in Fig. 3. In both monkeys, peripheral blood cells containing the SAG were increased by one log in response to the toremifene administration (from <0.1% to ~1%), although cells containing the non-expressing vectors remained at stable levels. The increase in SAG containing peripheral blood cells was transient. The accidental death of animal D1 precluded further follow-up data. On the other hand, in animal D2, the fraction of SAG-containing cells returned to the original levels after discontinuing the toremifene administration. Bone marrow-derived CFU were also examined in these two monkeys. Since the *ex vivo* transduction levels were very low in these two monkeys (Table 1), there were no provirus-positive CFU derived from bone marrow after transplantation. However, following administration of toremifene, a fraction of the CFU (2-3%) became positive for the SAG-provirus in each monkey, although there were still no CFU containing the control vector sequence (data not shown). Selective expansion was not observed in the other two monkeys that received hydroxytamoxifen (D3 and D4), yet the serum concentrations of hydroxytamoxifen did not reach the biologically optimal levels (at least 100 nM).[24]

Serious adverse effects were not observed in the animals after drug treatment, but there were some symptoms associated with high-dose administration of estradiol. Monkeys receiving estradiol (S1 and S2) showed genital swelling. One monkey (S2) showed abnormal osteogenesis resulting in thoracic deformation and further drug administration was discontinued.

Clonal integration analysis.

Unique vector insertion sites in individual CFU in animal S1 were identified using the inverse PCR technique.[38,39] DNA from 48 provirus-positive colonies at the first peak of increase in transduced cells (1 and 2 months post-transplantation; Fig. 2) and from 54 provirus-positive colonies at the second peak (10 and 11 months post-transplantation) in the animal S1 were analyzed. Thirty-five colonies yielded distinct bands on inverse PCR (Fig. 4A), and amplified DNA segments were sequenced. Overall, 14 unique flanking genomic sequences were identified at 4 time points (1, 2, 10, and 11 months post-transplantation) (Table 2). At least 7 clones contributed to the first peak and 9 to the second peak, clearly indicating no outgrowth of a single clonal population (Fig. 4B). Two of the clones were common sequences at intervals of 10 months, suggesting that true HSCs were transduced.

Discussion

The ability to selectively amplify cells containing potentially therapeutic genes *in vivo* would represent an important tool for the clinical application of HSC based gene transfer, circumventing many of the current limitations of this promising technology. We have examined the feasibility of such selection using a chimeric selective amplifier gene (SAG) *in vivo* in nonhuman primates. In the setting of a cynomolgus monkey transplantation protocol, levels of 30-50% of gene-modified CFU were reached *in vivo* when the SAG was used. These very high gene marking levels were attainable despite low initial marking levels. Although one may claim that this may be potentially due to technical issues, for instance, cross-colony contamination, the use of individual, well-separated CFU ensured analysis at the single cell level. The use of individual colonies indeed allowed clonal integration site analysis, and such analysis conducted in animal S1 suggested that the increase of *in vivo* marking was not an outgrowth of a single clonal population, but that multiple clones contributed to the increase of transduced cells. The unique insertions identified also ruled out cross contamination as an explanation of the high level marking observed. The clones detected at the second peak around 10 and 11 months after transplantation were likely derived from transduced, true long-term repopulating HSCs and their progeny may have expanded in response to the administered estrogen. Increased marking derived from multiple transduced clones in response to estrogen administration is encouraging for future applications of the SAG.

This *in vivo* expansion method may also circumvent other problems of HSC

gene therapy. One is the perceived necessity for myeloablation of recipients, which is associated with high systemic toxicity or potential damage to marrow stroma.[1] Even with nonmyeloablative conditioning, successful engraftment of transduced cells even at low levels may allow expansion to clinically relevant levels with this method.[40-42] Another problem is decline of transgene expression *in vivo*.[35,43] Since cells to be expanded in this system require transgene (SAG) expression, only cells expressing the transgene can be expanded, thus circumventing transgene silencing.

A highly stable and specific molecular switch is required to strictly regulate the cytokine receptor signal of SAGs, or a safety concern about leukemogenesis may be raised. Our chimeric gene product, either estrogen- or tamoxifen-responsive, can only be activated by a synthetic steroid metabolite with high stability and specificity such as tamoxifen.[28] Furthermore, a safety modification has been made to enhance the specificity of the molecular switch in the tamoxifen-responsive SAG (GCRTmR) compared to its prototype estrogen-responsive SAG (GCRER). In the tamoxifen-responsive SAG, a point mutation (G525R) was introduced into the estrogen receptor moiety so that the molecular switch no longer responds to endogenous estrogen, thus minimizing the background signal transduction generated by endogenous estrogen.[24] Until now, no leukemogenesis has been observed even in the monkeys (S1, S2 and D1) which received the prototype estrogen-responsive SAG (GCRER). Thus, leukemogenesis may be regarded as an unlikely event.

