RAPID COMMUNICATION

Retrovirally Marked CD34-Enriched Peripheral Blood and Bone Marrow Cells Contribute to Long-Term Engraftment After Autologous Transplantation

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We report here on a preliminary human autologous transplantation study of retroviral gene transfer to bone marrow (BM) and peripheral blood (PB)-derived CD34-enriched cells. Eleven patients with multiple myeloma or breast cancer had cyclophosphamide and filgrastim-mobilized PB cells CD34-enriched and transduced with a retroviral marking vector containing the neomycin resistance gene, and CD34-enriched BM cells transduced with a second marking vector also containing a neomycin resistance gene. After high-dose conditioning therapy, both transduced cell populations were reinfused and patients were followed over time for the presence of the marker gene and any adverse effects related to the gene-transfer procedure. All 10 evaluable patients had the marker gene detected at the time of engraftment, and 3 of 9 patients had persistence of the marker gene for greater than 18 months posttransplantation. The marker gene was detected in multiple lineages, including granulocytes, T cells, and B cells. The source of the marking was both the transduced PB graft and the BM graft, with a suggestion of better long-term marking originating from the PB graft. The steady-state levels of marking were low, with only 1:100 to 1:10,000 cells positive. There was no toxicity noted, and patients did not develop detectable replication-competent helper virus at any time posttransplantation. These results suggest that mobilized PB cells may be preferable to BM for gene therapy applications and that progeny of mobilized peripheral blood cells can contribute long-term to engraftment of multiple lineages. This is a US government work. There are no restrictions on its use.

Over the past decade, much interest has focused on the use of new gene-transfer technologies to correct a wide variety of somatic cell defects. The hematopoietic stem cell has been an obvious target because of its ability to permanently reconstitute the hematopoietic and immune systems after transplantation. Many different congenital and acquired disease states could theoretically be treated by introducing a new gene into stem cells. The best characterized and most highly developed technique being applied toward this goal involves retroviral gene transfer. In rodent models, investigators have shown efficient and reproducible gene transfer to a high percentage of long-term repopulating stem cells and achieved long-term expression of introduced genes in appropriate lineages. In large animal models, retroviral gene transfer has been much less efficient, although long-term expression of transferred genes has been shown. Efficient gene transfer to primitive human progenitor cells such as granulocyte-erythroid-monocyte-megakaryocyte colony-forming unit or long-term culture initiating cells has been reported, with gene transfer efficiencies greatly increased by exposing target cells to hematopoietic growth factors during transduction with viral vectors. Over the past 3 years, investigators have begun to apply retroviral gene transfer technology in preliminary human clinical trials.

In patients undergoing autologous transplantation as high-dose consolidation for multiple myeloma or breast cancer, we used retroviral vectors carrying the bacterial neomycin phosphotransferase gene to mark a fraction of their mobilized peripheral blood (PB) and bone marrow (BM) grafts. Our protocol had four purposes. First, we wished to investigate the efficiency of retroviral gene transfer to CD34-enriched hematopoietic cells collected from adults, and transduced under culture conditions optimized on the basis of animal models and in vitro human preclinical assays. This critical information would be applied to designing future therapeutic trials. Second, by using two different marking vectors in each patient, we could directly compare the use of mobilized peripheral blood (PB) cells versus BM cells as targets for retroviral gene transfer. Third, we could determine the kinetics and other characteristics of reconstitution after autologous transplantation and the engraftment potential of mobilized PB cells as compared with BM cells. Finally, if marked tumor cells were detected posttransplantation, the contribution of BM and mobilized PB to progression could be assessed.

We report here the gene marking results in eleven patients followed for at least 1 year after autologous transplantation of retrovirally-transduced BM and PB CD34-enriched cells. Two distinct marking vectors carrying the neomycin resistance gene were used to distinguish the PB from the marrow
gene-marking of blood and marrow CD34+ cells

The majority of patients have shown marking short-term after engraftment, and three patients showed persistence of the marker gene for at least 18 months. The marker gene has been detected in multiple lineages, in cells derived from both the PB and the BM grafts.

