Paclitaxel Chemotherapy after Autologous Stem-Cell Transplantation and Engraftment of Hematopoietic Cells Transduced with a Retrovirus Containing the Multidrug Resistance Complementary DNA (MDR1) in Metastatic Breast Cancer Patients

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ABSTRACT

The MDR1 multidrug resistance gene confers resistance to natural-product anticancer drugs including paclitaxel. We conducted a clinical gene therapy study to determine whether retroviral-mediated transfer of MDR1 in human hematopoietic cells would result in stable engraftment, and possibly expansion, of cells containing this gene after treatment with myelosuppressive doses of paclitaxel. Patients with metastatic breast cancer who achieved a complete or partial remission after standard chemotherapy were eligible for the study. Hematopoietic stem cells (HSCs) were collected by both peripheral blood apheresis and bone marrow harvest after mobilization with a single dose of granulocyte colony-stimulating factor (10 μg/kg/day). After enrichment for CD34+ cells, one-third of each collection was incubated ex vivo for 72 h with a replication-incompetent retrovirus containing the MDR1 gene (G1MD) in the presence of stem-cell factor, interleukin 3, and interleukin 6. The remaining CD34+ cells were stored without further manipulation. All of the CD34+ cells were reinfused for hematopoietic rescue after conditioning chemotherapy with ifosfamide, carboplatin, and etoposide regimen. After hematopoietic recovery, patients received six cycles of paclitaxel (175 mg/m2 every 3 weeks). Bone marrow and serial peripheral blood samples were obtained and tested for the presence of the MDR1 transgene using a PCR assay. Six patients were enrolled in the study and four patients received infusion of genetically altered cells. The ex vivo transduction efficiency, estimated by the PCR assay, ranged from 0.1 to 0.5%. Three of the four patients demonstrated engraftment of cells containing the MDR1 transgene. The estimated percentage of granulocytes containing the MDR1 transgene ranged from a maximum of 9% of circulating nucleated cells down to the limit of detection of 0.01%. One patient remained positive for the MDR1 transgene throughout all six cycles of paclitaxel therapy, whereas the other 2 patients showed a decrease in the number of cells containing the transgene to undetectable levels. Despite the low level of engraftment of MDR1-marked cells, a correlation was observed between the relative number of granulocytes containing the MDR1 transgene and the granulocyte nadir after paclitaxel therapy. No adverse reactions to the genetic manipulation procedures were detected. Therefore, engraftment of human HSCs transduced with the MDR1 gene can be achieved. However, the overall transduction efficiency and stable engraftment of gene-modified HSCs must be improved before MDR1 gene therapy and in vivo selection with anticancer drugs can be reliably used to protect cancer patients from drug-related myelosuppression.

INTRODUCTION

The demonstration of stable transfer of therapeutic genes into human cells capable of reconstituting the entire hematopoietic system is an important step in the evolution of gene manipulation from a laboratory technique to a clinical therapy. Although rodent studies have demonstrated that retroviral vectors can transfer genes into HSCs2 stably and efficiently, gene transfer into human HSCs in patients with cancer or leukemia has thus far had limited success. Clinical trials of high-dose...

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2 The abbreviations used are: HSC, hematopoietic stem cell; ICE, ifosfamide, carboplatin, and etoposide regimen; AGC, absolute granulocyte count; TM, transduction medium; IL, interleukin; SCF, stem-cell factor; nt, nucleotide; DHFR, dihydrofolate reductase.
chemotherapy with HSC support have demonstrated that human HSCs can be transduced ex vivo with retroviral vectors, and that these transduced cells can be detected in peripheral blood and bone marrow for prolonged periods of time after reinfusion (1–4). However, the level of gene marking in patients has been too low (<1%) for most therapeutic purposes.

Previous studies in patients with cancer and leukemia have used retroviral vectors containing the bacterial neomycin phosphotransferase (neo) gene, which was used as a marker of successful transduction and was not otherwise intended to have in vivo biological effects. However, the successful transfer of a gene that could impart a survival advantage to transduced cells and that could, therefore, allow selection and expansion after engraftment has the potential to overcome the low transduction efficiency evident in neo gene marking studies.

