs in Blood

Approximately 10 to 15 mL of peripheral-blood samples were collected before MSC infusion, immediately at the end of infusion (0 minutes), and 15 minutes and 60 minutes after infusion. RBCs were lysed with ammonium chloride buffer, and cells were washed with DPBS and plated in 35-mm plates with a 1:1 mixture of fresh DMEM-LG and culture-conditioned and filtered DMEM-LG. Media was changed twice a week starting at 72 hours. Cultures were scored positive if a layer of adherent cells ($>90$% confluent) with MSC morphology and surface expression of SH3 were detected.

Hematopoietic CFU Assay

Bone marrow mononuclear cells were grown in methylcellulose (Stem Cell Technologies, Vancouver, BC) containing a final concentration of 100 U/mL IL-3 (Sandoz Research Institute, Nutley, NJ), 2 U/mL erythropoietin (Amgen), 100 U/mL granulocyte-macrophage colony-stimulating factor (Immunex, Seattle, WA) and 0.1 mmol/L hemin (Sigma), as described previously. Cells were plated at a density of $1 \times 10^5$/mL in duplicate and grown at 37°C 5% CO$_2$. Twelve to 14 days later, colonies of greater than 50 cells were enumerated.

RESULTS

Patient Characteristics

The median age of the 32 enrolled patients was 47 years (range, 37 to 57 years) and the majority had stage IV breast cancer (Table 1). Four patients were taken off study. Two experienced disease progression after PBPC mobilization therapy and did not proceed to high-dose chemotherapy. Two other patients had breast cancer cells in their marrow aspirates that persisted in MSC cultures. These patients underwent high-dose chemotherapy and PBPC infusion but were not infused with MSCs.

MSC Cultures

Human bone marrow–derived MSCs were successfully isolated from a mean of 23.4 ± 5.9 mL of bone marrow aspirate from all 32 breast cancer patients, and all were successfully culture-expanded (Table 2). A morphologically homogenous population of fibroblast-like MSCs was de-

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
</tr>
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<tbody>
<tr>
<td>No. of patients</td>
<td>32</td>
</tr>
<tr>
<td>Age, years</td>
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</tr>
<tr>
<td>Median</td>
<td>47</td>
</tr>
<tr>
<td>Range</td>
<td>37–57</td>
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<tr>
<td>Stage at presentation</td>
<td></td>
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<tr>
<td>III</td>
<td>10</td>
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<tr>
<td>IV</td>
<td>22</td>
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<td>Prior chemotherapy regimens</td>
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<tr>
<td>One</td>
<td>11</td>
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<td>Two</td>
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<td>Prior radiotherapy</td>
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<tr>
<td>BM involvement</td>
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<tr>
<td>Tumor in PBPCs</td>
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<td>Off study</td>
<td>4</td>
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<tr>
<td>Disease progression</td>
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</tr>
<tr>
<td>Tumor in MSC culture</td>
<td>2</td>
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</table>

Abbreviations: BM, Bone marrow.
ected in primary cultures (Fig 1), which reached greater than 90% confluence in a median of 13 days (day of first passage). During late passages, MSCs had a larger appearance but remained homogenous. There was a good correlation ($r = 0.753$) between the number of mononuclear cells plated to initiate MSC cultures and the number of MSCs recovered at the first passage (Fig 2). A mean of $1.4 \pm 0.7 \times 10^5$ MSCs were recovered at the first passage from $1 \times 10^6$ input bone marrow mononuclear cells. MSCs that were recovered from the first passage were replated at $1 \times 10^6$ MSCs per 175-cm$^2$ flask. These cultures reached greater than 90% confluence in 7 days, which required weekly passages. MSC growth after the first passage was exponential, and the number of MSCs increased more than 2 logs for cultures maintained for six weekly passages (Fig 3). Mean MSC yield per 175-cm$^2$ flask was $4.5 \times 10^6$ MSCs (range, 1.9 to $8.8 \times 10^6$ MSCs) at the first passage and $3.4 \times 10^6$ MSCs (range, 2.0 to $5.0 \times 10^6$ MSCs) at the third passage. Due to culture laboratory space limitations, only that portion of MSCs that were predicted to yield two to three times the target MSC dose was maintained in culture. Potential total MSC yield was calculated on the basis of the actual expansion of MSCs in the cultured portion of MSCs from each passage and extrapolated to the total number of MSCs obtained from that passage (Table 3). Potential total MSC yield per kilogram of patient weight increased from a mean of $5.6 \times 10^6$ MSCs/kg after two passages to a mean of $36.3 \times 10^6$ MSCs/kg after six passages. Although the majority of patients' cultures contained at least the target number ($1 \times 10^6$/kg) of MSCs by day 21, median culture duration was 37 days (range, 20 to 50 days) due to obligatory delay between mobilization of PBPCs and institution of the high-dose chemotherapy. This duration was reduced to a median of 28.5 days by implementation of MSC cryopreservation.