**MATERIALS AND METHODS**

**Clinical procedures.** After informed consent, patients were enrolled in ongoing Institutional Review Board–approved autologous transplantation protocols for chemotherapy-responsive multiple myeloma or breast cancer at the Clinical Center of the National Institutes of Health (Bethesda, MD). As shown in Fig 1, patients received one dose of cyclophosphamide 4 g/m² intravenously followed by intravenous or subcutaneous filgrastim (Neupogen; Amgen, Thousand Oaks, CA) 10 µg/kg/d. Ten liters of whole blood was processed daily by apheresis using a Fenwal CS3000 blood cell separator (Deerfield, IL) or a Cobe Spectra device (Lakewood, CO), with the procedures initiated when the total leukocyte count exceeded 2,000 cells/µL. The first and third daily collections were cryopreserved without further processing; the second daily collection was used for genetic marking. After a 2- to 4-week rest period, the myeloma patients were treated with 5-fluorouracil (5FU), 15 mg/kg/d intravenously for 3 days. Ten days after the initiation of 5FU, at least 1 L of BM was procured from the posterior iliac crests by standard procedures. Breast cancer patients underwent marrow procurement without 5FU pretreatment. Albumin gradient separation was used to isolate a mononuclear cell fraction. Two thirds of the mononuclear cells were frozen without further processing, and one third of the cells were used for the genetic marking procedure. PB or BM mononuclear cells to be used for genetic marking were processed on the Ceptrate Stem Cell Concentrator (CellPro Inc, Bothell, WA) according to manufacturer’s instructions to obtain a CD34-enriched population of progenitor and stem cells. Myeloma patients received pretransplant conditioning therapy with melphalan 140 mg/m² and 1,200 rads of fractionated total body irradiation (TBI). Breast cancer patients received 16 g/m² ifosfamide over 4 days, 1,600 mg/m² carboplatin over 3 days, and 1,500 mg/m² etoposide over 3 days. Both transduced and nontransduced PB and BM cells were reinfused intravenously after thawing at 37°C.

**Viral vectors.** Two retroviral vectors, LNL6 and GINa.40, carrying an identical bacterial phosphotransferase gene conveying G418 neomycin resistance (neo) were used. Clinical grade supernatants procured from producer cell lines grown to confluence in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum were obtained from Genetic Therapy Inc (Gaithersburg, MD). All supernatants were shown to be free of replication-competent helper virus using the direct S + L-complementation focus assay, and some lots were also tested by coculture amplification on the mus dunni cell line. The vector titer of each aliquot of supernatant used ranged from 4.2 × 10⁸ to 2.1 × 10⁹ biologically-active particles/mL.

**Transduction with retroviral vectors.** CD34-enriched BM or PB cells were transduced in clinical grade supernatants at a density of 1 to 2 × 10⁶/mL for 72 hours at 37°C in 5% CO₂. Alternating patients had LNL6 versus GINa.40 used for the PB. If LNL6 was used for the PB, GINa.40 was used for the BM, and vice versa. Cultures were supplemented with 4 µg/mL protamine sulfate (Elkins-Sinn, Cherry Hill, NJ), 20 ng/mL interleukin-3 (IL-3; Sandoz, East Hanover, NJ), 100 ng/mL stem cell factor (Amgen) and 50 µg/mL gentamycin sulfate (GIBCO, Gaithersburg, MD). Cells from breast cancer but not myeloma patients were grown in 50 ng/mL IL-6 (Sandoz). Every 24 hours, cells were centrifuged and resuspended in fresh retroviral supernatant, protamine, and growth factors.
At the end of the culture period nonadherent cells were procured and cells adhering to the culture flask were dislodged by exposure to trypsin ( Gibco) for 5 minutes. All procured cells were counted, and aliquots were removed to assess viability, CD34 positivity, CFU-C number, G418 resistance, and transduction efficiency as assessed by polymerase chain reaction (PCR) for the neo gene. Cells were then cryopreserved.

