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Retroviral gene transfer into human hematopoietic cells: an *in vitro* kinetic study

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ABSTRACT

Background and Objective. **Successful gene therapy applications require optimized strategies to increase gene transfer efficiency into HPCs with long-term repopulating ability. One of the issues that needs to be clarified is how hematopoietic cells proliferate, differentiate and express the transgene after each cycle of transduction. We investigated the kinetics of cell expansion, CD34 antigen expression and transduction efficiency of human hematopoietic cells in culture conditions commonly used in retroviral gene transfer protocols.**

Design and Methods. **Purified CD34$^+$ cells from cord blood (n=5) or leukapheresis products (n=9) and a retroviral vector encoding an enhanced version of the green fluorescent protein (EGFP) were used. Target cells were exposed daily to vector-containing supernatants and a combination of interleukin 3 (IL-3), interleukin 6 (IL-6), stem cell factor (SCF) and Flt3-ligand (FL). Cell samples were harvested from the cultures and analyzed at 24 hour intervals for seven consecutive days.**

Results. **We found that CD34$^+$ cells proliferated and differentiated under our culture conditions. The number of genetically modified cells increased after each cycle of transduction. Median numbers of cells positive for both CD34 and EGFP increased steadily over the culture period, but after day four most of the EGFP$^+$ cells had a low CD34 expression.**

Interpretation and Conclusions. **Culturing and transducing CD34$^+$ cells for longer periods of time under these conditions might be detrimental for *ex vivo* gene transfer applications since the transduced cells are likely to have a decreased potential for long-term engraftment and repopulation *in vivo*.**

Key words: retroviral vectors, gene transfer, hematopoietic cells

Gene therapy holds promises for the treatment of a number of genetic and acquired disorders. Hematopoietic stem cells are ideal targets for gene transfer-based therapies since they can repopulate the hematopoietic and immune system for the entire life of the recipient. Measurements of the transduction efficiency of hematopoietic progenitor cells (HPCs) *in vitro* and many gene marking studies carried out *in vivo* have relied heavily on drug-resistance genes such as the neomycin phosphotransferase gene (*neo*).[1,2] Analysis of gene transfer efficiency using such genes requires tedious, time-consuming clonogenic assays, which often have high intrinsic variability. During the last few years, viral vectors encoding a variety of cell-surface markers, including a truncated version of the low affinity nerve growth factor receptor,[3,4] murine heat-stable antigen,[5] murine CD18 antigen,[6] and human CD24 antigen,[7] have been developed. All these reporter molecules allow direct determination of the transduction efficiency by flow cytometry after staining with specific labeled antibodies. The green fluorescent protein (GFP) and its variants have emerged as excellent markers for assessing gene transfer.[8] More recently, retroviral vectors containing an enhanced version of the molecule (EGFP) have been developed. Cells expressing these molecules become fluorescent, allowing direct monitoring of gene transfer efficiency by fluorescence microscopy, multiparametric analysis on phenotypically defined cell populations, cell sorting by FACS and cell tracking studies.[9-11]

The CD34 molecule is a surface marker defining an immature subset of HPCs which is enriched in long-term repopulating cells. Culture of these cells in the presence of stimulatory cytokines results in cell proliferation but also in progressive differentiation and a concomitant decrease in CD34 expression, loss of clonogenic ability and loss of engraftment and repopulating ability.[12,13] *Ex vivo* retroviral gene transfer into HPCs for clinical applications usually involves culturing HPCs with a combination of cytokines and the sequential addition of vector-containing supernatants. The number of cycles of infection and the length of exposure of HPCs to retroviral vectors varies according to the different authors and protocols used. In some cases the exposure is limited to six hours,[1] whereas in others, HPCs are cultured with viral supernatants for up to three weeks.[14,15]

Successful gene therapy applications require optimized strategies to increase gene transfer efficiency

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into HPCs with long-term repopulating ability. One of the issues that needs to be clarified is how hematopoietic cells proliferate, differentiate and express the transgene after each cycle of transduction. In *ex vivo* stem cell gene therapy, a balance between transduction efficiency, expansion and maintenance of an *immature* state is required for optimal results. In this study, we analyzed the kinetics of retroviral transduction into human HPCs in culture. Using a previously developed amphotropic retroviral vector containing the EGFP gene,[11] we simultaneously monitored the rates of cell expansion, transduction efficiency and CD34 expression in cultured human hematopoietic cells.