The observations obtained from the current study suggest that the increase of

transduced cells with the SAG was transient. Our present method may not result in expansion of transduced cells at the level of HSCs. It is possible that the LTR promoter does not express the SAG in HSCs. Alternatively, the G-CSF receptor signal generated by the SAG may not induce proliferation of HSCs.[44] Thus, the expansion of transduced cells might occur only within the differentiated progeny of transduced HSCs, not at the level of transduced HSCs themselves. However, even if the effect is transient, therapeutic effects can result from a transient increase of transduced cells in certain diseases such as chronic granulomatous disease, or tamoxifen can be administered repeatedly over time to maintain clinically relevant effects.

The levels of transduced cells in peripheral blood were much lower than the levels of transduced progenitor cells in bone marrow. As shown in the monkey S1, significant fractions (10% or more) of bone marrow-derived CFU contained the provirus for more than 1 year after transplantation (Fig. 2), indicating successful transduction of long-term repopulating HSCs. Nonetheless, very few provirus-containing cells were detected in peripheral blood after transplantation. Thus, there was considerable *in vivo* discrepancy of marking levels between progenitors and mature cells (at least one log). The discrepancy was likely caused at least in part by the GFP gene included in the vectors. In fact, some investigators have reported that immune response to xenogeneic GFP cleared GFP-transduced cells from peripheral blood in a nonhuman primate transplantation model.[45]

In the present study, half of six monkeys that received the SAG transduced

HSCs showed an increase of transduced cells *in vivo* (Table 1) suggesting that responses to exogenous estrogen may vary among monkeys. Little response in monkeys D3 and D4 may be explained by the low serum drug (hydroxytamoxifen) concentrations obtained. Although, in the other monkey S2, the serum drug concentrations reached biologically optimal levels (>100nM), an increase of transduced cells was not observed. We consider that a more potent SAG would be required to achieve *in vivo* expansion in all monkeys. We are currently testing further modified SAGs with the thrombopoietin receptor (Mpl) as a growth signal generator instead of the G-CSF receptor to overcome variable responses among monkeys.[20,44]

Materials and Methods

Cells and animals.

All cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Mediatech, Herndon, VA) supplemented with 10% fetal calf serum (FCS; Gibco, Gaithersburg, MD) and antibiotics (100 U/ml penicillin, Banyu, Tokyo, Japan and 0.1 mg/ml streptomycin, Meiji, Tokyo, Japan). Young cynomolgus macaques (*Macaca fascicularis*) which weighed around 3 kg were housed and handled in accordance with the Rules for Care and Management at the Tsukuba Primate Center and with the Guiding Principles for Animal Experiments using Non-Human Primates formulated by the Primate Society of Japan. The protocol of the experimental procedures was approved by the Animal Welfare and Animal Care Committee of the National Institute of Infectious Diseases (Tokyo, Japan).

Vector construction.

Construction of two selective amplifier genes (SAGs), GCRER and GCRTmR cDNAs, were previously described.[23,24] The GCRER cDNA is a prototype SAG composed of two genes; i) the murine G-CSF receptor (GCR) gene with deletion of the ligand-binding domain (amino acids 5-195 of the GCR) and with a point mutation (Y703F) to abolish the differentiation signal generated by the GCR,[27] and ii) the gene encoding the rat estrogen receptor (ER) hormone-binding domain (HBD).[23] The GCRTmR cDNA is a modified SAG. In the GCRTmR, a point mutation (G525R) was

introduced into the ER gene moiety of the GCRER to abolish responsiveness to endogenous estrogen but retain responsiveness to synthetic hormones such as tamoxifen (Tm).[24,28] Four mouse stem cell virus (MSCV)-based vector plasmids expressing the enhanced green fluorescent protein (designated simply GFP in this article; Clontech, Palo Alto, CA), expressing GCRER, expressing GCRTmR, and expressing both GCRER and GFP (GCRER/GFP) were constructed as described elsewhere (Fig. 1).[23,24]

