



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US93/11060 <b>(22) International Filing Date:</b> 15 November 1993 (15.11.93) <b>(30) Priority data:</b> 07/977,702 13 November 1992 (13.11.92) US <b>(60) Parent Application or Grant</b> (63) Related by Continuation US 07/977,702 (CIP) Filed on 13 November 1992 (13.11.92) <b>(71) Applicant (for all designated States except US):</b> BOARD OF REGENTS OF UNIVERSITY OF WASHINGTON [US/US]; Office of Technology Transfer, 4225 Roosevelt Way, N.E., Suite #301, Seattle, WA 98105 (US).		<b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only) :</b> PAPAYANNOPOULOU, Thalia [US/US]; 3336 Cascadia Avenue South, Seattle, WA 98144 (US). <b>(74) Agents:</b> HALEY, James, F., Jr. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020 (US). <b>(81) Designated States:</b> AU, CA, JP, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PERIPHERALIZATION OF HEMATOPOIETIC STEM CELLS  <b>(57) Abstract</b> <p>The invention provides methods for peripheralizing CD34<sup>+</sup> cells, including hematopoietic stem cells. In a first aspect, the method comprises the step of administering a blocking agent of VLA-4 antigen on the surface of CD34<sup>+</sup> cells. In a second aspect, the method comprises administering a blocking agent of VLA-4 antigen on the surface of CD34<sup>+</sup> cells and administering a stimulating agent of CD34<sup>+</sup> cell proliferation <i>in vivo</i>. The method according to the invention is useful in the treatment of cancer or AIDS, and in gene therapy.</p>		

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PERIPHERALIZATION OF HEMATOPOIETIC STEM CELLSField Of The Invention

The invention relates to the manipulation of  
5 hematopoietic stem cells. More particularly, the  
invention relates to methods for increasing the number  
of hematopoietic stem cells in peripheral blood.

BACKGROUND OF THE INVENTION

Hematopoietic stem cells are primitive,  
10 uncommitted progenitor cells that give rise to the  
lymphoid, myeloid and erythroid lineages of cells in  
blood. The stem cell population constitutes only a  
small proportion of the total cells in bone marrow and  
represents even a far more minuscule proportion of the  
15 cells in peripheral blood.

Stem cells have commonly been characterized  
by their surface antigenic determinants. Tsukamoto  
et al., U.S. Patent No. 5,061,620 (1991), teaches that  
a highly stem cell concentrated cell composition is  
20 CD34<sup>+</sup>, CD10<sup>-</sup>, CD19<sup>-</sup> and CD33<sup>-</sup>. Leon et al., Blood  
77:1218-1227 (1991), teaches that about one per cent of  
CD34<sup>+</sup> cells, or about 0.01% of the total marrow cell  
population, do not express differentiation antigens,  
such as CD33 (myeloid lineage), CD71 (erythroid  
25 lineage) or CD10 and CD5 (lymphoid B and T lineage),  
and that reduced expression of CD34 expression during

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maturation is associated with increased expression of the differentiation antigens.

Combinations of antigenic and functional characteristics have also been used to characterize stem cells. Sutherland et al., Proc. Natl. Acad. Sci. USA 87:3584-3588 (1990), teaches that primitive stem cells do not bind to soybean agglutinin, express high levels of CD34, form blast colonies with high plating efficiency and are enriched in long-term culture initiating cells (LTC-IC). Craig et al., Blood Reviews 6:59-67 (1992), teaches that the CFU-GM assay is the most widely used measure of the hematopoietic progenitor viability of a bone marrow or peripheral blood stem cell harvest, and correlates well with per cent CD34<sup>+</sup>. Spangrude, Immunology Today 10:344-350 (1989), teaches that stem cells accumulate low levels of rhodamine 123 relative to other bone marrow cell types. Chaudhary et al., Cell 66:85-94 (1991), teaches that stem cells express high levels of P-glycoprotein relative to other marrow cell types.

The ability to manipulate hematopoietic stem cells has become increasingly important in the development of effective chemotherapeutic and radiotherapeutic approaches to the treatment of cancer. Current approaches to chemotherapy and radiotherapy utilize bone marrow transplantation (BMT). BMT involves removing one to two liters of viable pelvic bone marrow containing stem cells, progenitor cells and more mature blood cells, treating the patient with chemotherapy or radiotherapy to kill tumor cells, and reintroducing bone marrow cells intravenously. BMT, however, suffers from many disadvantages. Harvesting of BM for BMT requires general anaesthesia, which increases both risk and cost. In addition, if cancer cells are present in the marrow and are not rigorously

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purged, recurrence of the disease is a distinct risk. Also, if widespread invasion of bone marrow by cancer cells (myeloma, Waldenstrom's macroglobulinemia) is present, peripheral blood cells are the only option for use in autologous transplantation (ABMT). Finally, patients who have undergone pelvic irradiation are not candidates for ABMT.

As a result of these difficulties, much interest has been developed in providing methods for obtaining stem cells from peripheral blood for autologous supply of stem cells to patients undergoing chemotherapy. Autologous supply of stem cells from peripheral blood would allow the use of greater doses of chemo- or radiotherapy, but with less risk than BMT. In addition, the use of stem cells from peripheral blood does not require anaesthesia to obtain the stem cells. Also, Lowry, *Exp. Hematol.* 20:937-942 (1992), teaches that cancer cells in the marrow tend not to peripheralize. The critical limitation in such a procedure, however, lies in the very small number of stem cells ordinarily present in peripheral blood. Lobo et al., *Bone Marrow Transplantation* 8:389-392 (1991), teaches that addition of peripheral blood stem cells collected in the absence of any peripheralization techniques does not hasten marrow recovery following myeloablative therapy. In contrast, Haas et al., *Exp. Hematol.* 18:94-98 (1990), demonstrates successful autologous transplantation of peripheral blood stem cells mobilized with recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF). Thus, increasing the number of stem cells in peripheral blood by peripheralization techniques is critical to the success of procedures utilizing peripheral blood as a source for autologous stem cell transplantation. Other cytokines may be useful in this regard. Rowe and

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Rapoport, J. Clin. Pharmacol. 32:486-501 (1992), suggests that in addition to GM-CSF, other cytokines, including macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF),  
5 erythropoietin, interleukins-1, -2, -3, -4 and -6, and various interferons and tumor necrosis factors have enormous potential.