Design and Methods

Hematopoietic cell samples

Leukapheresis products (LP) were obtained from patients with hematologic malignancies or solid tumors undergoing stem cell transplantation (n=9), and cord blood (CB) cells (n=5) were obtained from normal, full-term infants. Mobilization protocols included granulocyte colony-stimulating factor (G-CSF) alone or G-CSF plus chemotherapy. This study was approved by the Ethics Committee of our institution.

CD34⁺ cell selection

$CD34^+$ cells were isolated from LP and CB samples by an indirect immunomagnetic method (MiniMACS; Miltenyi Biotec, Inc, Sunnyvale, CA, USA) as previously described.[16] Briefly, mononuclear cells were incubated with a mouse IgG antihuman CD34 antibody (QBEND/10) for 20 minutes at 4ºC, washed and incubated for 30 minutes with a magnetic-labeled antibody recognizing the primary antibody (both antibodies were included within the MiniMACS kit). After washing, cells were passed through a column attached to an external magnet. Non-adsorbed cells were washed out and cells retained by the column were detached by flushing the column after removing the external magnetic field. Sample purity was assessed by FACS analysis after staining with a phycoerythrin (PE)-conjugated anti-CD34 antibody (Clone 8G12, Becton-Dickinson, San José, CA, USA). $CD34^+$-enriched cells were used directly or stored in liquid nitrogen.

Retroviral producer cell line

The retroviral vector used in this study was pSF-EGFP1, which contains a hybrid promoter specially designed to enhance EGFP expression in HPCs and has been described elsewhere.[11] Fresh aliquots of the amphotropic producer cell line PA317/EGFP1, yielding a viral titer of 2×10^6 infectious particles/mL, were thawed every four weeks and grown in Dulbecco's Modified Eagle medium supplemented with 4.5 g/L of glucose, 10% heat-inactivated fetal calf serum (FCS), 50 IU/mL penicillin, 50 µg/mL streptomycin, and 2 mM of L-glutamine. The medium was replaced at 70-90% of cell confluence by Iscove's Modified Dulbecco's medium supplemented with 12.5% FCS, 12.5% heat-inactivated horse serum, 1 mmol/L sodium pyruvate, 1 µM hydrocortisone, 0.1 mM 2-mercaptoethanol, 50 IU/mL penicillin, 50 mg/L streptomycin and 2 mM L-glutamine, and used 24 hours later to transduce the target cells.

Transduction of CD34⁺ cells

The transduction protocol used involved daily infection by vector-containing supernatant from day zero to day seven. Target cells were cultured in 25 cm^2 canted-neck tissue culture flasks (TPP, Trasadingen, Switzerland) at a cell density of $0.5\text{-}1\times10^5$/mL in 0.2 µm-filtered vector-containing supernatant. In addition, all cultures were supplemented daily with 4 µg/mL protamine sulphate, and 50 ng/mL each of recombinant human interleukin-3 (rhIL-3), recombinant human interleukin-6 (rhIL-6) (both kindly provided by Sandoz, Basel, Switzerland), recombinant human stem cell factor (rhSCF), and recombinant human rhFL (kindly provided by Amgen, Thousand Oaks, CA, USA). All cultures were maintained at 37ºC in 5% CO_2. In each experiment, a mock transduction was done in parallel using the same medium, growth factors and protamine sulphate but without addition of viral vectors. Ninety percent of the culture volume (9 mL) was harvested and replaced daily. One mL was used for cell counting, viability assessment (by trypan blue staining), and FACS analysis. The remaining cells were centrifuged at 1500 rpm for 10 minutes. Pelleted cells were resuspended in fresh filtered vector-containing supernatants supplemented with growth factors and protamine sulphate and added to the culture flasks.