### Table 2. MSC Culture Data

<table>
<thead>
<tr>
<th></th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting marrow, average ± SD (ml)</td>
<td>23.4 ± 5.9</td>
</tr>
<tr>
<td>Days to first passage</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>13</td>
</tr>
<tr>
<td>Range</td>
<td>12-16</td>
</tr>
<tr>
<td>Days in culture</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>37</td>
</tr>
<tr>
<td>Range</td>
<td>20-50</td>
</tr>
<tr>
<td>Number of MSCs infused</td>
<td></td>
</tr>
<tr>
<td>$1 \times 10^6$/kg</td>
<td>20</td>
</tr>
<tr>
<td>$1.2 \times 10^6$/kg</td>
<td>6</td>
</tr>
<tr>
<td>$&gt; 2 \times 10^6$/kg</td>
<td>2</td>
</tr>
<tr>
<td>Infused MSCs</td>
<td></td>
</tr>
<tr>
<td>Fresh harvest</td>
<td>20</td>
</tr>
<tr>
<td>Cryopreserved</td>
<td>8</td>
</tr>
<tr>
<td>Infusion time</td>
<td></td>
</tr>
<tr>
<td>T + 1</td>
<td>15</td>
</tr>
<tr>
<td>T-0, 1 hour after PBPCs</td>
<td>13</td>
</tr>
</tbody>
</table>

Abbreviation: T-0, day of PBPC infusion.

Fig 1. Phase contrast photomicrograph of cultured MSCs (magnification x100). (A) Single fusiform adherent cells early in the primary culture. (B) Late (day 13) in the primary culture, showing confluent patches of MSCs immediately before first passage. (C) Confluent layer of fifth-passage MSCs.
There was no significant correlation between either the number of MSCs obtained at the first passage or MSC expansion (as total estimated MSC yield divided by the number of passages) and the number of prior chemotherapy regimens (one prior chemotherapy regimen v two or more, $P = .2$ and .5, respectively) or prior radiotherapy treatments (one prior treatment v two or more, $P = .7$ and .6, respectively). In addition, we found no correlation between the number of CD34$^+$ cells collected per leukopheresis and the number of MSCs harvested at first passage ($r = 0.18$).

There was a weak correlation between the number of CD34$^+$ cells collected per leukopheresis and the MSC expansion (as total estimated MSC yield, divided by the number of passages) ($r = 0.379$) and the number of MSCs per 175-cm$^2$ flask at the first passage ($r = 0.282$).

There was no evidence of bacterial, fungal, or Mycoplasma contamination in any of the 3,029 flasks processed. Cell viability was determined by trypan blue staining at the end of the harvest and before infusion and was greater than 95% in every infusate at both time points. Cells were characterized by flow cytometry using human MSC-specific monoclonal antibodies that react with surface antigens of MSCs designated SH2, SH3, and SH4 before infusion. Every harvest revealed a homogenous population of cells with high side and forward scatter and high expression of SH antigens (> 95% of cells) by flow cytometry (Fig 4).

There was no detectable difference in the staining of MSCs with the SH2, SH3, and SH4 MSC-specific antibodies after two passages versus six passages. There was no significant contamination of the MSC harvests with hematopoietic cells (CD45$^+$ or CD14$^+$). Detached MSCs appeared as large round cells (two to three times larger then neutrophils on a cytospin preparation) with a large nucleus and a lacy cytoplasm (Fig 5).