Sample processing. Mononuclear or CD34-enriched and transduced cells from PB or BM were plated in methylcellulose as described, with or without 1.2 mg/mL active G418 ( Gibco/ BRL, Gaithersburg, MD). DNA for PCR analysis was prepared from cell pellets of pretransplantation and posttransplantation CD34-enriched cells by digestion in 5 mmol/L TRIS, pH 8.0, 0.45% Nonidet P-40, 0.45% Tween 20 and proteinase K. Posttransplantation PB (whole blood, mononuclear cells, and granulocytes) and BM samples were collected at 1- to 3-month intervals for the first year and every 6 months thereafter. PB was separated into mononuclear and granulocyte fractions via centrifugation over lymphocyte separation media (LSM, Organon Teknika, Durham, NC), and the granulocyte fraction was separated over LSM a second time, yielding granulocyte and mononuclear cell fractions that had a mean purity of 98.5% (range, 95% to 100%) as assessed by differential counts on 500 cells. Contaminating cells in the granulocyte fraction were monocytes. DNA was isolated using an ONCOR nonorganic DNA extraction kit (Gaithersburg, MD). PB and BM mononuclear cells were stained with anti-Leu-5b(CD2-fluorescein isothiocyanate [FITC]) and anti-Leu-12(CD19-phycocerythrin [PE]), BM cells with anti-HPCA-2(CD34-FITC) and anti-Leu-17(CD38-PE) (Becton Dickinson, San Jose, CA). Concurrent staining with appropriate isotype controls was done for every sample. Cells were sorted on a Coulter Epics Elite instrument (Coulter Electronics, Hialeah, FL) and sorted cell populations were pelleted and DNA extracted as described above. Reanalyzed via fluorescence-activated cell sorting (FACS) of sorted cell populations showed greater than 95% to 98% purity for sorted B- and T-cell populations, and 85% to 90% purity for CD34+ cells.  

PCR analysis. One microgram purified DNA from blood samples or 20 μL of directly digested cell pellet DNA was added to buffer, Taq polymerase, and deoxynucleotide triphosphates as specified in the Gene Amp PCR kit (Perkin-Elmer Cetus, Norwalk, CT), then divided equally between a PCR tube containing an outer pair of nested primers for the neo gene and one containing β-actin primers in the presence of 10 μCi/mL 32P deoxyctydine triphosphate (32pDCTP) (Amersham, Arlington Heights, IL). Outer neo primers were 5'-CAG ATT GTC TGT GTG GC and 5'-GGC CAG ACT GTT ACC ACT CC. β-actin primers were 5'-CAT TGT GAT GGA CTC CCG AGA CGC and 5'-CAT CTC CTC CTC GAA GTG TAG AGC. Amplification conditions were 95°C for 2 minutes, 95°C for 1 minute, 60°C for 1.5 minutes, and 72°C for 2 minutes, with 20 cycles for the outer neo amplification, and 23 cycles for the β-actin amplification, followed by a final 10-minute 72°C extension for both reactions. The outer neo PCR products were purified using a Promega Wizard PCR Prep kit (Madison, WI), then amplified in the presence of 10 μCi/mL 32pDCTP using the Gene Amp kit components as directed and a set of internal neo primers: 5'-CGG ATC GCT ACC AAG CAG TAC and 5'-GAG CGA ATG GCC TCT CCA CC. The inner neo PCR conditions were 95°C for 1 minute, 60°C for 1.5 minutes, and 72°C for 2 minutes for 20 cycles, followed by extension at 72°C for 10 minutes. The final PCR products were separated on 5% polyacrylamide gels. The expected band size was 683 bp for the neo product and 232 bp for the β-actin product. Negative controls in every reaction set included no DNA and DNA extracted from normal PB concurrently with the test samples. Positive controls for the β-actin were log dilutions of DNA from normal PB or BM and, for the neo, DNA from the G1Na.40 cell line containing a single-copy neo gene diluted into normal BM DNA. PCR products positive for the neo gene were run on a 5% denaturing gel (Long Ranger, Malvern, PA) to separate the 16-bp difference between LNL6 versus G1Na.40-derived PCR products.