FACS analysis of CD34⁺ cells

The transduction efficiency in the $CD34^+$ cells was assessed directly by FACS analysis. Cells contained in 1 mL of the cultures (10% of the total culture volume) were counted using a Neubauer hemocytometer. A minimum of 5×10^4 cells were washed with 1% bovine serum albumin in phosphate-buffered saline, and stained with phycoerythrin (PE)-conjugated anti-CD34 antibody for 20 min at room temperature. Cells were washed and analyzed using an EPICS XL cytometer (Coulter Electronics, Hialeah, FL, USA) equipped with an argon ion laser tuned at 488 nm. A PE-conjugated mouse isotypic antibody served as a control. A first gate including only the living cells was used to estimate the percentage of $CD34^+$ cells. A second gate on $CD34^+$ cells was used to analyze the double positive, CD34 and green fluorescent cell population. At least 5,000 events were analyzed from each sample.

Statistical analysis

Data are presented as the mean ± standard error of the mean. Statistical significance of differences between groups was analyzed by the two-tailed Student's t-test and the ANOVA test for repeated measures using the software SPSS v.6.1 (SPSS Inc, Chicago, IL, USA).

Results

Expansion of hematopoietic cells in vitro *and level of CD34⁺ expression*

Purified CD34⁺ cells (>80% purity) were cultured for seven days, as described in the Materials and Methods section. Estimated cell counts at 24-hour intervals are shown in Figure 1. Cell viability along the seven days of culture was greater than 90%. Total nucleated cells expanded an average of 6.7 fold (range, 1.3-22.2) for LP samples and 14.6 fold (range, 11-27.5) for CB samples by day seven under the experimental culture conditions (p=0.01). Absolute numbers of CD34⁺ cells expanded about two fold over the seven days of culture with no statistically significant differences between LP and CB. However, since the high rates of total cell expansion seen after day four is basically due to the generation of CD34 negative cells, the percentage of CD34⁺ cells declined quickly after day four regardless of the origin of the sample (CB or LP) (Figure 2).

Levels of CD34 antigen expression declined steadily over the seven days of culture, with no statistically significant differences between LP and CB. FACS analysis of CD34 and EGFP expression of a representative experiment (a CB sample) at different timepoints is shown in Figure 3. Cells expressing high levels of CD34 antigen (above the third logarithm of fluorescence intensity) decreased progressively, and had completely disappeared by day five.

Transduction efficiency at different time intervals

The percentage of total cells expressing EGFP increased progressively after sequential exposures to the vector-containing supernatants during the culture period, and was higher in CB samples than in LP samples (p=0.007) (Figure 4). Regarding the CD34⁺ cell population, there were no significant differences in the transduction rates between CB and LP samples. When cell counts were normalized to 10^5 CD34⁺ cells plated on day zero, the median absolute numbers and the percentages of CD34⁺ cells expressing EGFP increased progressively over the culture period similarly in both types of samples (Figure 5).

To investigate whether the increased transduction level observed after each cycle of infection was due to the daily addition of vector-containing supernatant, cell samples were transduced either on day 0 (only), on days 0 and 1, on days 0, 1 and 2, and on days 0, 1, 2, and 3 etc, and analyzed on day 7. Levels of gene transfer in each case analyzed on day 7 were comparable to those observed 24 hours after the last cycle of transduction (data not shown).

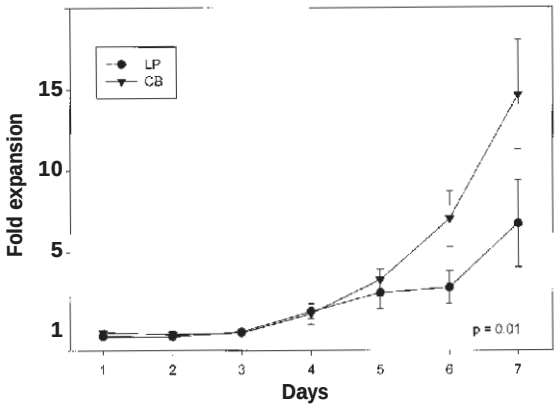

Figure 1. Time profile of cell expansion. Samples of the cultures (1 mL) were harvested every day for cell counts. Data points represent mean daily estimations of the fold expansion of total nucleated cells (from day 0) and bars represent standard errors. *CB: cord blood; LP: leukapheresis product.*