Breast cancer therapy offers an ideal context in which to determine the utility of such a gene therapy strategy. High-dose chemotherapy followed by autologous bone marrow and/or peripheral blood progenitor transplantation has been increasingly used in the treatment of various malignancies including breast cancer. Hematopoietic toxicity of high-dose chemotherapy is diminished with the use of HSC support, and clinical studies have demonstrated that the treatment of breast cancer patients with high-dose chemotherapy with HSC support is associated with high clinical response rates (5). In addition, pilot studies have suggested that high-risk stage II breast cancer patients treated with high-dose chemotherapy and HSC support may have increased disease-free survival and overall survival relative to historical controls (6).

Although high-dose chemotherapy regimens used for consolidation therapy are associated with high clinical response rates in metastatic breast cancer, the majority of patients ultimately develop progressive disease (5). The potential of gene therapy to impart the chemotherapy resistance of HSCs would allow the use of high-dose therapy earlier in the overall course of therapy and the safe escalation of antitumor cancer chemotherapy after autologous transplantation. Therefore, a breast cancer treatment regimen that uses high-dose chemotherapy and HSC cell support, followed by further administration of active antitumor cancer drugs, is a rational setting for the study of the transfer of genes that confer resistance to breast cancer drugs into human HSCs. In this setting, the in vivo effect of antitumor cancer therapy on drug-resistance genes transferred into HSCs can be determined in a therapeutic milieu.

Patients. Patients with histologically confirmed metastatic breast cancer who achieved a complete response or a partial response after three to five cycles of standard induction chemotherapy were eligible for this study. Patients with evidence of metastatic disease in bone (by bone scan or X-ray) at the time of diagnosis or in the central nervous system (by high-dose computed tomography scan of the head) or bone marrow (by histological and cytological evaluation of bone marrow biopsy and/or aspirate) at the time of study entry were excluded from the study. Eligibility criteria included normal renal, cardiac, hepatic, and pulmonary function.

The protocol was approved by the National Cancer Institute Institutional Review Board, the NIH Recombinant DNA Committee, and the United States Food and Drug Administration. All of the patients signed separate informed consents for the chemotherapy to treat their breast cancer and for procedures and risks related to the gene therapy. The schema is shown in Fig. 1. After recovery from induction chemotherapy, patients were treated with cyclophosphamide (4 g/m²) over 4 h × 1 dose. Mesna (800 mg/m²) was given i.v. admixed with cyclophosphamide and then every 3 h over 30 min starting 3 h after the completion of cyclophosphamide, for a total of 12 doses. Normal saline hydration at 3000 ml/m²/day was started 4 h before, and continued for 24 h after, treatment with cyclophosphamide. Patients received filgrastim 10 µg/kg/day on days 2–15 and underwent apheresis when their WBC increased to greater than 5000 cells/µl after reaching the nadir level. Peripheral blood cells were collected using a Fenwal CS3000 (Baxter Corp, Deerfield, IL) or a Cobe Spectra (COBE, Lakewood, CO). Patients underwent a bone marrow harvest at least 4 weeks after treatment with cyclophosphamide.

After bone marrow harvests, patients were treated with ICE chemotherapy consisting of: (a) ifosfamide (3500 mg/m²) i.v. over 2 h daily × 4 days on days 1, 2, 3, and 4; (b) mesna (700 mg/m²) admixed with ifosfamide, then given as a 3-h infusion starting at the completion of the i.v. ifosfamide in (a) and every 3 h thereafter for six doses; (c) carboplatin (600 mg/m²) over 24 h daily × 3 days on days 1, 2, and 3; and (d) etoposide (250 mg/m²) over 2 h every 12 h on days 1, 2, and 3 for a total of six doses (17). Unmanipulated and G1MD-transduced hematopoietic cells from both the peripheral apheresis collection and the bone marrow harvest were thawed and reinfused on day 7. Patients received filgrastim (10 µg/kg/day) starting on day 7 and continuing until achieving an AGC greater than 2000 cells/µl. Patients were treated with six cycles of paclitaxel (175 mg/m²)
Salt Lake City, UT), 10% DMSO (Tera Pharmaceuticals, medium containing 50% human AB serum, 40% Plasmalyte-A and 15 units/ml heparin (Fujisawa, Deerfield, IL). The TM consisted of three parts DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% fetal bovine serum (Biowhittaker, Walkersville, MD) and 1 part PBS harvest CD34 selection