Another approach to autologous transplantation is to purify stem cells from peripheral  
10 blood using immunoaffinity techniques. These techniques hold promise not only for autologous stem cell transplantation in conjunction with chemotherapy, but also for gene therapy, in which purified stem cells are necessary for genetic manipulation to correct  
15 defective gene function, then reintroduced into the patient to supply the missing function. However, Edgington, Biotechnology 10:1099-1106 (1992), teaches that current procedures require three separate four  
20 hour sessions to process enough cells in the absence of peripheralization. DePalma, Genetic Engineering News, Vol. 12, May 1, 1992, teaches that this can be improved by treatment with G-CSF for peripheralization.

These studies underscore the importance of developing new methods to effect the peripheralization  
25 of hematopoietic stem cells. One possibility is to search for new ways to release stem cells from the bone marrow environment into the periphery. Unfortunately, little is known about the types of molecular interactions that hold hematopoietic stem cells in the  
30 marrow environment in vivo. Recently, some in vitro studies have been undertaken to look at the role of integrins, fibronectin, and other surface antigens in binding between stem cells and bone marrow stromal cells.

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Integrins are a large family of integral membrane glycoproteins having over 16 heterodimeric members that mediate interactions between cells, interactions between cells and the extracellular matrix, and interactions involved in embryonic development and regulation of T-cell responses. Among integrins, the VLA-5 ( $\alpha^5\beta_1$ ) complex is widely distributed and functions as a receptor for fibronectin. The VLA-4 ( $\alpha^4\beta_1$ ) complex is expressed at substantial levels on normal peripheral blood B and T cells, thymocytes, monocytes, and some melanoma cells as well as on marrow blast cells and erythroblasts. Ligands for VLA-4 are vascular cell adhesion molecule-1 (VCAM-1) and CS-1, an alternately spliced domain within the Hep II region of fibronectin. Another group of integrins (CDIIa/CD18, CDIIb/CD18, and CDIIc/CD18) share the common  $\beta_2$  chain and are variably expressed on peripheral T cells, monocytes, and mature granulocytes. Ligands for  $\beta_2$ -integrins include members of the Ig superfamily (ICAM-1 and ICAM-2) found on activated endothelial cells.

Teixido et al., J. Clin. Invest. 90:358-367 (1992), teaches that in an in vitro model, interactions between VLA-4/VCAM-1, VLA-5/fibronectin and  $\beta_2$ -integrin/ICAM-1 are all important for adhesion between bone marrow stromal cells and cells expressing high levels of CD34. Simmons et al., Blood 80:388-395 (1992), teaches that in an in vitro model, adhesion between stromal cells and CD34<sup>+</sup> cells was predominantly dependent on the VLA-4/VCAM-1 interaction and was largely inhibited by monoclonal antibodies to either VLA-4 or VCAM-1, with fibronectin playing a minor role in binding. Williams et al., Nature 352:438-441 (1991), using in vivo mouse studies, teaches that adhesion of murine hematopoietic stem cells to stromal

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cell extracellular matrix (ECM) is partly promoted by proteolytic fragments of fibronectin containing an alternatively spliced region of the IIICS domain, and suggests that the interaction is likely to be mediated by VLA-4. All of these studies utilized antibodies to prevent adherence between stem cells and their microenvironment. However, none have analyzed whether such interactions are reversible, or perturbable after adherence has taken place. These results indicate the need for further studies to determine what interactions between the bone marrow environment and hematopoietic stem cells are responsible for keeping the stem cells within that environment in vivo and whether such interactions can be perturbed to effect peripheralization of stem cells.

There is, therefore, a need for new methods for peripheralizing stem cells, both for scientific investigatory purposes for understanding the processes of peripheralization and homing, and for the development of better methods of peripheralization for autologous stem cell transplantation in the course of cancer treatment or gene therapy. Preferably, such methods should produce even higher levels of stem cells in peripheral blood than existing methods provide.

#### BRIEF SUMMARY OF THE INVENTION

In a first aspect, the invention provides a novel method for increasing the number of hematopoietic stem cells and CD34<sup>+</sup> cells in peripheral blood, which is also known as "peripheralization" or "mobilization" of hematopoietic stem cells and CD34<sup>+</sup> cells. This method comprises the step of administering a blocking agent of VLA-4 antigens on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells. Various agents can be used to mediate such blocking, including anti-VLA-4 or anti-



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VCAM-1 antibodies which may optionally be single chain, humanized or chimeric, Fab, Fab', F(ab')<sub>2</sub> or F(v) fragments thereof, heavy or light chain monomers or dimers thereof, or intermixtures of the same, soluble  
5 fibronectin, CS-1 peptides or fibronectin peptides containing the amino acid sequence EILDV or conservatively substituted amino acid sequences, or soluble VCAM-1, bifunctional VCAM-1/Ig fusion proteins or VCAM-1 peptides.

10 In another aspect, the invention provides a novel method for peripheralizing hematopoietic stem cells and CD34<sup>+</sup> cells with more predictable greater effectiveness than cytokine treatment alone provides. According to this aspect of the invention, the method comprises  
15 administering a blocking agent of VLA-4 antigens on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells, as in the first aspect of the invention, in combination with a stimulating agent of hematopoietic stem cell proliferation. The step of administering a stimulating  
20 agent of hematopoietic stem cell proliferation can be carried out by using a cytokine, preferably G-CSF, stem cell factor, totipotent stem cell factor, stem cell proliferation factor or GM-CSF, but alternatively M-CSF, erythropoietin, interleukins-1, -2, -3, -4, -6, or  
25 11.

In another aspect, the invention provides an improved method of transplanting peripheral blood stem cells into a patient who has undergone chemotherapy or radiotherapy for cancer. In this method, prior to the  
30 administration of myeloablative chemotherapy or radiotherapy, stem cells are peripheralized from the patient's bone marrow by administration of an agent that mediates blocking of VLA-4 antigens on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells. This  
35 agent may be administered alone, or preferably in

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conjunction with an agent that stimulates proliferation of stem cells. The peripheralized stem cells are then collected from peripheral blood by leukapheresis. Stem cells are then enriched from the collected  
5 peripheralized blood by immunoadsorption using anti-CD34 antibodies. Optionally, the enriched stem cells are then expanded ex vivo by culturing them in the presence of agents that stimulate proliferation of stem cells. Following administration of myeloablative  
10 chemotherapy or radiotherapy, the enriched, and optionally expanded stem cells are then returned to the patient's circulating blood and allowed to engraft themselves into the bone marrow.