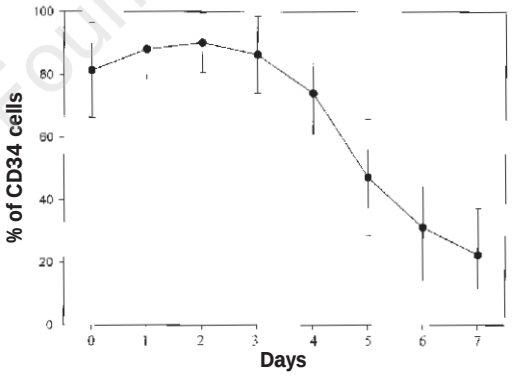

Figure 2. Time profile of CD34 expression. Purified CD34+ cells were transduced daily and analyzed at different timepoints. The percentage of cells expressing the CD34 antigen was monitored daily by flow cytometry. Data points represent the mean of the percentages of cells expressing CD34 levels above the isotypic control. Bars indicate the standard errors.

Discussion

Stem cell gene therapy protocols using retroviral vectors are aimed at generating sufficient numbers of transduced cells able to result in a level of long-term engraftment and long-term expression of the transgene that is of therapeutic benefit. Current vection. This makes conditions for optimal retroviral gene transfer into HPCs similar in many aspects to the conditions used for stem cell expansion, which often result in myeloid differentiation and progressive loss of the immature phenotype and functional ability after a few days in culture. In addition, murine hematopoietic cells have been reported to acquire an engraftment defect when cultured in vitro in the presence of IL-3, IL-6, IL-11 and SCF both in normal and in myeloablated mice.[18,19] However, it is generally accepted that hematopoietic progenitors which have been manipulated, expanded and transduced *ex vivo* can engraft in several *in vivo* models.[20-23] Purified

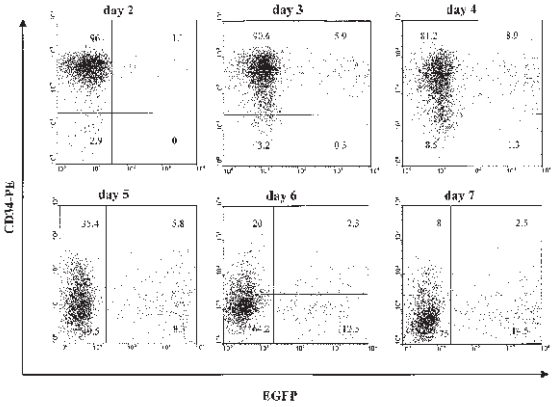

Figure 3. Dot plots of FACS analysis of a representative experiment, from a cord blood sample, at different days of culture. A total of 5,000 events in the live cell gate (defined according to light scatter properties) were analyzed in each plot. CD34 expression (Y axis) is plotted against EGFP expression (X axis). The top right squares correspond to cells that are both CD34 and EGFP positive. Numbers inside squares represent the percentages of cells. Cells expressing high levels of CD34 (above the third log of fluorescence) were almost completely lost by day five. Plots of days 0 and 1 have been omitted since levels of CD34 expression on days 0 and 1 were very similar to those observed on day 2, and transduction levels on day 1 were very low.

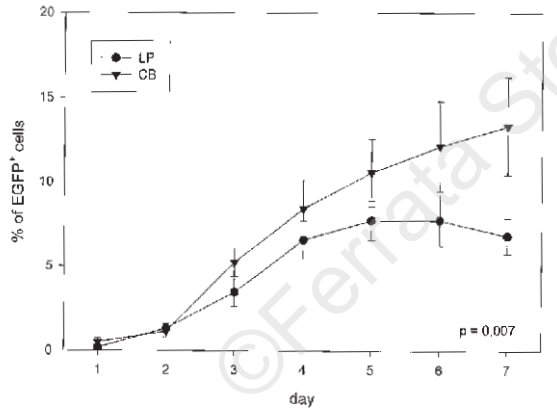

Figure 4. Transdution rates on different days. Datapoints represent means of the percentages of total cells expressing EGFP. Bars represent standard errors.
CB: cord blood; LP: leukapheresis product.