Detection of helper virus. Posttransplantation PB mononuclear cell DNA was screened by PCR for recombinant helper virus genomes as described. Positive control log dilutions of DNA from the G1Na.40 packaging line into normal human BM DNA were run concurrently. The primers used to amplify a segment of the amphotropic envelope gene were 5'-CTG TCT CCC TGC GGA GTA CTT and 5'-GGA GTA GGG GTA ATG GCA GAG AGC. Conditions for amplification were 95°C for 2 minutes, then 25 cycles of 95°C for 1 minute, 60°C for 1 minute, 72°C for 1.5 minutes, followed by extension at 72°C × 10 minutes.

RESULTS

Protocol design and clinical outcomes. Eleven patients were entered onto the gene marking protocol over a 1-year period. Their characteristics are summarized in Table I. Figure 1 and Materials and Methods detail the protocol design. CD34-enriched PB and BM were used instead of unfractionated BM for several reasons. First, the volume of retroviral supernatant necessary to transduce unfractionated PB or BM from an adult, at the multiplicity of infection optimal for retroviral transfer (at least 10 particles/cell), would have been prohibitive (20 to 50 L per patient cell collection per exposure). Second, in the primate model, we have shown that CD34-enriched BM is equivalently susceptible to retroviral gene transfer as unfractionated BM. Third, neither breast cancer nor multiple myeloma cells express CD34 antigen, and, thus, CD34 selection serves as a purging modality for participating patients.

In all patients, one third of the mobilized PB collections were CD34-selected and transduced with either LNL6 or G1Na.40 marking vectors. CD34-enrichment of PB resulted in postimmunoabsorption fractions that averaged 53.2% (± 29.2%) CD34+ cells, with an average CD34+ yield of 69.1%. After transduction, the CD34+ percentage increased to 64.7% (± 26.5%) (P < .005), and the total number of CD34+ cells increased by 10%. In 10 of 11 patients, one third of the BM mononuclear cell fraction was CD34-enriched and transduced with the marking vector, which was not used on the patient’s respective PB cells. After immunoabsorption, the BM fractions were 65.4% (± 11.5) CD34+, with an average CD34+ yield of 32.6%. After transduction, the CD34+ percentage increased to 73.3% (± 5.2) (P < .05), and the total number of CD34+ cells increased by 20%. Table 1 gives the total number of CD34+ PB and BM cells transduced and infused for each patient. On average, 8.80 × 10^5 and 2.58 × 10^4 transduced CD34+ cells/kg from the PB and BM, respectively, were reinfused. These numbers represent approximately one sixth of the total CD34+ cells infused into each patient because of cell loss during mononuclear cell processing, CD34 immunoabsorption, and transduction. There was large interpatient variability in the number of CD34+ cells reinfused, most likely because of differences in prior chemotherapy and underlying marrow reserve. CD3 T cells were depleted 2.5 to 4 logs in both the BM and PB grafts by the CD34-enrichment procedure.

Patients engrafted on schedule compared with patients transplanted on the same or similar clinical protocols without
gene marking, with neutrophil counts reaching 500 on a mean of day 12, and platelets reaching 20,000 on day 13. There were no toxic events attributable to the marking procedure, such as acute reactions during infusions of transduced cells, unusual infections, or later development of second malignancies. One patient (BC#3) died before engraftment with venous occlusive disease of the liver and renal failure attributed to chemotherapy toxicity. Because of concern over adverse consequences of replication-competent helper virus generation, posttransplantation PB mononuclear cell DNA was analyzed by semiquantitative PCR for the frequency of the transferred neo gene; this assay produced similar estimates of transduction efficiency as compared with CFU-C neo percentages (data not shown).