PBPC harvest CD34 selection 0

2/3 non-transduced 1/3 MDR1-transduced (up to 1 x 10^6 CD34+ cells/kg)

bone marrow harvest CD34 selection

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![Diagram](image_url)

**Fig. 1 A.** The schema of the clinical study, showing induction, mobilization, transplantation with stem-cell rescue, and subsequent paclitaxel chemotherapy. **B.** A diagram of the PCR assay showing the position of the primers used to detect the MDR1 transgene and the relative positions of their annealing sequences on the human genome. The numbering system shows that of the G1MD vector. The MDR1 cDNA starts at nt 1479 and ends at nt 5339. The MDR1 ATG start codon is at position 1491.

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over 3 h every 3 weeks) after hematopoietic recovery. No filgrastim was given after paclitaxel.

**G1MD Retroviral Transduction of CD34+ Cells.** For both the peripheral blood aphereses and the bone marrow harvests, the collected mononuclear cells were CD34+ enriched using a Ceprate stem-cell concentrator column (Cellpro, Bothell, WA) according to the manufacturer’s instructions (18). An aliquot (5 x 10^6) of CD34 selected cells was examined for breast cancer cell contamination by immunohistochemistry using a panel of antikeratin antibodies. All of the CD34+ samples were negative for tumor cell contamination with the limit of detection being 1 tumor cell in 10^5 CD34+ cells.

Two-thirds of the CD34+ cells were placed in freezing medium containing 50% human AB serum, 40% Plasmalyte-A (Baxter, Deerfield, MI), 10% DMSO (Tera Pharmaceuticals, Salt Lake City, UT), 10 μg/ml Darnase (Genentech, Thousand Oaks, CA), and 15 units/ml heparin (Fujisawa, Deerfield, IL). The cells were cryopreserved in a controlled-rate freeze and stored in liquid nitrogen.

One-third of the CD34+ cells were centrifuged at 290 x g for 10 min and resuspended in TM at a concentration of 1–1.5 x 10^6 cells/ml. The TM consisted of three parts DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% fetal bovine serum (Biowhittaker, Walkersville, MD) and 1 part supernatant from the amphotropic MDR1 retroviral producer cell line G1MD clone 5 (Genetic Therapy Inc., Gaithersburg, MD). TM was also supplemented with 4 μg/ml proteamine sulfate (Fujisawa, Deerfield, MI), 20 ng/ml IL-3 (Novartis, NJ), 50 ng/ml IL-6 (Novartis), and 100 ng/ml SCF (Amgen, Thousand Oaks, CA). Cells were cultured in 650-cm^2 flasks (Costar, Cambridge, MA) for 18–24 h at 37°C in 5% CO. At 24 h and 48 h, the cells were collected by centrifugation at 745 x g for 10 min, resuspended in fresh TM (containing fresh G1MD supernatant), and incubated at 37°C in 5% CO. Cells were harvested at 72 h by centrifugation at 745 x g for 10 min. Aliquots were taken for PCR analysis of G1MD vector DNA and for testing for bacterial and fungal contamination. The remaining cells were resuspended in freezing medium and stored in liquid nitrogen.

**Detection of Transgenes in Hematopoietic Cells.** PCR analyses were performed to detect G1MD vector DNA in the following samples: (a) in CD34+ cells after the 72-h transduction; (b) in bone marrow cells obtained before the first paclitaxel cycle and after the last paclitaxel cycle; and (c) in peripheral blood samples before each of six cycles of paclitaxel therapy and after the last cycle of paclitaxel.

Cell pellets from bone marrow aspirates were prepared by incubation of the cells in a hypotonic RBC lysis solution (Gentra Systems, Inc. Research Triangle Park, NC) for 10 min at room temperature, followed by centrifugation for 10 min at 2000 x g. Mononuclear and polymorphonuclear cell fractions were isolated from each of the peripheral blood samples using double-gradient centrifugation. Whole blood was layered onto Histopaque-1077 (Sigma, St. Louis, MO) medium which had been carefully layered over an equal volume of Histopaque-1119. After centrifugation at 700 x g for 30 min, the two cell layers at each interphase were carefully aspirated. Mononuclear cells and platelets were found at the plasma/Histopaque1077 interface; granulocytes were found at the 1077/1119 interface. The two cell samples were collected by centrifugation for 10 min at 700 x g and resuspended in HEPES-buffered saline (Biofluids, Rockville, MD). Aliquots of cell preparations were counted with a hemocytometer and stained with Hema 3 stain (Biochemical Sciences, Inc.) to document the purity of the collection. The remaining cells were concentrated by centrifugation and resuspended in a cell lysis solution (Genta Systems, Inc.). All of the samples were stored at ~80°C until DNA extraction.