In another aspect, the invention provides an  
15 improved method of transplanting peripheral blood stem cells into a patient who has undergone myeloablative chemotherapy or radiotherapy for AIDS. This method involves the same steps as described for transplanting peripheralized stem cells into a patient who has  
20 undergone chemotherapy or radiotherapy for cancer. In addition, this method further optionally involves administration to the patient of anti-HIV agents, such as antivirals such as AZT, soluble CD4, and CD4-directed blockers of the AIDS virus or antisense or  
25 antigene oligonucleotides, both before and after the return of the enriched and optionally expanded stem cells to the patient's circulating blood. This step serves a "mopping up" function to prevent residual virus from infecting the progeny of the newly returned  
30 stem cells.

In another aspect, the invention provides an improved method for carrying out gene therapy in patients having various genetic and acquired diseases. In this method, stem cells are peripheralized from the  
35 patient's bone marrow by administration of an agent

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that mediates blocking of VLA-4 antigens on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells. As in the method previously described herein, this agent may be administered alone or in conjunction with an agent that stimulates proliferation of stem cells. Peripheral blood is then collected by leukapheresis. Stem cells are then enriched from the collected peripheral blood by immunoadsorption using anti-CD34 antibodies. Optionally, the enriched stem cells are then expanded ex vivo by culturing them in the presence of agents that stimulate proliferation of stem cells. The enriched and optionally expanded stem cells are then transduced with an amphotropic retroviral vector, or other suitable vectors, that expresses a gene that ameliorates the genetic or acquired disease. Optionally, the vector may also carry an expressed selectable marker, in which case successfully transduced cells may be selected for the presence of the selectable marker. The transduced and optionally selected stem cells are then returned to the patient's circulating blood and allowed to engraft themselves into the bone marrow.

It is an object of the invention to provide a method for peripheralizing hematopoietic stem cells and CD34<sup>+</sup> cells as an experimental model for investigating hematopoiesis, homing of stem cells to the bone marrow, and cytokine-induced peripheralization of stem cells. It is a further object of the invention to provide a method for optimizing peripheralization of hematopoietic stem cells and CD34<sup>+</sup> cells to provide stem cell-enriched peripheral blood for autologous transplantation following chemo- or radiotherapy. It is a further object of the invention to provide a method for peripheralizing CD34<sup>+</sup> cells to maximize the yield of purified hematopoietic stem cells and

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progenitor cells from peripheral blood, either for autologous transplantation of the stem cells following chemo- or radiotherapy, or for use in gene therapy. It is a further object of the invention to provide a method for peripheralizing stem cells and CD34<sup>+</sup> cells without risk of causing cytokine-induced cell differentiation of normal stem cells or proliferation of contaminating leukemia cells. It is a further object of the invention to provide a peripheralization technique that has predictable timing for the peak of progenitor content in peripheral blood for scheduling leukapheresis.

The invention satisfies each of these objects by providing a method for peripheralizing stem cells and CD34<sup>+</sup> cells by administering a blocking agent of VLA-4 antigen on the surface of hematopoietic stem cells. This effect can be increased by the use of such blocking agents in conjunction with approaches to amplify stem cells to produce a synergistic effect.

#### 20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows profiles of both total white blood cells and CFU in peripheral blood before and after treatment of macaques (panels A and C) or a baboon (panel B) with anti-VLA-4 antibodies (murine monoclonal antibody HP1/2). Dashed lines represent total white blood cell counts, as recorded on the right vertical axes. Cross-hatched boxes represent CFU-GM, as recorded in the left vertical axes. Black boxes represent BFUe, as represented on the left vertical axes. Downward-pointing arrows represent points of administration of antibody. Horizontal axes represent days before and after first administration of anti-VLA-4 antibody.

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Figure 2 shows profiles of both total white blood cells and CFU in peripheral blood before and after treatment of an animal with the anti-CD18 monoclonal antibody 60.3. All symbols are as in

5 Figure 1.

Figure 3 shows results of combined treatment with G-CSF and anti-VLA-4 monoclonal antibody HP1/2. In panel A, symbols are as in Figure 1, except that narrow downward-pointing arrows represent points of  
10 G-CSF administration, bold downward-pointing arrows represent points of antibody administration, and dotted lines (with triangles) represent total lymphocyte counts. In panel B, the same symbols show the results for a control animal treated with GCSF alone.

15 Figure 4 shows high proliferative potential (HPP) progenitors (colonies over 0.5 mm in diameter of compact growth) resulting from combined treatment with GCSF and HP 1/2 antibody (panel A) or GCSF alone (panel B). Symbols are as in Figure 3.

20 Figure 5 shows the nucleotide sequences encoding the variable regions of the heavy and light chains of anti-VLA-4 murine monoclonal antibody HP 1/2. Panel A is the nucleotide sequence encoding the variable heavy region, with the first nucleotide  
25 representing the beginning of the first codon. Panel B is the nucleotide sequence encoding the variable light region, with the first nucleotide representing the beginning of the first codon.

Figure 6 shows results of combined treatment  
30 with 5-fluorouracil and anti-VLA-4 murine monoclonal antibody HP1/2. Symbols are as described for Figure 3. Panel A shows the combined results, whereas Panel B shows the results of 5-fluorouracil treatment alone.

Figure 7 shows the nucleotide sequences of  
35  $V_H$ - and  $V_K$ -encoding regions having CDR-encoding

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sequences from murine HP1/2 transplanted therein. Panel A shows the transplanted  $V_H$  sequence. Panel B shows the transplanted  $V_K$  sequence.

Figure 8 shows the nucleotide sequences encoding the variable regions of the heavy and light chains of the humanized anti-VLA-4 antibody hHP1/2. Panel A is the nucleotide sequence encoding the  $V_H$  region. Panel B is the nucleotide sequence encoding the  $V_K$  region.

Figure 9 shows results of treatment with humanized anti-VLA-4 antibody hHP1/2. Symbols are as described for Figure 3.

Figure 10 shows results of treatment with murine Fab fragments of anti-VLA-4 antibody HP1/2. Symbols are as described for Figure 3.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to the manipulation of hematopoietic stem cells. More particularly, the invention relates to the peripheralization of hematopoietic stem cells and other  $CD34^+$  cells.