In vitro assessment of transduction efficiency. Aliquots of cells were assayed for transduction efficiency at the end of the 72-hour transduction period by two different methods. Cells were plated in methylcellulose cultures with and without G418, and the percentages of CFU-C (colony-forming units) resistant to G418 (neo) were calculated. Table 1 gives the individual values for each transduction. Overall mean efficiency was 21.4%. Efficiencies of transduction were similar for target cells obtained from breast cancer versus myeloma patients (18.4% vs 23.7%), but somewhat lower for PB versus BM target cells (14.5% vs 29.2%). However, efficiencies were variable from patient to patient, perhaps because of large differences in the titers of various clinical supernatant lots and differences in the susceptibilities of individual patient’s target cells to transduction. There was no correlation of CFU-C transduction efficiency with the purity of the CD34-enriched population. Cell pellets from the end of transduction were also assayed by semiquantitative PCR for the frequency of the transferred neo gene; this assay produced similar estimates of transduction efficiency as compared with CFU-C neo percentages (data not shown).

Posttransplantation analysis for the marker gene. DNA samples prepared from BM, PB, granulocyte and mononuclear PB fractions, and in some patients, sorted T and B cells were analyzed by semiquantitative PCR for the neo gene at the time of engraftment (days 15 through 30), and then every 3 months posttransplantation for up to 24 months. Representative PCR analyses on patient MM#2 are shown in Fig 2. Each set of patient samples collected had a normal donor PB sample processed in parallel to control for any contamination that could have been introduced during processing. Thus, patient samples were only scored positive for the transferred gene if the control DNA samples were negative. Only one patient sample on one occasion had to be excluded from analysis for this reason. Patient MM#2 had positive PB, granulocyte, and BM signals on day 88 (Fig 2A) at a level of about 1:1,000 cells. On day 466 (Fig 2B), she had positive signals in all fractions, again at a level of about 1:1,000 cells, and on day 585 (Fig 2C) she had positive signals in PB, BM, mononuclear cells, as well in sorted T cells, but at somewhat lower levels (1:10,000).

Figure 3 summarizes the neo marking data over time in all patients. At the time of engraftment, the neo gene could be detected in PB and/or BM samples from 10/10 evaluable patients, at levels estimated between 1:5,000 and 1:100 cells
positive. Between 3 and 9 months posttransplantation, 5 of 10 patients had positive signals, and by greater than 9 months posttransplantation, 3 of 9 patients still had positive signals, 2 consistently and 1 intermittently. The loss of the marker gene over time in some patients suggests that only committed progenitors rather than true long-term engrafting cells were successfully transduced, or that engraftment of the manipulated cells was only transient.

In the three patients with positive neo signals greater than 1 year after transplantation (MM#2, MM#3, and BC#5), we estimate that between 1 in 10,000 and 1 in 1,000 cells contain the marker gene. These low levels can not be routinely detected by assaying individual CFU-C from posttransplantation BM for G418 resistance, and we have scored only rare positive CFU-C posttransplantation using this assay. BM cells were sorted via FACS for CD34+ cells and analyzed by PCR between 9 and 12 months in patient MM#3, and were found to be positive at a similar level. One in 10,000 cells containing the neo gene is also at the limit of detection for the nested PCR assay, explaining why some samples may be intermittently positive by repeated PCR assays (Fig 3). Fluctuations in the clones contributing to hematopoiesis...
over time could bring the number of neo positive cells in and out of detectable PCR range, explaining why some patients (notably BC#5) have had intermittently positive signals over time.