DNA was isolated from the granulocytes, monocytes, and bone marrow cell pellets using the Puregene DNA isolation kit (Genta Systems, Inc.). The frozen samples were thawed and incubated with RNase A for 15 min at 37°C followed by the addition of protein precipitation solution according to the manufacturer’s directions. After centrifugation at 15,000 x g for 3 min, an equal volume of isopropyl alcohol was added to the supernatant. The sample was again centrifuged at 15,000 x g for 1 min; the DNA pellet was washed once with 70% alcohol and resuspended in DNA hydration solution, and the concentration was determined by UV spectrophotometry.

Care was taken to avoid potential contamination of patient specimens with PCR products. DNA extraction and PCR assays were performed in separate rooms. Vector DNA was detected with a PCR assay using MDR1 primers (5'-TGA AAC AAA ACG ACA GAA TAG TAA C-3' and 5'-AAT ACT AAC AGA ACA TCC TCA AAG C-3'). These primers span nt 3726–4424 on the G1MD (MDR1) cDNA sequences begin at position 1479 in the G1MD vector, and the ATG start site is position 1491; Ref. 15). The size of the amplified product is 699 bp (see Fig.
Engraftment with MDRI-transduced Hematopoietic Cells

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Date of metastatic disease</th>
<th>Prior adjuvant chemotherapy</th>
<th>Induction chemotherapy</th>
<th>Disease sites</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>12/94</td>
<td>None</td>
<td>FLAC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Breast, axillary, and SCV node</td>
<td>Alive, NED 5/98</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>7/95</td>
<td>CEF</td>
<td>CT</td>
<td>Chest wall</td>
<td>Alive, NED 4/98</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>2/96</td>
<td>None</td>
<td>A</td>
<td>Breast, axillary and SCV nodes</td>
<td>Progression 3/97; DOD 6/9/97</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>8/96</td>
<td>None</td>
<td>CA, cytoxan and Adriamycin, and fluorouracil</td>
<td>Breast, axillary and SCV nodes</td>
<td>Progression 10/9/97; DOD 1/3/98</td>
</tr>
</tbody>
</table>

<sup>a</sup> FLAC, fluorouracil, leucovorin, Adriamycin, and cytoxan; CEF, cytoxan, epirubicin, and fluorouracil; A, Adriamycin; CT, cytoxan and paclitaxel; CA, cytoxan and Adriamycin; SCV node, supraclavicular node; DOD, died from disease; NED, no evidence of disease.

The characteristics of the four patients who received G1MD-transduced CD34+ cells are shown in Table 1. Two additional patients were entered on the trial but were removed from the study before receiving any G1MD-transduced cells. One patient did not mobilize sufficient numbers of CD34+ cells, and the other patient developed progressive disease after stem-cell mobilization and, therefore, was not eligible to receive high-dose chemotherapy with stem-cell support. Four patients were treated with ICE chemotherapy and received the G1MD-transduced CD34+ cells as well as the stored, nonmanipulated cells. All of these four patients recovered from the autologous transplantation and were subsequently treated with six cycles of paclitaxel after hematopoietic reconstitution.

RESULTS

The characteristics of the four patients who received G1MD-transduced CD34+ cells were as follows: Two patients (patients 1 and 2) underwent both bone marrow harvest and peripheral stem-cell apheresis, while one patient (patient 3) developed progressive disease after transplantation and was subsequently treated with six cycles of paclitaxel after hematopoietic reconstitution.

The total number of CD34+ cells isolated from peripheral blood and bone marrow harvests and the estimated G1MD transduction efficiency of each patient sample is shown in Table 2. Patients 3 and 4 had only transduction of peripheral blood CD34+ cells because of inadequate harvests from their bone marrow BM procedures and limited retroviral supernatant. For all of the patients, the mean total number of CD34+ cells reinfused was 3.25 x 10<sup>6</sup> kg (range, 2.32–4.90 x 10<sup>6</sup> kg). On
Nested G1MD PCR reaction are shown in the middle row. This is from the dilution series of the control cells. The results of the G1MD vector. This assay, although less sensitive than the shows the results obtained after the first PCR reaction for the top row for each patient sample received G1MD-transduced CD34+ cells for the presence of 1. The assay is capable of detecting up to a single copy of G1MD vector DNA in 30,000 cells (0.003%).