In a first aspect, this invention provides a method for peripheralizing hematopoietic stem cells and  $CD34^+$  cells, comprising the step of administering a blocking agent of VLA-4 antigens on the surface of hematopoietic stem cells and  $CD34^+$  cells. For purposes of this invention, the term "blocking agent of VLA-4 antigens" is intended to mean an agent that is capable of interfering with interactions between VLA-4 antigens and either VCAM-1 or fibronectin on the surface of stromal cells or in the extracellular matrix (ECM). As demonstrated herein, such blocking of VLA-4 antigens causes peripheralization of stem cells and  $CD34^+$  cells. This demonstration utilized a monoclonal antibody against VLA-4 as a blocking agent. Those skilled in

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the art will recognize that, given this demonstration, any agent that can block VLA-4 antigens can be successfully used in the method of this invention. Thus, for purposes of this invention, any agent capable of blocking VLA-4 antigens on the surface of hematopoietic stem cells is considered to be an equivalent of the monoclonal antibody used in the examples herein. For example, this invention contemplates as equivalents at least peptides, peptide mimetics, carbohydrates and small molecules capable of blocking VLA-4 antigens on the surface of CD34<sup>+</sup> cells or hematopoietic stem cells.

In a preferred embodiment, the blocking agent that is used in the method of this invention to block VLA-4 antigens on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells is a monoclonal antibody or antibody derivative. Preferred antibody derivatives include humanized antibodies, chimeric antibodies, single chain antibodies, Fab, Fab', F(ab')<sub>2</sub> and F(v) antibody fragments, and monomers or dimers of antibody heavy or light chains or intermixtures thereof. The successful use of monoclonal antibody OKT3 to control allograft rejection indicates that, although humanized antibodies are preferable, murine monoclonal antibodies can be effective in therapeutic applications. Monoclonal antibodies against VLA-4 are a preferred blocking agent in the method according to this invention. Human monoclonal antibodies against VLA-4 are another preferred blocking agent in the method according to the invention. These can be prepared using in vitro-primed human splenocytes, as described by Boerner et al., J. Immunol. 147:86-95 (1991). Alternatively, they can be prepared by repertoire cloning as described by Persson et al., Proc. Natl. Acad. Sci. USA 88:2432-2436 (1991) or by Huang and

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Stollar, J. of Immunol. Methods 141:227-236 (1991).  
Another preferred blocking agent in the method of the present invention is a chimeric antibody having anti-VLA-4 specificity and a human antibody constant region.

5 These preferred blocking agents can be prepared according to art-recognized techniques, as exemplified in U.S. Patent No. 4,816,397 and in Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984). Yet another preferred blocking agent in the method of this

10 invention is a humanized antibody having anti-VLA-4 specificity. Humanized antibodies can be prepared according to art-recognized techniques, as exemplified in Jones et al., Nature 321:522 (1986); Riechmann, Nature 332:323 (1988); Queen et al., Proc. Natl. Acad. Sci. USA 86:10029 (1989); and Orlandi et al., Proc. Natl. Acad. Sci. USA 86:3833 (1989). Those skilled in the art will be able to produce all of these preferred blocking agents, based upon the nucleotide sequence encoding the heavy and light chain variable regions of

20 HP1/2 [SEQ. ID. NOS. 1 and 2], as shown in Figure 5, using only well known methods of cloning, mutagenesis and expression (for expression of antibodies, see, e.g., Boss et al., U.S. Patent No. 4,923,805). Two other preferred blocking agents are single chain

25 antibodies, which can be prepared as described in U.S. Patent No. 4,946,778, the teachings of which are hereby incorporated by reference; and biosynthetic antibody binding sites, which can be prepared as described in U.S. Patent No. 5,091,513, the teachings of which are

30 hereby incorporated by reference. Those skilled in the art will recognize that any of the above-identified antibody or antibody derivative blocking agents can also act in the method of the present invention by binding the receptor for VLA-4, thus acting as agents

35 for blocking the VLA-4 antigen on the surface of



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hematopoietic stem cells, within the meaning of this term for purposes of this invention. Thus, antibody and antibody derivative blocking agents according to this invention, as described above, include embodiments  
5 having binding specificity for VCAM-1 or fibronectin, since these molecules appear to either be important in the adhesion between stem cells and stromal cells or the extracellular matrix or interfere with traffic of stem cells through other tissues and blood.

10 In another preferred embodiment, the blocking agents used in the method according to this invention are not antibodies or antibody derivatives, but rather are soluble forms of the natural binding proteins for VLA-4. These blocking agents include soluble VCAM-1,  
15 bifunctional VCAM-1/Ig fusion proteins, or VCAM-1 peptides as well as fibronectin, fibronectin having an alternatively spliced non-type III connecting segment and fibronectin peptides containing the amino acid sequence EILDV or a similar conservatively substituted  
20 amino acid sequence. These blocking agents will act by competing with the stromal cell- or ECM-bound binding protein for VLA-4 on the surface of stem cells.

In this method according to the first aspect of the present invention, blocking agents are  
25 preferably administered parenterally. The blocking agents are preferably administered as a sterile pharmaceutical composition containing a pharmaceutically acceptable carrier, which may be any of the numerous well known carriers, such as water,  
30 saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, or combinations thereof. Preferably, the blocking agent, if an antibody or antibody derivative, will be administered at a dose between about 0.1 mg/kg body weight/day and about  
35 10 mg/kg body weight/day. For non-antibody or antibody

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derivative blocking agents, the dose range should preferably be between molar equivalent amounts to these amounts of antibody. Optimization of dosages can be determined by administration of the blocking agents, followed by CFU-GM assay of peripheral blood, or assay of CD34<sup>+</sup> cells in peripheral blood. The preferred dosage should produce an increase of at least 10-fold in the CFU-GM counts in peripheral blood.

In a second aspect, the present invention provides a method for peripheralizing hematopoietic stem cells that is far more effective than cytokine treatment alone. According to this aspect of the invention, the method comprises the step of administering a blocking agent of VLA-4 antigens on the surface of hematopoietic stem cells in combination with the step of administering a stimulating agent of hematopoietic stem cell proliferation in vivo. The step of administering a blocking agent of VLA-4 antigens on the surface of hematopoietic stem cells is carried out in exactly the same fashion that is described for the first aspect of the invention. The step of administering a stimulating agent of hematopoietic stem cell proliferation in vivo is preferably carried out through the administration of cytokines.