These vector plasmids were transfected into the BOSC23 ecotropic packaging cell line by the calcium phosphate method as previously described.[46] Supernatants were harvested 48-60 hours after transfection and were used to infect PG13 packaging cells using standard techniques[47] to generate retroviruses pseudotyped with the gibbon ape leukemia virus envelope. Stable producer clones were established by limiting dilution. The titers of GFP, GCRER, GCRTmR, and GCRER/GFP vectors used in these studies were $1x10^6$, $5x10^5$, $5x10^5$, and $5x10^5$/ml, respectively, as assessed by viral RNA dot-blot according to the method described elsewhere.[47] Two non-expressing retroviral vectors G1PLI and G1PLII were previously described.[30] Both supernatants of G1PLI and G1PLII used in this study had titers of $5x10^5$/ml as assessed by viral RNA dot-blot.

Transduction and transplantation.

Animals were treated with recombinant human SCF (50 or 200 μg/kg; Amgen, Thousand Oaks, CA) and recombinant human G-CSF (10, 50, or 100 μg/kg; Chugai, Tokyo, Japan) subcutaneously daily for 5 days, immediately followed by 50 ml bone

marrow aspiration. The nucleated cell fraction was obtained by red blood cell lysis with ACK buffer (155mM NH_4Cl, 10mM $KHCO_3$, and 0.1mM EDTA; Wako, Osaka, Japan). Enrichment for $CD34^+$ cells was performed using the immunomagnetic beads (Dynal, Lake Success, NY). The purity of $CD34^+$ cells obtained using this technique ranged from 90 to 95% as assessed with another monoclonal anti-CD34 antibody (clone 563; PharMingen, San Diego, CA). Mean CFU enrichment was 48-fold as assessed by colony-forming progenitor assays performed before and after enrichment. Transduction was conducted for a total of 96 hours at a starting density of $1\text{-}5x10^5$ cells/ml with daily replacement of vector supernatants and cytokines. Transduction cultures were supplemented with 50 ng/ml recombinant human interleukin-6 (IL-6; Ajinomoto, Osaka, Japan), 100 ng/ml recombinant human thrombopoietin (Kirin, Tokyo, Japan), 100 ng/ml recombinant human SCF (Amgen), and 100 ng/ml recombinant human Flt-3 ligand (FL; Research Diagnostics, Flanders, NJ). However, in the initial two monkeys (C1 and C2), 20 ng/ml recombinant human interleukin-3 (IL-3; Kirin) was included in the transduction cultures. Thrombopoietin was not included in the transduction cultures for the initial three monkeys (C1, C2, and S1). RetroNectin™ (Takara, Shiga, Japan) was used to coat culture plates before transduction and was used as directed.[48] After the 96-hour transduction period, transduced cells were washed and resuspended in phosphate-buffered saline (PBS) containing 10% autologous serum, and reinfused into each monkey following 500 cGy (all monkeys except S2) or 550 cGy (S2) total body irradiation daily for the preceding two days.

Estrogen or tamoxifen administration.

In the monkey S1, estradiol (E2) pellets (17-β-estradiol 0.2g/pellet, release time 60 days; Innovative Research of America, Sarasota, FI) were implanted twice subcutaneously (0.2g/body at day 231 and another 2g/body at day 287 post-transplantation). Weekly intramuscular injection of E2 was administered to monkey S2 (50mg/body once a week starting from day 47 to day 68 post-transplantation). Daily oral administration of toremifene (a derivative of tamoxifen, Nihon Kayaku Co., Ltd., Tokyo, Japan)[37] was administered to monkeys D1 (0.02mg/body/day at days 88-94, 0.2mg/body/day at days 95-101, 2mg/body/day at days 102-108, and 20 mg/body/day at days 109-115 post-transplantation) and D2 (100 mg/body/day from day 63 to day 108 post-transplantation). The dose escalation of toremifene in the animal D1 was conducted to elucidate the appropriate dose of administration to monkeys and it was found that at least 20 mg/body/day was necessary to achieve the biologically optimal serum concentrations (>100nM) of toremifene. Daily oral administration of hydroxytamoxifen (an active metabolite of tamoxifen; Sigma, St. Louis, MO) was administered to monkeys D3 (10 mg/body/day starting from day 39 to day 78 post-transplantation) and D4 (10 mg/body/day starting from day 32 to day 59 post-transplantation). Serum concentrations of E2 were measured by an ELISA kit (IMx Estradiol kit, Abbott Laboratory, Abbott Park, IL) as directed. Serum concentrations of toremifene and hydroxytamoxifen were measured by hyperperformance liquid chromatography (HPLC)

(NAC, Tokyo, Japan).