As shown in Fig. 3, one of the four patients (patient 2) had no detectable G1MD marking in granulocytes obtained from peripheral blood after hematopoietic reconstitution or at any time after treatment with six cycles of paclitaxel even using the nested PCR reaction. Each of the other three patients had evidence for G1MD marking in peripheral blood granulocytes at the time of hematopoietic reconstitution. Patient 1 demonstrated detectable G1MD marking in granulocytes until cycle 5 of paclitaxel, but the transgene became undetectable before the last cycle of paclitaxel. In this patient, the highest level of G1MD marking (~1%) was noted at the time of reconstitution, and it decreased gradually over the next 15 weeks of therapy. Patient 3 showed G1MD vector marking in granulocytes at the time of hematopoietic reconstitution and throughout all of the six cycles of paclitaxel. This patient exhibited the highest level of G1MD marking in granulocytes (~9%). G1MD vector DNA was present in granulocytes obtained from patient 4 after each of the first two cycles of paclitaxel. However, after cycle 3 of paclitaxel, G1MD marking in granulocytes fell to undetectable levels in this patient. The highest level of G1MD marking in patient 4 was only 0.01%.

None of the four patients exhibited any evidence of an increase in G1MD vector marking level in granulocytes after the treatment with paclitaxel. Even in patient 3, in whom the high level of G1MD marking was observed, the level of G1MD marking remained unchanged for approximately three cycles of paclitaxel and then decreased during the last two cycles of therapy.

PCR analysis for G1MD vector DNA was also performed on monocytes isolated before each cycle of paclitaxel and at the end of therapy (Fig. 4). The results obtained from analyses of peripheral blood monocytes is similar to that obtained from granulocytes (see Fig. 3). Thus, patient 1 lost G1MD marking in monocytes after cycle 3 (granulocyte marking was lost after cycle 5); patient 2 had undetectable G1MD marking at any time after hematopoietic reconstitution and paclitaxel therapy; patient 3 had G1MD marking in monocytes throughout all of the six cycles of paclitaxel; and patient 4 became negative for G1MD marking before cycle 4 of paclitaxel (same as granulocyte marking).

The relation of relative MDRI gene marking of granulocytes to AGC nadirs after each cycle of paclitaxel therapy is shown in Fig. 5. The mean AGC nadir during the nine cycles in which the MDRI transgene could not be detected was 354/μl. In

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**Table 2** Reinfused cells

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>PB CD34+ posttransduction (× 10^6/kg)</th>
<th>PB CD34+ transduction efficiency (G1MD+)</th>
<th>PB CD34+ nontransduced (× 10^6/kg)</th>
<th>BM CD34+ posttransduction (× 10^6/kg)</th>
<th>BM CD34+ transduction efficiency (G1MD+)</th>
<th>BM CD34+ nontransduced (× 10^6/kg)</th>
<th>Total no. cells reinfused (× 10^6/kg)</th>
<th>Estimated no. of G1MD-transduced cells reinfused (× 10^6/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.51</td>
<td>0.3</td>
<td>3.2</td>
<td>0.24</td>
<td>0.3</td>
<td>0.89</td>
<td>4.84</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>0.5</td>
<td>1.68</td>
<td>0.12</td>
<td>0.1</td>
<td>0.41</td>
<td>2.56</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>1.60</td>
<td>0.2</td>
<td>1.64</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.24</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
<td>0.2</td>
<td>1.78</td>
<td>ND</td>
<td>ND</td>
<td>0.8</td>
<td>2.34</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*PB, peripheral blood; BM, bone marrow; ND, not determined.*