Preferred cytokines for stimulating hematopoietic stem cells to proliferate include granulocyte colony-stimulating factor (G-CSF), stem cell factor, totipotent stem cell factor (TSCF), stem cell proliferation factor (SCPF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), erythropoietin, interleukin-1, -2, -3, -4, -6, and -11. Most preferred are G-CSF, stem cell factor and GM-CSF, because all three of these are known to cause

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proliferation of stem cells. The ability of G-CSF and GM-CSF to stimulate proliferation of progenitors is well established (see, e.g., Metcalf, Nature 339:27-30 (1989)), as is their ability to cause peripheralization of hematopoietic stem cells (see, e.g., Haas et al., Exp. Hematol. 18:94-98 (1990) and Blood 72:2074 (1988)). This ability has also been established for stem cell factor (Andrews et al., Blood 80:920-927 (1992)). In addition, the enormous potential of these other cytokines identified herein has been recognized (see Rowe and Rapoport, J. Clin. Pharmacol. 32:486-501 (1992)). For purposes of this invention, stimulation of hematopoietic stem cells to proliferate can be carried out by any cytokine that is capable of mediating such proliferation in vivo. Thus, for purposes of this invention, any cytokine that can stimulate hematopoietic stem cells to proliferate in vivo is considered to be equivalent to G-CSF, stem cell factor and GM-CSF, which are also considered to be equivalent to each other. In addition, the use of chemotherapeutic agents alone can lead to the peripheralization of progenitors. Such agents can also be combined with VLA-4 blocking agents in the method according to the present invention.

In this method according to the second aspect of the invention, cytokines are preferably administered parenterally. The cytokines are preferably administered as a sterile pharmaceutical composition containing a pharmaceutically acceptable carrier, which may be any of the numerous well known carriers, such as water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, or combinations thereof. Preferably, the cytokine, if G-CSF, will be administered at a dose between about 1  $\mu\text{g}/\text{kg}$  body weight/day and about 50  $\mu\text{g}/\text{kg}$  body weight/day, most

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preferably at about 10-15  $\mu\text{g}/\text{kg}$  body weight/day. Most preferably, cytokines will be administered over a course of from about four to about ten days. Optimization of dosages or the combination of cytokines (e.g., G-CSF and kit ligand) can be determined by administration of the cytokine and administration of the blocking agents, followed by CFU-GM assay of peripheral blood. The preferred dosage should produce an increase of at least 5-fold in the CFU-GM counts per milliliter of peripheral blood, compared with cytokines alone.

According to this aspect of the present invention, the step of administering a blocking agent of VLA-4 antigens on the surface of hematopoietic stem cells or  $\text{CD34}^+$  cells and the step of administering stimulating agents for proliferation of these cells can be carried out concomitantly or sequentially. In a preferred embodiment, the steps are carried out sequentially, preferably administering stimulating agents of  $\text{CD34}^+$  or hematopoietic stem cell proliferation being the first step.

In a third aspect, this invention provides an improved method of transplanting peripheral blood stem cells into a patient who has undergone chemotherapy or radiotherapy for cancer. In this method, prior to the administration of chemotherapy or radiotherapy, stem cells are peripheralized from the patient's bone marrow by administration of an agent that mediates blocking of VLA-4 antigens on the surface of hematopoietic stem cells and  $\text{CD34}^+$  cells. The blocking agents used in this method are preferably selected from those blocking agents described in the discussion of the first aspect of the invention. This agent may be administered alone, or in conjunction with an agent that stimulates proliferation of stem cells. The proliferation

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stimulating agents optionally used in this method are preferably selected from those proliferation stimulating agents described in the discussion of the second aspect of the invention. The peripheralized stem cells are then collected from peripheral blood by leukapheresis. Stem cells are then enriched from the collected peripheralized blood by CD34 affinity chromatography such as immunoabsorption using anti-CD34 antibodies. Such stem cell enrichment is known in the art and has been described, for example, by Berenson, Transplantation Proceedings 24:3032-3034 (1992) and the references cited therein. Optionally, the enriched stem cells are then expanded ex vivo by culturing them in the presence of agents that stimulate proliferation of stem cells. This ex vivo expansion can be carried out using, alone or in combination, any of the proliferation stimulating agents described in the discussion of the second aspect of the invention. Such ex vivo expansion of CD34<sup>+</sup> cells from peripheral blood is known in the art and has been described, for example, by Bruggar et al., Blood 81:2579-2584 (1993). Following administration of chemotherapy or radiotherapy, the enriched and optionally expanded stem cells are then returned to the patient's circulating blood and allowed to engraft themselves into the bone marrow.

The value of using peripheralized stem cells for transplantation after chemotherapy or radiotherapy for cancer is recognized in the art and has been described in numerous references, including Bensinger et al., Blood 81:3158-3163 (1993); Chao et al., 81:2031-2035 (1993); Kessinger and Armitage, Blood 77:211-213 (1991); Gale et al., Bone Marrow Transplantation 9:151-155 (1992); and Siena et al., Blood 74:1904-1914 (1989). The present method

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according to the invention provides an improvement in the transplantation of stem cells from peripheral blood by increasing the concentration of such stem cells in the peripheral blood, thereby greatly improving the  
5 likelihood of success of the transplantation.

In a fourth aspect, the present invention provides an improved method of transplanting purified peripheral blood stem cells into a patient who has undergone myeloablative chemotherapy or radiotherapy  
10 for AIDS. This method involves the same steps as described for transplanting peripheralized stem cells into a patient who has undergone chemotherapy or radiotherapy for cancer. In addition, this method further optionally involves administration to the  
15 patient of anti-HIV agents, such as antivirals such as AZT, soluble CD4, and CD4-directed blockers of the AIDS virus or antisense or antigene oligonucleotides, both before and after the return of the enriched and optionally expanded stem cells to the patient's  
20 circulating blood. This step serves a "mopping up" function to prevent residual virus from infecting the progeny of the newly returned stem cells.

The myeloablative chemotherapy or radiotherapy will generally be expected to destroy any  
25 cells in the blood that are infected by HIV. The "mopping up" step thus serves to remove any residual virus that otherwise could possibly infect the progeny of the stem cells transplanted into the patient after such therapy. Several agents can be useful in such a  
30 "mopping up" step. For example, CD4-directed anti-HIV agents and analogs have been shown to prophylactically prevent infection of uninfected CD34<sup>+</sup> cells by HIV. Similarly, anti-HIV oligonucleotides have been shown to prevent HIV infection of uninfected cells, for example  
35 in U.S. Patent No. 4,806,463, the teaching of which are

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hereby incorporated by reference. Such oligonucleotides have been shown to prevent virus escape for up to a 100 day test period. See Lisziewicz et al., Proc. Natl. Acad. Sci. USA 90:3860-3864 (1993).