Colony Assays and PCR.

Cells were plated in α-minimum essential medium (MEM, Gibco) containing 1.2% methylcellulose (Shinetsu Kagaku, Tokyo, Japan) supplemented with 2 U/ml recombinant human erythropoietin (EPO; Roche Diagnostics, Mannheim, Germany), 100 ng/ml recombinant human IL-3 (PeproTech, London, UK), 100 ng/ml interleukin-11 (IL-11; PeproTech), 100 ng/ml recombinant human SCF (Biosource, Camarillo, CA), 20% FCS (Intergen, Purchase, NY), 1% bovine serum albumin (Sigma), $5x10^{-5}$M 2-mercaptethanol (Sigma), and antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin). At day 14, colonies containing greater than 50 cells were counted, and individual colonies were plucked into 50 μl of distilled water, digested with 20 μg/ml proteinase K (Takara) at 55°C for 1 hour followed by 99°C for 10 min, and assessed for vector (GFP, SAG, or non-expressing vector) sequence by nested PCR. The outer primer set was 5'-GCC ACA AGT TCA GCG TGT CC-3' and 5'-AGC TCG ATG CGG TTC ACC AG-3' for GFP-expressing vectors, 5'-GTA CAT TCG CTC TGA CTC CAC TCA G-3' and 5'-CAA TAA GCC CAT CAT TGA GGC TTC AC-3' for SAG-expressing vectors, and 5'-TCC ATC ATG GCT GAT GCA ATG CGG C-3' and 5'-GAT AGA AGG CGA TGC GCT GCG AAT CG-3' for non-expressing vectors. Amplification conditions for outer PCR were 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min with 20 cycles. The outer PCR products were purified by the MicroSpin S-400

HR Columns (Amersham, Piscataway, NJ). The inner primer set was 5'-TAC GGC AAG CTG ACC CTG AAG-3' and 5'-TGT CGC CCT CGA ACT TCA CCT-3' for GFP-expressing vectors, 5'-CCA CCC CTA GCC CTA AAT CTT ATG-3' and 5'-GGT GGT TCA GCA TCC AAT AAG G-3' for SAG-expressing vectors, and 5'-ATA CGC TTG ATC CGG CTA CCT G-3' and 5'-GAT ACC GTA AAG CAC GAG GAA G-3' for non-expressing vectors. Amplification conditions for inner PCR were 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min with 20 cycles. Simultaneous PCR for β-actin sequence was also performed to document DNA amplification of the sample on each colony. The primer set for β-actin was 5'-CAT TGT CAT GGA CTC TGG CGA CGG-3' and 5'-CAT CTC CTG CTC GAA GTC TAG GGC-3'. Amplification conditions for β-actin PCR were 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min with 30 cycles. The sizes of the final PCR products were 246, 315, 370 and 234 bp for GFP, SAG, non-expressing vector, and β-actin sequences, respectively. PCR products were separated on 1% agarose gels. The transduction efficiency of CFU was calculated by dividing the number of positive colonies for vector sequence by the number of positive colonies for β-actin sequence.

Semi-quantitative PCR.

Heparinized peripheral blood was drawn from each monkey at the indicated times. Nucleated cells were obtained by ACK lysis of red blood cells. Cellular DNA was extracted by the QIAamp Blood Kit (Qiagen, Valencia, CA) and 250 ng of DNA

was used for each PCR. The standards consisted of DNA extracted from the GCRER/GFP-vector producer clone (which has two copies of the vector sequence per cell) or the G1PLII-vector producer clone (which has three copies of the vector sequence per cell) serially diluted with control cynomolgus genomic DNA. The negative control consisted of DNA extracted from nontransduced cynomolgus peripheral blood. Simultaneous PCR for β-actin sequence was also performed to document initial DNA amounts of samples. PCR for vector (GFP, SAG, or non-expressing vector) sequence was performed in a nested way using the same primers as described in the above section *"Colony Assays and PCR"*, but the inner PCR was carried out in the presence of SYBR Green using an ABI PRISM 7700 sequence detection system with the buffers, nucleotides, and Taq polymerase from the SYBR Green PCR Core Reagents Kit (PE Applied Biosystems, Foster City, CA) as directed. Amplification of the inner PCR was carried out using a 2-step PCR method with a denaturing temperature of 95°C and an annealing temperature of 60°C. All semi-quantitative PCR was certified each time to yield linear amplifications in the range of the intensity of the positive control series (.0001~1.0 copy/cell, correlation coefficient > .98). For calculating the transduction efficiencies, the Ct value of the vector sequence was normalized based on the Ct value of the internal control β-actin sequence on the same sample as directed in the manufacturer's protocol.