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**Fig. 2** PCR analysis of *ex vivo* transduction of CD34 selected cells. CD34+ cells were incubated for 72 h with G1MD retroviral supernatant in medium containing SCF, IL-3, and IL-6 as described. PCR analysis for G1MD vector DNA was performed as described in “Patients and Methods.” For semiquantitative estimates of transduction efficiency, the MDR1 PCR signal in the G1MD vector was normalized to the level of actin DNA. On the left side of each panel, the dilution series, showing the relative signal strength of genomic DNA from a clonal cell line transduced with a single copy of the G1MD vector serially diluted in DNA extracted from CD34+ cells. PBPC, peripheral blood progenitor cell apheresis; BM, bone marrow harvest.
contrast, the mean AGC nadir during the six paclitaxel cycles associated with the highest levels of the MDR1 gene marking was 3.5-fold higher at 1243/μl. In the seven cycles in which there was an intermediate level (<0.01%) of G1MD marking in granulocytes, the mean AGC nadir was also intermediate at 552/μl. Using Spearman (nonparametric) correlation analysis, a moderate-to-strong correlation (r values ranging from 0.4 to 0.8) was found between G1MD levels and AGC nadir for each cycle of therapy. Although this analysis suggests a possible association between MDR1 marking in granulocytes and subsequent toxicity from paclitaxel treatment, the 22 evaluable chemotherapy cycles reflect the experience of only four patients. Furthermore, four of the six samples with the highest level of G1MD marking (>0.01) shown in Fig. 5 are from one patient (patient 3). Although patient 3 demonstrated high levels of G1MD gene marking in granulocytes (up to 9% G1MD gene copy number), this apparent association between paclitaxel-induced hematopoietic toxicity and G1MD marking in granulocytes must be regarded as tentative.

**DISCUSSION**

In this study, we have shown that patients with metastatic breast cancer can be reconstituted with MDR1-transduced cells after high-dose chemotherapy with stem-cell support. The transduction efficiency with G1MD vector after a 72-h incubation of CD34+ cells in the presence of SCF, IL-3, and IL-6 was 0.1–0.5%. This transduction efficiency is somewhat lower than our previous clinical trials results using neo retroviral vectors (LN6 and G1Na) and using the same transduction condition of 72-h incubation of CD34+ cells using the same cytokines (1). In that trial, neo gene transduction efficiency of CD34+ cells ranged from 4 to 77% (mean, 21%; Ref. 1). Although the transduction efficiency of the G1MD vector in the current study is lower, the titer of the clinical grade neo vectors (4.2 × 10^3 to 2.1 × 10^6 biologically active particles/ml) is somewhat higher than the titer of the clinical grade G1MD vector used in the current trial (3.1–6.4 × 10^5 biologically active particles/ml), and the total volume of retroviral supernatant was 4- to 6-fold lower in the current trial because of the limited availability of vector.

Despite the reinfusion of a limited number of G1MD-marked CD34+ cells, three of the four patients in this trial had evidence of G1MD marking in both granulocytes and monocytes after engraftment. Indeed, high levels (~9%) of G1MD marking in granulocytes of patient 3 at engraftment were observed after reinfusion of only 3.2 × 10^3 G1MD-marked cells/kg. Because the number of G1MD gene-marked CD34+ cells reinflused in the patient on this trial was < 0.1% of the total number of reinfused cells, the relatively high level of G1MD gene marking in patients suggests a potential benefit of G1MD transduction on progenitor cell repopulating ability. However, it should also be noted that one patient in this trial did not engraft with G1MD-transduced hematopoietic cells. Furthermore, in two previous studies of MDR1 gene transfer into hematopoietic cells, more than one-half of the patients failed to engraft with MDR1 gene-modified hematopoietic cells (21, 22).

Recently, Bunting et al. (23) compared the repopulating ability of murine bone marrow cells transduced with a Harvey-based retroviral vector containing either MDR1 (HaMDR1) or a mutant DHFR gene in the presence of SCF, IL-3, and IL-6. Competitive repopulation studies indicated that the murine bone marrow cells transduced with MDR1 and then expanded in culture for up to 12 additional days in the presence of cytokines were far more capable of both in vitro progenitor cell expansion and stable engraftment than either DHFR-transduced or mock-transduced cells (23).