5 Accordingly, this method according to the invention should provide a new therapeutic approach to AIDS.

In a fifth aspect, this invention provides an improved method for carrying out gene therapy in patients having any of a variety of genetic and  
10 acquired diseases. In this method, stem cells are peripheralized from the patient's bone marrow by administration of an agent that mediates blocking of VLA-4 antigens on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells. The blocking agents used in  
15 this method are preferably selected from those blocking agents described in the discussion of the first aspect of the invention. As in the method previously described herein, this agent may be administered alone or in conjunction with an agent that stimulates  
20 proliferation of stem cells. The proliferation stimulating agent optionally used in this method is preferably selected from those proliferation stimulating agents described in the discussion of the second aspect of the invention. Peripheral blood is  
25 then collected by leukapheresis. Stem cells are then enriched from the collected peripheral blood by immunoadsorption using anti-CD34 antibodies. Such stem cell enrichment is known in the art and has been described, for example, by Berenson, Transplantation  
30 Proceedings 24:3032-3034 (1992) and the references cited therein. Optionally, the enriched stem cells are then expanded ex vivo by culturing them in the presence of agents that stimulate proliferation of stem cells. This ex vivo expansion can be carried out using, alone  
35 or in combination, any of the proliferation stimulating

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agents described in the discussion of the second aspect of the invention. Such ex vivo expansion of CD34<sup>+</sup> cells from peripheral blood is known in the art and has been described, for example, by Bruggar et al., Blood 5 81:2579-2584 (1993). The enriched and optionally expanded stem cells are then infected with an amphotrophic retroviral vector, or other appropriate vector, that expresses a gene that ameliorates the genetic or acquired disease. Optionally, the vector 10 may also carry an expressed selectable marker, in which case successfully transduced cells may be selected for the presence of the selectable marker. The transduced and optionally selected stem cells are then returned to the patient's circulating blood and allowed to engraft 15 themselves into the bone marrow. The usefulness of approaches to using stem cells from peripheral blood for retroviral-mediated gene transfer and subsequent transplantation into a patient is recognized in the art and has been described, for example, by Bragni et al., 20 Blood 80:1418-1422 (1992). The present method according to the invention provides an improvement in the transplantation of stem cells from peripheral blood by increasing the concentration of such stem cells in the peripheral blood, thereby greatly improving the 25 likelihood of success of the retroviral transfection and subsequent transplantation and allows for repeated administration of genetically engineered cells in patients with partially ablative regimens and receiving agents that promote proliferation of transduced cells. 30 Such stem cell enrichment is known in the art and has been described, for example, by Berenson, Transplantation Proceedings 24:3032-3034 (1992) and the references cited therein.

The instant invention is useful for many 35 purposes. The methods of peripheralizing hematopoietic



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stem cells or CD34<sup>+</sup> cells is of value in scientific research dedicated to understanding the molecular interactions and molecular signals involved in the homing of these cells to bone marrow, as well as their trafficking in response to certain infections and trauma. This invention also provides sources of peripheral blood that is enriched in CD34<sup>+</sup> and hematopoietic stem cells, thus making the methods of the invention useful for therapeutic applications involving autologous transplantation of these cell types following chemotherapy or radiotherapy or in the course of gene therapy. The present invention provides many advantages over the current exclusively cytokine-based techniques. For example, peripheralization can be obtained without risk of cytokine-induced cell differentiation of normal cells or proliferation of contaminating leukemia cells and can be combined with cytotoxic agents. In addition, in the method of the invention, the timing of the peak of progenitors in peripheral blood is consistently between about 24 and about 72 hours from first injection of antibody, thus making the most beneficial timing for leukapheresis more predictable.

The efficacy of specific embodiments of methods according to both aspects of the instant invention is demonstrated in the examples. According to the first aspect of the invention, monoclonal antibodies against VLA-4 were administered to both macaques and a baboon. These antibodies, mouse monoclonal HP1/2, have previously been described by Pulido et al., J. Biol. Chem. 266:10241 (1991), and are known to block VLA-4 antigen on various cell surfaces. In the present case, administration of these antibodies resulted in as much as a 80-fold increase (average of 40-fold) in CFU-GM present in peripheral blood. The

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well known CFU-GM assay is the most widely used measure of the hematopoietic progenitor viability of a PBSC harvest and correlates well with per cent CD34<sup>+</sup> cells present in peripheral blood (see Craig et al., Blood Reviews 6:59-67 (1992)). Thus, these results demonstrate that, in a primate, administering a blocking agent of VLA-4 antigen on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells results in peripheralization of the hematopoietic stem cells and CD34<sup>+</sup> cells. These results should be applicable to humans as well.

According to the second aspect of the invention, monoclonal antibodies against VLA-4 were administered to a macaque after five days of treatment with G-CSF. It is well known that G-CSF can stimulate hematopoietic stem cells and CD34<sup>+</sup> cells in vivo (see Metcalf, Nature 339:27-30 (1989)). G-CSF alone caused an increase in CFU-GM present in peripheral blood by days 4 and 5 of treatment. After discontinuation of G-CSF treatment and commencement of treatment with anti-VLA-4 antibodies, the number of CFU-GM in peripheral blood increased even more dramatically. It will be recognized by those skilled in the art that G-CSF alone does not cause the type of post-treatment increases in CFU-GM that were observed in the present case, as confirmed by a control experiment using G-CSF alone. Thus, these results demonstrate that, in a primate, administering a blocking agent of VLA-4 antigen on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells in combination with administering a stimulating agent for proliferation of these cells has a synergistic effect. There is no reason to believe that these results will not apply equally well to humans.

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Although not wishing to be bound by theory, Applicant believes that administering a blocking agent of VLA-4 antigens on the surface of hematopoietic stem cells and CD34<sup>+</sup> cells causes peripheralization of these  
5 cells by mediating release of the cells from the marrow environment via disruption of interactions between VLA-4 and its microenvironmental ligands, such as fibronectin and/or VCAM-1 on stromal cells or in the ECM. Administering stimulating agents of hematopoietic  
10 stem cell and CD34<sup>+</sup> cell proliferation is believed to cause peripheralization at least in part via sheer increase in the numbers of these cells. Thus, it is believed that administering a blocking agent of VLA-4 antigens in combination with a stimulating agent of  
15 stem cell proliferation effect peripheralization by complementary mechanisms. The observed synergistic effect between anti-VLA-4 antibodies and G-CSF supports this interpretation. In addition, the observed synergistic effect between anti-VLA-4 antibodies and  
20 5-fluorouracil further confirms this interpretation. Since these mechanisms appear to be complementary, the observed synergistic effect should be observed, regardless of whether administration of the blocking agent of VLA-4 antigens and stimulation of  
25 proliferation are carried out concomitantly or in sequence.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not intended to be limiting in  
30 nature.