human CD34+ cells proliferate when cultured in the presence of combinations of growth factors.[24] Under these conditions the total number of mononuclear cells, CFU-GMs and CD34+ cells can increase several fold but the level of CD34 expression declines and cells with an immature phenotype are progressively replaced by more differentiated cells.[13] Furthermore, some authors have reported a decreased clonogenic

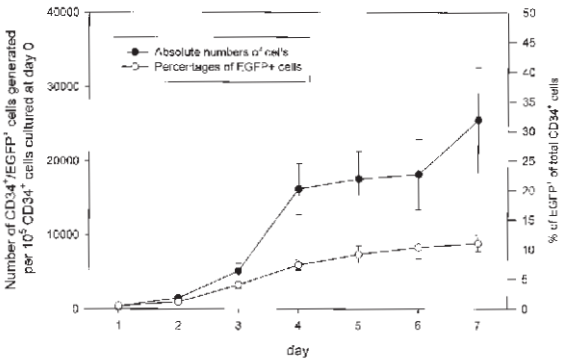

Figure 5. Time profile for the generation of transduced CD34+ cells. Data points represent daily estimations of the absolute number of CD34+ / EGFP+ cells per 10^5 CD34+ cells cultured on day 0 (●) and percentages of EGFP+ cells of total CD34+ cells (○). Bars represent standard errors (n=14).

potential of cultured CD34+ cells undergoing one or more cell divisions in comparison with cells that did not divide.[25]

We used an amphotropic retroviral vector containing the EGFP gene[11] to investigate the kinetics of retroviral gene transfer into human hematopoietic cells under our particular culture conditions. We considered the loss of CD34 expression as a surrogate marker of hematopoietic cell differentiation since it has been demonstrated that this loss is associated with an increased expression of differentiation antigens such as CD33 and CD13.[13] Fresh samples gave similar results to frozen and thawed samples (data not shown). In addition, a wide variability in the rates of cell expansion, differentiation and transduction was found between individual samples regardless their origin. Factors that may account for such differences include intrinsic variability among the different samples, chemotherapy treatments received prior to the mobilization regimen (LP samples), purity of the CD34+ sample, and variability in the retroviral titers of the supernatants used for transduction.[26,28] In our experiments, it is unlikely that the use of different viral stocks could account for any differences, since we previously demonstrated that viral titers of this producer cell line (PA317/EGFP1) are very stable over time. In addition, we also previously showed that transduction efficiency of EGFP detected by FACS analysis correlates very well with detection of EGFP by CFUs assays, thus obviating the need for the latter method.[11]

As expected, the total number of mononuclear cells and the percentage of transduced cells increase after repeated cycles of transduction, whereas the percentage of CD34+ cells and their level of CD34 expression declined rapidly after 4 days in culture. CB cells

yielded higher gene transfer and expansion rates, which is consistent with previous reports.[29,30] However, there were no statistically significant differences in the generation of CD34+/EGFP+ cells between both types of samples, probably due to small differences in the loss of CD34 expression between CB and LP samples. Interestingly, the expansion rates correlated with transduction efficiency at day seven (data not shown), which is in concordance with previous observations that mitosis is required for efficient gene transfer by conventional retroviral vectors. Results of transduction experiments performed on day 0, days 0 and 1, days 0, 1 and 2 etc, and analyzed on day 7, indicate that repeated cycles of infection are required to increase transduction efficiency, and that analysis of EGFP expression 24 hours after transduction does not underestimate gene transfer rates. In this regard, it has been reported that EGFP can be detected as soon as nine hours after retroviral transduction.[31] We believe that differences in the level of EGFP expression observed between day two and day seven may be explained, at least in part, by cytoplasmatic accumulation of EGFP, as has also been described using other genetic markers.[32] The above mentioned results are consistent with the observation that absolute CD34+ cell expansion is maximal at day seven under similar culture conditions,[13] and that loss of CD34 expression is associated with a loss of clonogenic potential.[12,33] This suggests that, for *in vivo* applications, transducing hematopoietic cells *ex vivo* for more than four days can be disadvantageous under similar experimental conditions. We conclude that expansion and transduction efficiency into human CD34+ cells in short-term cultures increase after each cycle of infection, but the rapid loss of CD34 expression seen after four days of culture can make it impractical, for clinical applications, to transduce human hematopoietic cells for longer periods of time, unless culture conditions are improved. Further *in vivo* studies addressing engraftment and the repopulating potential of transduced cells are necessary to optimize retroviral-mediated gene therapy.