In all three patients with positive neo signals 1 year or longer posttransplantation, the granulocyte lineage has been positive for the gene. The short circulation and survival times of granulocytes mean that the positive PCR signals were not simply caused by prolonged survival of transduced terminally differentiated cells, but to continued production of daughter cells from primitive long-term engrafting cells or less likely from G-CFU or GM-CFU that remained quiescent for prolonged periods before contributing to hematopoiesis. Purified B cells have been positive in patient MM#3 intermittently between days 180 and 450, and purified T cells have been positive in patient MM#2 up to almost 2 years posttransplantation. (Fig 2)

One major objective of this study was to compare the gene-transfer potential of mobilized PB to BM target cells. A 16-bp sequence present at the 5' end of the neo gene in G1Na.40 but not LNL6 allowed the amplified PCR products to be distinguished on denaturing gels, as shown in Fig 4. In patient MM#2 (Fig 4A), the PB graft was transduced with G1Na.40 and the BM graft was transduced with LNL6. At the time of engraftment, a stronger G1Na.40 signal was detected in both PB and BM, and a very weak LNL6 signal was present in the BM sample. Over time, the LNL6 signal disappeared, and the G1Na.40 PB graft-derived signal accounted for all of the long-term marking. Patient MM#3 (Fig 4C) also had LNL6 transducing the BM graft and G1Na.40 transducing the PB graft. Over time, both grafts contributed to marking, with LNL6 and G1Na.40 bands present at equivalent levels in most samples. Patient BC#5 (Fig 4B) instead had LNL6 vector used to transduce the PB graft, and a granulocyte sample obtained late after transplantation was positive only for the PB (LNL6)-derived signal.

Analysis of tumor cells present posttransplantation. Three breast cancer patients have relapsed posttransplantation.

Fig 3. Summary of PCR results on all patient samples posttransplantation. Samples were only scored as positive or negative if β-actin PCR on the sample gave a signal consistent with amplification of 500 ng of DNA. Samples were only scored as positive if both reagent and concurrently extracted normal PB controls were negative for the neo signal. (○), positive Neo signal; (●), negative Neo signal.
Fig 4. Separation of PCR products on an 8% denaturing sequencing gel to determine source of Neo marking. (A) shows patient MM#2; (B), patient BC#5; (C), patient MM#3; (D), control mixtures of single-copy LN6 and G1Na.40 DNA at the indicated percentage ratios. Control, concurrently extracted PB DNA; LN, LN6 vector; G1, G1Na.40 vector; MNC, purified peripheral blood mononuclear cells; and GRAN, purified peripheral blood granulocytes.

Discussion

We have shown that both mobilized PB and BM CD34-enriched cells can contribute to engraftment long-term after autologous transplantation in adults with advanced malignancies. There has been heightened interest over the past several years in using mobilized PB “stem” cells as an alternative to BM in autologous and, more recently, in allogeneic transplantation. The question of whether PB contains true long-term repopulating stem cells with properties similar to steady-state marrow has been controversial, and is of critical importance, especially if PB is to be used for allogeneic transplantation, where recovery of endogenous hematopoiesis is not certain. The use of two different marking vectors allowed us for the first time to compare BM and PB-derived engraftment in the same patient. In this study, we showed that PB grafts contributed to long-term (greater than 18-month) myeloid and lymphoid engraftment in several patients. These data support the recent enthusiasm for initial clinical trials using mobilized PB cells in allogeneic transplantation. Proof of true stem cell engraftment in these patients would require demonstration that the insertion site of the marker gene in different lineages was identical, but the low efficiency of gene transfer precluded this type of analysis.

We wished to determine the relative potential of BM versus PB CD34-enriched cells as targets for retroviral gene transfer. Our group, as well as others, have shown that retroviral gene transfer to PB versus marrow target cells as assessed by in vitro progenitor assays is equivalent or superior. In the dog model, progeny of retrovirally-marked G-CSF—mobilized PB cells were detected at low levels for more than 6 months posttransplantation. In our study, we found at least equivalent long-term marking from mobilized PB as compared with BM CD34-enriched target cells. Larger studies will be necessary to quantify the relative efficiency of gene transfer into these two populations of target cells. Mobilized PB is a very attractive target for gene therapy applications. A large number of primitive CD34+ cells or even more primitive long-term culture initiating cells...
can be collected from PB after growth factor or growth factor plus chemotherapy mobilization as compared with BM, allowing collection of a potentially expanded target cell population for gene transduction. Repeated cycles of mobilization, collection, transduction, and transplantation would thus be feasible using PB, and is an approach to increase the number of gene-corrected cells in a patient. High-cell doses have allowed engraftment without prior ablation in the murine model, and this would obviously be desirable in human applications directed at nonmalignant diseases such as Gaucher disease or Fanconi anemia.