Clonal integration analysis.

Individual colony DNA extracted as described *"Colony Assays and PCR"* was resuspended in 200 μl PBS and mixed with 200 μl buffered phenol-chloroform-isoamyl alcohol (25:24:1; vol/vol). The aqueous phase was precipitated by the addition of 2 μg glycogen (Boehringer Mannheim GmbH, Mannheim, Germany), 18 μl of 10 mol/l ammonium acetate, and 500 μl absolute ethanol, and this was incubated at –20°C overnight. DNA was centrifuged at 10,000 rpm for 20 minutes, rinsed in 70 % ethanol, and dried on the bench-top for 1 hour. DNA pellets were resuspended in 25 μl H_2O.

The clonal identity of vector proviral integrants was determined using inverse PCR by the method previously described.[39] To 20 μl DNA from each sample, 10 μl React 2 Buffer (Gibco), and 2μl (20U) TaqI restriction enzyme (Gibco) was added with H_2O to a total volume 100μl. Samples were digested for 2 hours at 65°C with the readdition of 2 μl of TaqI after the first hour of incubation. A 16-μl sample was then ligated by the addition of 4 μl 5X T4 ligase buffer (Gibco) and 1 μl T4 ligase (Gibco) at 16°C for a minimum of 6 hours. The first round of amplification of 10-μl circularized DNA used the primers 5'-AGG AAC TGC TTA CCA CA and 5'-CTG TTC CTT GGG AGG GT in Perkin-Elmer (Foster City, CA) PCR buffer. The first cycle was at 95 °C denaturation for 5 minutes, 50 °C annealing for 2 minutes, and 72 °C extension for 4 minutes. The subsequent 29 cycles were identical, except that the denaturation time was reduced to 1 minute. Nested PCR was then performed on 2 μl of the amplified product with primers 5'-TCC TGA CCT TGA TCT GA and 5'-CTG AGT GAT TGA CTA CC using the same reaction condition and cycles. Resultant PCR products were separated

on a 1% agarose gel (Gibco) with ethidium bromide. Bands were purified from the agarose gel and subjected to DNA sequence analysis. Amplified PCR products were ligated directly into the TA vector (Invitrogen, Carlsbad, CA) and sequenced using an automated sequencer and Taq polymerase (Perkin-Elmer). Sequences were aligned using DNAStar (DNAStar, Madison, WI).

Acknowledgments

We thank Fumiko Ono, Hayato Narita, and Khoji Hanari for handling and care of monkeys. We thank John F. Tisdale for critical reading of the manuscript. We are grateful to Ikunoshin Kato for supplying RetroNectin™. We acknowledge Nippon Kayaku's supply of toremifene, Amgen's supply of SCF, Ajinomoto's supply of IL-6, Chugai's supply of G-CSF, and Kirin's supply of IL-3 and thrombopoietin. This study was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Y.H. and K.O.), the Ministry of Health, Labor and Welfare of Japan (K.O.), Takeda Science Foundation (K.O.), Grant-in-Aid of the Japan Medical Association (K.O.), Yamanouchi Foundation for Research on Metabolic Disorders (K.O.), Jichi Medical School Young Investigator Award (Y.H.), the Mochida Memorial Foundation for Medical and Pharmaceutical Research (Y.H.), and Senri Life Science Foundation (Y.H.).

Figure Legends

Figure 1. Vector construction. The GCRER is a prototype of the selective amplifier gene (SAG) and is composed of the G-CSF receptor (GCR) gene and the estrogen receptor (ER) gene hormone-binding domain (HBD). In the GCRER, the G-CSF-binding domain (amino acids 5-195) was deleted from the GCR gene to abolish responsiveness to G-CSF, and a point mutation (Y703F) was introduced to knock out the differentiation signal generated by the GCR. In the GCRTmR, another point mutation (G525R) was introduced into the ER gene moiety of the GCRER. GCRTmR no longer reacts to endogenous estrogen but responds to tamoxifen. The GCRER/GFP vector is a bicistronic vector expressing the GCRER gene (as the first cistron) and GFP gene (as the second cistron). A vector expressing only the GCRER, GCRTmR, or GFP gene was also constructed. All vectors are derived from the mouse stem cell virus (MSCV) and pseudotyped with the gibbon ape leukemia virus envelope. TM, trans-membrane domain; TA, trans-activating domain; IRES, internal ribosome entry site; LTR, long terminal repeat.