Contributions and Acknowledgments

JBr performed the experiments of isolation and transduction of CD34+ cells, FACS analysis, and contributed to the writing of the manuscript. TP contributed to the isolation and transduction of hematopoietic cells, and FACS analysis. AL contributed to the experiments and the writing of the manuscript. JP helped with the FACS analysis. JG revised the final form of the manuscript. JBa designed the study, contributed to the experiments of transduction and FACS analysis and the writing of the paper. The authors thank Gemma Capmany for providing purified CD34+ cells from cord blood samples, Joan Bertrán, Anna Bigas, José Antonio Cancelas, Michael Lynch and Rob Ploemacker, for their critical reviews.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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References

1. Brenner MK, Heslop HE, Rill D, et al. Transfer of marker genes into hematopoietic progenitor cells. Cytokines Mol Ther 1996; 2:193-200.
2. Emmons RV, Doren S, Zujewski J, et al. Retroviral gene transduction of adult peripheral blood or marrow-derived CD34+ cells for six hours without growth factors or on autologous stroma does not improve marking efficiency assessed in vivo. Blood 1997; 89:4040-6.
3. Mavilio F, Ferrari G, Rossini S, et al. Peripheral blood lymphocytes as target cells of retroviral vector-mediated gene transfer. Blood 1994; 83:1988-97.
4. Phillips K, Gentry T, McCowage G, Gilboa E, Smith C. Cell-surface markers for assessing gene transfer into human hematopoietic cells. Nature Med 1996; 2: 1154-6.
5. Conneally E, Bardy P, Eaves CJ, et al. Rapid and efficient selection of human hematopoietic cells expressing murine heat-stable antigen as an indicator of retroviral-mediated gene transfer. Blood 1996; 87:456-64.
6. Eipers PG, Krauss JC, Palsson BO, Emerson SG, Todd RF III, Clarke MF. Retroviral-mediated gene transfer in human bone marrow cells grown in continuous perfusion culture vessels. Blood 1996; 86:3754-62.
7. Pawliuk R, Kay R, Lansdorp PM, Humphries RK. Selection of retrovirally transduced hematopoietic cells using CD24 as a marker of gene transfer. Blood 1994; 84:2868-77.
8. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. Science 1994; 263:802-5.
9. Persons DA, Allay JA, Allay ER, et al. Retroviral-mediated transfer of the green fluorescent protein gene into murine hematopoietic cells facilitates scoring and selection of transduced progenitors in vitro and identification of genetically modified cells in vivo. Blood 1997; 90:1777-86.
10. Bierhuizen MFA, Westerman Y, Visser TP, Dimjati W, Wognum AW, Wagemaker G. Enhanced green fluorescent protein as selectable marker of retroviral-mediated gene transfer in immature hematopoietic bone marrow cells. Blood 1997; 90:3304-15.
11. Limon A, Briones J, Puig T, et al. High-titer retroviral vectors containing enhanced green fluorescent protein (EGFP) for efficient expression in hematopoietic cells. Blood 1997; 90:3316-21.
12. Lu L, Xiao M, Shen RN, Grigsby S, Broxmeyer HE. Enrichment, characterization, and responsiveness of single primitive CD34+++ human umbilical cord blood hematopoietic progenitors with high proliferative and replating potential. Blood 1993; 81:41-8.