Our study did not identify the source of relapse post autologous transplantation in patients with multiple myeloma or breast cancer. We could not detect the marker gene in breast cancer samples assayed at the time of relapse. CD34-bright plasma cells remaining in the marrow of myeloma patients after transplantation were also negative for the neo gene, although none of the myeloma patients were studied at the time of clinical relapse or progression. The CD34-enrichment process has been shown to serve as a 2- to 4-log purge of myeloma and breast cancer tumor cells, so very few tumor cells were exposed to retroviral vector during transduction. There have been reports of gene-marked relapses in patients with acute leukemia, chronic leukemia, or neuroblastoma after autologous transplantation with whole BM, but these types of tumor cells have been shown to be transducible in vitro under the conditions used for the clinical trials, and these studies, unlike ours, were primarily focused on answering questions about the source of relapse.

The overall efficiency of retroviral gene transfer in this clinical trial was too low to be considered useful for therapeutic applications. Two other clinical gene-transfer studies have reported higher marking efficiencies, but there were important differences in the patient populations studied. Investigators at St Jude Children's Research Hospital (Memphis, TN) transduced autologous BM from children undergoing transplantation for acute leukemia or neuroblastoma. They used the same vectors and source of supernatants, and in some cases fractions of the same production lots that we used in our study. Levels of marked marrow progenitors and PB mononuclear cells averaged 5% at 12 to 18 months posttransplantation and, although some have fallen over more prolonged periods of observation, remain a log higher than what we report here (M. Brenner, personal communication, December 1994). Two patient-specific factors may have contributed to the higher efficiencies they observed: the younger age of St Jude patients (range, 2-19 years) and the prompt collection of autologous marrow after high-dose induction chemotherapy for relapsed tumor. In contrast, our patients were middle-aged adults that had been heavily treated with multiple cycles of moderate-dose myelosuppressive therapy and may have sustained permanent stem cell depletion or damage. Higher numbers of primitive cells may have been available and in cycle in the St Jude patients, and thus, susceptible to retroviral gene transfer. The other difference between the studies involved the transduction conditions: St Jude used a brief 6-hour exposure to vector without inclusion of hematopoietic growth factors, a transduction procedure that in vitro and in vivo preclinical studies found very inefficient. A second published gene-marking study of patients undergoing autologous transplantation for chronic myeloid leukemia also reported higher transduction efficiencies using a 6-hour exposure to virus, but many of the neo-positive cells were residual leukemic cells and cannot be equated with normal hematopoietic elements.

We are currently extending our protocol to examine this simplified 6-hour transduction protocol in an adult patient population. We are also testing the inclusion of an autologous marrow stromal support layer during transduction as another approach to improving the efficiency of gene transfer, because stromal cells or stromal matrix molecules can substitute for or enhance the effects of exogenous growth factors, and may improve gene transfer into primitive cells in animal models. We would stress the necessity of testing transduction modifications in human clinical marking trials, because no in vitro assays have yet proven predictive of gene-transfer efficiency to human in vivo long-term repopulating cells. As an example, gene-transfer efficiencies to CFU-C assessed at the end of transduction were much higher in our study than in the St Jude study, yet long-term levels of the marker gene assessed in patients after transplantation were instead much greater in their patients, and did not correlate with CFU-C transduction levels. Results generated over the next several years from these and other gene-marking trials, as well as from preliminary trials with therapeutic intent for Gaucher Disease, Fanconi anemia, and human immunodeficiency virus infection will indicate whether or not retroviral gene transfer to hematopoietic stem cells will be a feasible and effective approach to therapy for these and other disorders.

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