In the last eight patients on the trial, MSCs were cryopreserved a median of 28.5 days (range, 20 to 30 days) after the start of culture. In these samples, viability after

<table>
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<th>Table 3. Human MSC Expansion Potential</th>
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<td><strong>Passages</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
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</tbody>
</table>

*Indicates total number of weekly passages.
†n = number of individual patients’ MSC cultures.
‡Only a portion of cells were replated, and the total MSC yield at the next passage was calculated based on the expansion of the portion cultured. Exponential growth rates were calculated based on the growth curves shown in Fig 3 ($e^{\cdot\cdot\cdot}$: $r$, growth rate; $t$, time).
Thawing determined by trypan blue staining was 84% ± 6%. Thawed MSCs were infused into patients within 10 minutes. A small aliquot of these cells were returned to in vitro cultures to analyze their proliferation. Approximately $1 \times 10^6$ thawed MSCs from all eight cryopreserved units reached greater than 90% confluence in a 175-cm$^2$ flask within 7 days. This is the same duration required by $1 \times 10^6$ fresh MSCs to reach same degree of confluence.

Breast Cancer Contamination

At enrollment, four patients had breast cancer contamination in their marrow aspirate as determined by immunocytochemical analysis after staining with a cocktail of breast cancer-specific antibodies\(^8\) (Table 4). In two of these patients, cultured MSCs contained no detectable breast cancer cells and were infused into patients as described below. In the other two patients, breast cancer cells were detectable at first passage of MSCs in numbers 1 to 1.5 log lower than those found in the starting bone marrow aspirate. Nevertheless, these MSCs were not infused into these two patients as mandated by the clinical protocol.

PBPC and MSC Infusion

Patients received PBPC infusion containing a median of $13.9 \times 10^6$ CD34$^+$ cells/kg (range, 1.5 to $39 \times 10^6$ CD34$^+$ cells/kg). The 28 assessable patients also received 1 to
2.2 × 10^6 autologous MSCs/kg 1 or 24 hours after PBPC infusion. The MSC cell dose chosen was empiric and was influenced by safety consideration at the beginning of the study. On determination of safety, the dose was increased from 1 × 10^6 to 2 × 10^6 MSCs/kg. Patients whose MSCs were cryopreserved received 10% additional cells to compensate for potential cell loss during the cryopreservation procedure. The total number of MSCs infused was 51 to 174 × 10^6. Given that each confluent flask had 2.5 to 5 × 10^6 MSCs, it required harvesting a minimum of 10 flasks for all patients. In 15 patients, MSCs were given on the next day after PBPCs, and in the remaining 13 patients, MSC infusion occurred 1 hour after PBPCs (Table 2). In 20 patients, MSCs were harvested fresh on the day of infusion and were administered within 1 hour. In the remaining eight patients, a target dose of MSCs were harvested and cryopreserved, and when thawed, the cells were infused immediately. There was no immediate or delayed toxicity related to IV MSC infusion. None of the patients experienced allergic reactions or respiratory symptoms.

Detection of Clonogenic MSCs in Blood

MSCs were not detected in the blood at baseline in any patient. Clonogenic MSCs were detected in venous blood up to 1 hour after infusion of autologous MSCs in 13 (62%) of 21 patients analyzed. MSCs that were recovered from the venous blood had morphology and surface marker expression (SH2, SH3, and SH4) that was identical to those recovered from bone marrow (Fig 6). Circulating MSCs were detected at the end of infusion in eight (38%) of 21 patients, 15 minutes later in 10 patients (48%), and 1 hour later in three patients (14%) (Fig 7). When seven patients who received 1.2 to 2.2 × 10^6 cryopreserved MSCs were analyzed separately, circulating MSC detection rates were 0% (0 of seven patients) before infusion, 85% (six patients) at the end of infusion, 43% (three patients) at 15 minutes, and 14% (one patient) at 1 hour.

Hematopoietic Engraftment and Clinical Outcome

Hematopoietic engraftment was prompt in all patients, with median neutrophil recovery (> 500/μL) in 8 days (range, 6 to 11 days) and platelet count recovery greater than 20,000/μL and greater than 50,000/μL unsupported in 8.5 days (range, 4 to 19 days) and 13.5 days (range, 7 to 44 days), respectively. Bone marrow CFU concentrations recovered to 70% of baseline by day 42 (Fig 8). All patients were discharged from the hospital. There was only one patient who died within the first 100 days of transplantation from unknown cause. This patient was evaluated on day T+21 and was free of symptoms and clinical findings. Median follow-up of the remaining patients is 9 months (range, 4 to 22 months). Three patients died as a result of disease progression. Of the 24 remaining patients, 11 are without evidence of disease, three have stable disease, and 10 have relapsed.