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Example 1Peripheralization Of Stem Cells  
Using An Anti-VLA-4 Antibody

Three macaques and one baboon were injected  
5 intravenously with anti-VLA-4 mouse monoclonal antibody  
HP1/2 (1 mg/kg body weight/day) for four consecutive  
days. At various time points during and after  
completion of treatment, peripheral blood was collected  
and mononuclear cells were collected using a  
10 conventional Ficoll-Hypaque separation procedure.  
Total white blood cells were calculated from the number  
of mononuclear cells recovered per milliliter of blood.  
CFU-GM and BFUe were determined according to  
conventional assays (see, e.g., Papayannopoulou et al.,  
15 Science 224:617 (1984)). The results of these studies  
are shown for two macaques (panels A and C) and one  
baboon (panel B) in Figure 1. These results  
demonstrate that treatment of these primates with an  
anti-VLA-4 monoclonal antibody causes a small increase  
20 (up to 2-fold) in the total white blood cell count,  
peaking at about 2 to 4 days after beginning of  
treatment. More importantly, the total CFU-GM per ml  
blood increased much more dramatically (about 40-fold),  
also peaking at about 2 to 4 days after beginning of  
25 treatment. In another macaque, a CFU-GM increase of  
about 8-fold was observed after a single injection of  
antibody (data not shown). Given the well established  
use of the CFU-GM assay to measure the repopulating  
potential of hematopoietic progenitors and the  
30 correlation between CFU-GM and percentage CD34<sup>+</sup>, these  
results establish that the anti-VLA-4 antibodies cause  
peripheralization of stem cells.

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Example 2Failure of CD18 Blocking Agents to Cause  
Peripheralization of Stem Cells

The antigen CD18 is present on stem cells and  
5 is widely believed to be important in interactions  
involving stem cells. To test whether blocking agents  
for CD18 could cause peripheralization of stem cells,  
another macaque was treated with a monoclonal antibody  
against CD18. Antibody was delivered by intravenous  
10 injection for three days at a dosage of 2mg/kg of body  
weight/day. The results of this control experiment are  
shown in Figure 2. Total white blood cell counts did  
increase with this treatment, consistent with previous  
experiments with rabbits. However, total GFU-GM showed  
15 no increase after treatment with anti-CD18 monoclonal  
antibodies. Thus, even though CD18 is widely believed  
to be important in interactions involving stem cells,  
blocking agents of CD18 do not lead to  
peripheralization of stem cells or progenitor cells.  
20 These results confirm that the peripheralization of  
stem cells observed upon treatment with anti-VLA-4  
monoclonal antibody was indeed due to specific blocking  
of VLA-4.

Example 3

25           Synergistic Peripheralization Of Stem Cells  
              Resulting From Treatment With Both  
Anti-VLA-4 Antibody In Combination With G-CSF

A baboon was treated with recombinant human  
G-CSF twice daily for five consecutive days. Each  
30 G-CSF treatment consisted of intravenous injection of  
15 micrograms G-CSF per kilogram of body weight. After  
the five days of G-CSF administration, the baboon  
received two injections, spaced one day apart, of anti-  
VLA-4 monoclonal antibody (HP1/2). Each injection

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contained 1 milligram antibody per kilogram body weight. Total white blood cells and CFU-GM were determined as described in Example 1. The results are shown in Figure 3. As shown in panel A of that figure, G-CSF resulted in the expected increase in CFU-GM by days 4 and 5 of treatment, along with a marked increase in total white blood cells. Surprisingly, after the administration of anti-VLA-4 antibody beginning after the last day of a 5 day G-CSF treatment, yet another marked increase in CFU-GM was observed, this time without any increase in total white blood cells. This second increase resulted in about a six-fold improvement in the number of CFU-GM, relative to G-CSF alone. A control animal treated with G-CSF alone according to the same protocol showed a continuous decline in peripheral blood CFU after cessation of treatment (see figure 3, panel B). These results indicate that treatment with anti-VLA-4 antibody was responsible for this second increase in CFU-GM. Thus, combined treatment with anti-VLA-4 antibody and G-CSF results in a synergistic effect, causing far greater increases in CFU-GM than treatment by either G-CSF or anti-VLA-4 antibodies alone.

#### Example 4

##### 25        Analysis Of High Proliferative Potential Cells In Peripheral Blood Following Combined Treatment With G-CSF And Anti-VLA-4 Antibody

In the experiments described in Example 3, high proliferative potential (HPP) cells were also counted. HPP cells are cells that give rise to colonies that are macroscopically visible, over 0.5 mm in diameter with dense, compact growth on the analysis grid. Presence of these cells is associated with greater repopulation capacity and such cells are believed to be earlier progenitors. The results are

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shown in Fig. 4. The observed disparity in peripheral blood HPP cells between G-CSF treatment alone and G-CSF treatment in combination with anti-VLA-4 antibodies is even greater than the disparity observed for CFU-GM.

5 These results suggest that the combined treatment not only produces more progenitors, but also produces earlier progenitors having potentially greater repopulation capacity.

#### Example 5

10 Synergistic Peripheralization Of Stem Cells  
Resulting From Treatment With Anti-VLA-4  
Antibody In Combination With 5-Fluorouracil

A baboon was treated with the chemotherapeutic agent 5-fluorouracil at a dosage of 100 mg per kilogram body weight. Beginning five days later, the baboon received four injections, spaced one day apart, of anti-VLA-4 monoclonal antibody (HP1/2). Each injection contained one milligram antibody per kilogram body weight. Total white blood cells and CFU-GM were determined as described in Example 1. The results are shown in Figure 6. As shown in panel B of that figure, 5-fluorouracil alone produced a modest increase in CFU-GM at days 11 and 12. Administration of anti-VLA-4 antibody after the 5-fluorouracil, however, resulted in a dramatic further increase in CFU-GM, an increase of greater than ten times that produced by 5-fluorouracil alone. These results indicate that combined treatment with anti-VLA-4 antibody and 5-fluorouracil produces a synergistic effect, causing far greater increases in CFU-GM than treatment with either agent alone. Moreover, when taken together with the G-CSF/ anti-VLA-4 antibody results, these results strongly support the theory that the observed synergism results from stimulation of proliferation of progenitors by one agent and release

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of the progenitors from the marrow by another. Thus, these results strongly suggest that such a synergistic effect can be produced by any agent that can stimulate proliferation, in conjunction with any agent that can  
5 bring about release from the marrow.