The mechanism whereby MDR1 transduction may enhance hematopoietic engraftment is not clear. Recent studies have indicated that MDR1 overexpression can protect cells from undergoing apoptosis (24, 25). Thus, MDR1 overexpression may reduce the susceptibility of ex vivo expanded HSCs to undergo programmed cell death. Conversely, because MDR1 is a marker of early progenitor cells, its expression may play a role in decreasing the primitive cell’s propensity to differentiate into committed progenitor cells (23). Nevertheless, the relatively high level of G1MD marking observed in patients in the current clinical trial (up to 9% G1MD copy number in patient 3), despite the limited number of G1MD-transduced CD34+ cells reinflused, supports the hypothesis that MDR1 gene overexpression may enhance the repopulating ability of HSCs in patients.

Of some concern was the finding by Bunting et al. (23) that mice transplanted with ex vivo expanded, HaMDR1-transduced hematopoietic cells developed a myeloproliferative disorder characterized by high peripheral WBC counts and splenomegaly. However, these workers also noted that animals transplanted with MDR1-transduced cells that were reinflused immediately after transduction did not develop any evidence of a myeloproliferative syndrome. This latter observation is consistent with several previous studies (10, 13, 26) that demonstrated that animals could be safely transplanted with MDR1-transduced hematopoietic cells that were not expanded in culture after transduction. Furthermore, transgenic animals overexpressing MDR1 in bone marrow cells developed normally and without evidence of any hematopoietic disorder (7). Thus, overexpression of MDR1 in bone marrow cells by itself does not result in myeloproliferation. Conversely, continued passage of HaMDR1-transduced hematopoietic cells ex vivo in the presence of early acting cytokines is apparently a prerequisite for any subsequent risk of myeloproliferation in transplanted animals. It must also be noted that the animal studies by Bunting et al. (23) used a Harvey-based retroviral vector (HaMDR1) and not the Maloney-based G1MD vector used in the clinical trial described in this report. The possible contribution of vector-derived sequences to cellular proliferation will need to be addressed (23).

It is also important to note that none of the patients in the
clinical study described in this report developed elevated WBC counts or any evidence of a myeloproliferative disorder after transplantation with MDR1-transduced cells. This is consistent with other MDR1 gene transfer clinical trials, which indicated that patients can be engrafted safely with MDR1-marked hematopoietic cells (21, 22). Hesdorfer et al. (22) used a 72-h incubation of CD34+ cells with a Harvey-based MDR1 retroviral vector in the presence of cytokines (SCF, IL-3, and IL-6) in fibronectin-coated plates. These investigators found MDR1 retroviral vector marking in bone marrow samples from 2 of 7 patients at the time of hematopoietic reconstitution from high-dose chemotherapy. No data were presented from this trial on long-term MDR1 marking or the results after subsequent treatment of patients with MDR1 chemotherapy. Hanania et al. (21) reported results on 20 patients who received MDR1-transduced cells after high-dose chemotherapy. At hematopoietic recovery, these investigators found detectable MDR1-vector marking in bone marrow samples from 5 of 8 patients who received CD34+ cells transduced ex vivo using a 72-h transduction protocol that used autologous stroma and additional cytokines (IL-3 and IL-6). In contrast, they found no evidence of MDR1-vector marking in bone marrow samples from 5 of 8 patients who received CD34+ cells transduced using a 6-h coculture with retroviral supernatant in the absence of cytokines. In addition, Fahman et al. (27) examined the effects of paclitaxel chemotherapy in 10 patients immediately after hematopoietic reconstitution from high-dose chemotherapy and infusion of MDR1-vector-transduced CD34 cells. Although three of these patients had evidence of MDR1-vector marking in bone marrow cells at reconstitution and before paclitaxel therapy, none of their patients had evidence by PCR of MDR1 vector DNA in hematopoietic cells after paclitaxel therapy despite repeated testing over time. The authors concluded that only short term reconstituting cells had been modified by MDR1 vector in their study. It should also be emphasized that each of the clinical trials to date, including the one described in this report, used nonexpanded MDR1 transduced cells. No hematopoietic abnormalities have been reported thus far in patients transplanted with MDR1 transduced cells.