13. Koizumi K, Sawada K, Sato N, et al. Transitional changes in immunophenotypic subpopulations of human peripheral blood CD34+ cells expanded in vitro. Exp Hematol 1994; 22:1171-8.
14. Bienzle D, Abrams-Ogg ACG, Kruth SA, et al. Gene transfer into hematopoietic stem cells: Long-term maintenance of in vitro activated progenitors without marrow ablation. Proc Natl Acad Sci USA 1994; 91: 350-4.
15. von Kalle C, Kiem HP, Goehle S, et al. Increased gene transfer into human hematopoietic progenitor cells by extended in vitro exposure to a pseudotyped retroviral vector. Blood 1994; 84:2890-7.
16. Cancelas JA, Querol S, Canals C, et al. CD34+ cell positive selection from mobilized peripheral blood by an indirect immunomagnetic method: effect of the type of mobilization and assessment of tumor depletion ability. J Hematother 1995; 4:531-8.
17. Rao SS, Peters SO, Crittenden RB, Stewart FM, Ramshaw HS, Quesenberry PJ. Stem cell transplantation in the normal nonmyeloablated host: relationship between cell dose, schedule, and engraftment. Exp Hematol 1997; 25:114-21.
18. Kittler ELW, Peters SO, Crittenden RB, et al. Cytokine-facilitated transduction leads to low level engraftment in nonablated hosts. Blood 1997; 90:865-72.
19. Peters SO, Kittler ELW, Ramshaw HS, Quesenberry PJ. Ex vivo expansion of murine marrow cells with interleukin-3 (IL-3), IL-6, IL-11 and stem cell factor leads to impaired engraftment in irradiated hosts. Blood 1996; 87:30-7.
20. Williams DA, Lemischka IR, Nathan DG, Mulligan RC. Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse. Nature 1984: 310:476-80.
21. van Beusechem VW, Valerio D. Gene transfer into hematopoietic stem cells of nonhuman primates. Hum Gene Ther 1996; 7:1649-68.
22. Serrano F, Varas F, Bernad A, Bueren JA. Accelerated and long-term hematopoietic engraftment in mice transplanted with ex vivo expanded bone marrow. Bone Marrow Transplant 1994; 14:855-62.
23. Varas F, Bernad A, Almendral JM, Bueren JA. Relevance of myeloablative conditioning in the engraftment of limiting numbers of normal and genetically marked lympho-hematopoietic stem cells. Bone Marrow Transplant 1994; 18:981-9.
24. Haylock DN, To LB, Dowse TL, Juttner CA, Simmons PJ. Ex vivo expansion and maturation of peripheral blood CD34+ cells into the myeloid lineage. Blood 1992; 80:1405-12.
25. Traycoff CM, Orazi A, Ladd AC, Rice S, McMahel J, Srour EF. Proliferation-induced decline of primitive hematopoietic progenitor cell activity is coupled with an increase in apoptosis of ex vivo expanded CD34+ cells. Exp Hematol 1998; 26:53-62.
26. Douer D, Ramezani L, Mohrbacher AF, et al. The influence of patient-related factors on ex vivo retroviral-mediated gene transfer into mobilized peripheral blood myeloid progenitors. Hum Gene Ther 1998; 9:1061-8.
27. Dunbar CE, Cottler-Fox M, O'Shaughnessy JA, et al. Retrovirally marked CD34-enriched peripheral blood and bone marrow cells contribute to long-term engraftment after autologous transplantation. Blood 1993; 85:3048-57.
28. Bregni M, Di Nicola M, Siena S, et al. Mobilized peripheral blood CD34+ cells express more amphotropic retrovirus receptor than bone marrow CD34+ cells. Haematologica 1998; 83:204-8.
29. Lansdorp PM, Dragowska W, Mayani H. Ontogeny-related changes in proliferative potential of human hematopoietic cells. J Exp Med 1993; 178:787-91.
30. Al-Lebban ZS, Henry JM, Jones JB, Eglitis MA, Anderson WF, Lothrop CD Jr. Increased efficiency of gene transfer with retroviral vectors in neonatal hematopoietic progenitor cells. Exp Hematol 1990; 18:180-4.
31. Levy JP, Muldoon RR, Zolotukhin S, Link Jr CJ. Retroviral transfer and expression of a humanized, red-shifted green fluorescent protein gene into human tumor cells. Nat Biotechnol 1996; 14:610-4.
32. Cheng L, Du C, Lavau C, et al. Sustained gene expression in retrovirally transduced, engrafting human hematopoietic stem cells and their lympho-myeloid progeny. Blood 1998; 92:83-92.
33. Corrias MV, Scuderi F, Pasino M, et al. Growth factors increase retroviral transduction but decrease clonogenic potential of umbilical cord blood CD34+ cells. Haematologica 1998; 83:580-6.