Figure 2. *In vivo* expansion of transduced hematopoietic progenitor cells in animal S1. $CD34^+$ cells from the animal S1 were transduced with a retroviral vector expressing the estrogen-responsive SAG (GCRER). The transduced cells were reinfused into the monkey that was myeloablated by total body irradiation. After transplantation, individual bone marrow-derived colonies were examined for the provirus by PCR. The

percentage of the provirus-containing cells is shown over time (months) after transplantation. The arrows indicate time points of subcutaneous implantation of estradiol (E2) pellets.

Figure 3. Dual genetic marking study showing selective expansion of SAG-transduced cells in peripheral blood. Bone marrow $CD34^+$ cells were harvested and split into two equal aliquots; one aliquot was transduced with an SAG-expressing retroviral vector (GCRER or GCRTmR) and the other aliquot was transduced with a control non-expressing vector (PLII). Both aliquots were reinfused simultaneously into each myeloablated monkey. The animals (D1 and D2) received toremifene (a derivative of tamoxifen) posttransplantation and *in vivo* marking levels (% of provirus-containing cells) in peripheral blood derived from the two populations are shown. The estrogen-responsive SAG (GCRER) was used in the monkey D1 and the tamoxifen-responsive one (GCRTmR) was used in the monkey D2. Shaded bars indicate the periods during which biologically optimal serum levels (>100 nM) of toremifene were obtained. Animal D1 suffered an accidental death and no further follow-up data could be obtained.

Figure 4. The vector insertion site analysis among individual CFU in the animal S1 by the inverse PCR technique. (**A**) Cellular DNA containing integrated provirus was digested with TaqI, which cuts twice within the proviral backbone and again in the

flanking genomic DNA. The LTR-containing fragments were self-ligated and underwent 2 rounds of amplification by inverse PCR using nested LTR-specific primers (see Materials and Methods). A representative gel showing positive inverse PCR products from 10 CFUs is shown. M: Molecular marker. Lanes 1-3: 1-month sample, lane 4: H_2O, lanes 5 to 11: 10-month samples. **(B)** PCR bands were isolated, cloned, and sequenced to confirm and identify the genomic DNA flanking the LTR (see Table 2). Overall 14 unique flanking genomic sequences were found at 4 time points (1, 2, 10, and 11 months posttransplantation). At least 7 clones contributed to the first peak and 9 clones contributed to the second peak, clearly indicating no outgrowth of a single clonal population. Two of the clones were common sequences at intervals of 10 months.

References

1. Dunbar CE *et al.* Retrovirally marked CD34-enriched peripheral blood and bone marrow cells contribute to long-term engraftment after autologous transplantation. *Blood* 1995; **85:** 3048-3057.
2. Hanazono Y, Dunbar CE. Genetic marking. In: Forman SJ, Blume KG, Thomas ED (ed). Hematopoietic Cell Transplantation, 2nd Edition. Blackwell Science, Inc.: Malden, MA, 1999, pp 89-96.
3. Hanazono Y, Terao K, Ozawa K. Gene transfer into nonhuman primate hematopoietic stem cells: implications for gene therapy. *Stem Cells* 2001; **19:** 12-23.
4. Sorrentino BP *et al.* Selection of drug-resistant bone marrow cells *in vivo* after retroviral transfer of human MDR1. *Science* 1992; **257:** 99-103.
5. Hanania EG *et al.* Results of MDR-1 vector modification trial indicate that granulocyte/macrophage colony-forming unit cells do not contribute to posttransplant hematopoietic recovery following intensive systemic therapy. *Proc Natl Acad Sci USA* 1996; **93:** 15346-15351.
6. Hesdorffer C *et al.* A phase I trial of retroviral-mediated transfer of the human MDR-1 gene as marrow chemoprotection in patients undergoing high-dose chemotherapy and autologous stem cell transplantation. *J Clin Oncol* 1998; **16:** 165-172.
7. Moscow JA *et al.* Engraftment of MDR1 and NeoR gene-transduced hematopoietic cells after breast cancer chemotherapy. *Blood* 1999; **94:** 52-61.