Previous murine studies have indicated that MDR1-transduced hematopoietic cells could be selectively expanded in animals treated with MDR1 chemotherapy (10, 13). In the current clinical trial, three of the four patients demonstrated G1MD vector sequences in peripheral blood for 10, 16, and 21 weeks after the infusion of cells. Although this is the first study to detect MDR1 vector-modified hematopoietic cells in patients after chemotherapy, we did not detect any expansion of G1MD-marked granulocytes in peripheral blood during paclitaxel therapy. There are several possible explanations for the progressive loss of hematopoietic cells containing the MDR1 transgene during successive chemotherapy cycles: (a) it is possible that the transduced MDR1 gene in these patients was not expressed at sufficiently high enough levels to protect progenitor cells from paclitaxel toxicity. Because of the low level of transduction, it was not possible to directly measure P-glycoprotein function in...
the MDR1-transduced cells; (b) it is conversely possible that the dose of paclitaxel used, 175–200 mg/m², was not sufficiently toxic to confer a clear survival advantage to the G1MD-transduced HSCs; and (c) because retroviral integration requires cell division, it is possible that the incubation of CD34⁺ cells with SCF, IL-3, and IL-6 resulted not only in increased CD34⁺ cell proliferation and retroviral transduction but also resulted in the differentiation of dividing, gene-transduced cells into cells with finite proliferation and self-renewal potential. These cells would produce MDR1 transgene-containing hematopoietic cells for a limited number of cell divisions until ultimately undergoing apoptosis.

Although the numbers of patients in this study is limited, we did observe an association between higher G1MD marking levels and higher AGC nadirs in patients during the posttransplant paclitaxel therapy (Fig. 5). These results suggest that MDR1 gene therapy may be able both to ameliorate hematopoietic toxicity and to enhance chemotherapy dose intensity. One would not necessarily expect that the relatively small numbers of cells that contain the MDR1 transgene would have a discernible clinical impact. Clearly, these observations must be regarded as tentative at this time. Because many factors contribute to chemotherapy-induced hematopoietic toxicity, additional clinical studies with more patients and improved MDR1 gene transduction of HSCs will be needed to address the potential clinical benefit of this approach.

In order for this strategy to be effective, efforts must be made to improve the efficiency of retroviral gene transfer into HSCs capable of engraftment and self-renewal in patients. In this trial, as well as in previous neo gene marking studies (1–3), the majority of the CD34⁺ cells that patients received were frozen immediately after harvest and were not incubated ex vivo in the retroviral TM. It is possible that the low level of gene marking achieved in these trials may be due to preferential engraftment of the nonmanipulated hematopoietic progenitor cells. Animal studies have indicated that nonmanipulated progenitor cells preferentially engraft in animals compared with hematopoietic cells transduced ex vivo (28). Future trials should explore whether MDR1 gene transfer into hematopoietic progenitor cells can be accomplished without the need for the infusion of nonmanipulated hematopoietic cells using nonmyeloablative conditioning regimens.

MDR1 is one of many genes that confer resistance to antineoplastic agents. Other groups have shown that hematopoietic cells transduced with other drug-resistance genes can protect from hematopoietic toxicity or can be selectively expanded after treatment with appropriate antineoplastic agents including genes encoding O⁶-methylguanyly methyltransferase, aldehyde dehydrogenase, and mutant DHFR (29–39). Although overexpression of MDR1 results in the development of resistance to many of the agents most commonly used to treat breast cancer...
including taxanes, anthracyclines, and *Vinca* alkaloids, the transfer of other drug-resistance genes alone or in combination may prove useful in protecting HSCs from cytotoxic effects of anticancer therapy.

Approaches to increase the efficiency of gene transfer into a self-renewing stem-cell population also need to be explored. For example, recent studies suggest that HSCs harvested using SCF and G-CSF resulted in increased marking with retroviral vectors *in vitro* (40). Other studies have indicated that the inclusion of other cytokines including FLT3 ligand (41, 42) or various extracellular matrix molecules (43) during the *ex vivo* transduction incubation with retroviral vector can increase the efficiency of gene transfer into CD34+ cells *in vitro*. In addition, retroviral vectors that have an envelope protein derived from the gibbon ape leukemia virus (GALV) may have a higher gene transfer efficiency in hematopoietic cells of baboons (44, 45). Clinical studies will be needed to assess the ability of SCF, FLT3 ligand, or other factors to increase the efficiency of retroviral gene transfer into HSCs capable of long-term engraftment in patients.

**REFERENCES**


Paclitaxel Chemotherapy after Autologous Stem-Cell Transplantation and Engraftment of Hematopoietic Cells Transduced with a Retrovirus Containing the Multidrug Resistance Complementary DNA (MDR1) in Metastatic Breast Cancer Patients

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