DISCUSSION

This report is the first describing that autologous MSCs can be successfully isolated, ex vivo culture-expanded, and infused IV without toxicity into advanced breast cancer patients at the time of PBPC transplantation. We have optimized MSC culture expansion methods to generate large numbers of autologous MSCs in a relatively short period of time for clinical use with a therapeutic intent. The culture technique was simple, yielding greater than 1 × 10^6 MSCs/kg patient weight in 3 to 4 weeks and could be efficiently carried out in a single institution without microbiologic contamination. Optimized culture conditions did not promote growth or survival of detectable contaminating...
breast cancer cells. In contrast to our earlier report, we infused autologous MSCs after myeloablative chemotherapy to promote survival and engraftment with PBPCs. Despite the MSCs large size and ex vivo culture with fetal calf serum–containing medium, there were no infusion-related immediate or delayed toxicities associated with administration of up to $2.2 \times 10^6$ MSCs/kg. Furthermore, hematopoietic reconstitution was rapid, particularly in platelet counts, in the majority of patients. Our results indicate that this form of novel cellular therapy is feasible and may have a number of beneficial clinical effects in the setting of hematopoietic stem-cell transplantation and should be studied in randomized trials.

MSCs seem to constitute an essential part of the marrow microenvironment and support hematopoiesis. A number of investigators have demonstrated that the bone marrow microenvironment is damaged because of the effects of alkylating agents and radiation, which diminishes its hematopoietic support function. We propose that culture-expanded MSCs can be used to improve the rate and quality of hematopoietic engraftment by regenerating the marrow microenvironment, particularly in patients who previously received stroma-damaging therapy.

Breast cancer patients treated with high-dose chemotherapy generally experience complete and rapid neutrophil and platelet engraftment when supported with mobilized PBPCs.
MESENCHYMAL STEM-CELL TRANSPLANTATION

Although culture-expanded MSCs can be safely infused into patients after high-dose chemotherapy, their distribution, survival, and participation in tissue function is largely unknown. Recipients of unmanipulated allogeneic bone marrow transplants were shown to regenerate their marrow stroma from autologous cells.26 These results were interpreted by the relative resistance of the stromal elements to myeloablative therapy, which allows regeneration of autologous stroma. In addition, the number of stromal precursors in the bone marrow graft is likely to be small, and the homing efficiency of these cells is unknown. Murine stromal cells were infused into mice by a number of investigators27-29 after radiation therapy and were found to facilitate hematopoietic recovery. Stromal cells of the COL1A1 transgenic mice were found 30 to 150 days later in marrow, spleen, bone, lung, and cartilage of syngeneic mice and constituted 1.5% to 12% of the cells.30 Similarly, genetically marked canine MSCs were infused into autologous as well as dog leukocyte antigen–identical litter-mate dogs after 9.2 Gy of total-body irradiation along with unmodified bone marrow or PBPCs.31,32 Green fluorescence protein gene–marked canine MSCs were found predominantly in the marrow of sternum, rib, and limbs at 6 and 14 weeks postinfusion. More recently, xenotransplantation models are being developed to determine the transplantability and homing of human MSCs in animals. Preliminary results show survival of human MSCs in immunocompromised mice33 and preimmune fetal sheep34 weeks after transplantation. A multicenter study investigating the safety of allogeneic culture-expanded MSC infusion in humans is currently underway, and genotypic differences between the donor and the recipient should allow us to determine the distribution of these cells in vivo. In addition, studies with marker- or therapeutic-gene transduced MSCs are being developed in the autologous setting to investigate distribution and homing of MSCs, as well as to use them as cellular vehicles for delivery of exogenous gene products.28

In summary, autologous MSCs can be isolated, rapidly expanded to large numbers, and infused into patients undergoing high-dose chemotherapy and autologous PBPC transplantation. Therapeutic potential of MSCs should be further investigated in the clinical setting.

ACKNOWLEDGMENT

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REFERENCES