#### Example 6

##### Preparation Of A Humanized Anti-VLA-4 Antibody

The complementarity determining regions (CDRs) of the light and heavy chains of the anti-VLA-4  
10 monoclonal antibody HP1/2 were determined according to the sequence alignment approach of Kabat et al., 1991, 5th Ed., 4 vol., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, NIH, USA. The CDRs of murine HP1/2 V<sub>H</sub> correspond to  
15 the residues identified in the humanized V<sub>H</sub> sequences disclosed herein as amino acids 31-35 (CDR1), 50-66 (CDR2) and 99-110 (CDR3), which respectively correspond to amino acids 31-35, 50-65 and 95-102 in the Kabat alignment. The CDRs of murine HP1/2 V<sub>K</sub> correspond to  
20 the residues identified in the humanized V<sub>K</sub> sequences disclosed herein as amino acids 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3), and to the same residues in the Kabat alignment. The Kabat NEWM framework was  
25 chosen to accept the heavy chain CDRs and the Kabat REI framework was chosen to accept the kappa chain CDRs. Transplantation of the CDRs into the human frameworks was achieved by using M13 mutagenesis vectors and synthetic oligonucleotides containing the HP1/2  
CDR-encoding sequences flanked by short sequences  
30 derived from the frameworks. The V<sub>H</sub> mutagenesis vector, M13VHPCR1 contains the NEWM framework and has been described by Orlandi et al., Proc. Natl. Acad. Sci USA 86:3833-3837 (1989). The V<sub>K</sub> mutagenesis vector, M13VKPCR2 contains essentially the REI framework and is



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identical to the M13VKPCR1 vector described by Orlandi et al., except that there is a single amino acid change from Val to Glu in framework 4. Transplanted product was recovered by PCR and cloned into M13mp19 for  
5 sequencing. The transplanted  $V_H$  sequence [SEQ. ID NO:3] is shown in Figure 7, panel A. In addition to the CDR grafting, this product encodes the murine amino acids at positions 27-30 and an Arg to Asp change at position 94. The transplanted  $V_K$  sequence [SEQ. ID  
10 NO:4] is shown in Figure 7, panel B.

Additional modifications were introduced via the two step PCR-directed mutagenesis method of Ho et al., Gene 77:51-59 (1989). For the  $V_H$  sequence, position 24 (Kabat numbering) was changed from Val to  
15 Ala and position 75 (Kabat numbering) was changed from Lys to Ser, then amino acid positions 27-30 and 94 were mutated back to the NEWM sequences. The final humanized  $V_H$  sequence [SEQ. ID NO:5] is shown in Figure 8, panel A. For the  $V_K$  sequence, the same two  
20 step PCR-directed mutagenesis approach was used to introduce additional modifications. The final humanized  $V_K$  sequence [SEQ. ID NO:6] is shown in Figure 8, panel B.

The entire  $V_H$  and  $V_K$  regions of humanized  
25 HP1/2 were cloned into appropriate expression vectors. The appropriate human IgG1, IgG4 or kappa constant region was then added to the vector in appropriate reading frame with respect to the murine variable regions. The vectors were then cotransduced into YB2/0  
30 ray myeloma cells (available from ATCC), which were then selected for the presence of both vectors. ELISA analysis of cell supernatants demonstrated that the humanized antibody produced by these cells was at least equipotent with murine HP1/2. The cell line expressing

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this humanized antibody was deposited with the ATCC on November 3, 1992 and given accession number CRL 11175.

Example 7

5 Peripheralization Of Stem Cells Resulting From Treatment With Humanized Anti-VLA-4 Antibody

Humanized anti-VLA-4 antibodies prepared according to Example 6 were tested for peripheralizing stem cells. The baboon model was used again with three daily antibody injections. The results are shown in 10 Figure 9. As previously shown for murine antibody, the humanized anti-VLA-4 antibody produces a large increase in peripheralized CFU. Thus, humanized VLA-4 antibodies are capable of causing peripheralization of stem cells and progenitor cells in the same manner as 15 the murine monoclonal antibody HP1/2. This result suggests that the humanized antibody may also be capable, like the monoclonal antibody, of acting synergistically in combination with G-CSF for peripheralizing stem cells.

20 Example 8

Peripheralization Of Stem Cells Resulting From Treatment With Anti-VLA-4 Murine Fab Fragment

Fab fragments from the murine antibody HP1/2 were tested for their ability to peripheralize stem 25 cells and progenitor cells. The experiment was performed by administration of 1 mg/kg of Fab fragment twice daily for three days. In this instance, a modest effect (compared with humanized or monoclonal antibody) was observed, due to the rapid clearance of Fab 30 fragments. Though modest, the observed characteristic BFU-e increase validates this result. This result demonstrates that anti-VLA-4 antibody Fab fragments are capable of causing peripheralization of stem cells and

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progenitor cells. This suggests that anti-VLA-4 Fab fragments may be capable of acting synergistically in combination with G-CSF for peripheralizing stem cells. In addition, since the Fab fragments are not known to  
5 have any effector function other than binding antigen, this result suggests that any blocking agent that can bind VLA-4 and thereby block its interaction with VCAM-1 will be capable of peripheralizing stem cells, and in doing so, of acting synergistically with factors  
10 that promote stem cell proliferation.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Papayannopoulou, Thalia (USA only)  
Board of Regents, U.  
Washington (except USA)
- (ii) TITLE OF INVENTION: PERIPHERALIZATION OF HEMATOPOIETIC STEM  
CELLS
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: c/o FISH & NEAVE
  - (B) STREET: 1251 Avenue of the Americas
  - (C) CITY: New York
  - (D) STATE: New York
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 10020
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 07/977,702
  - (B) FILING DATE: 13-NOV-1992
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Haley Jr., James F.
  - (B) REGISTRATION NUMBER: 27,794
  - (C) REFERENCE/DOCKET NUMBER: B173CIP
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (212) 596-9000
  - (B) TELEFAX: (212) 596-9090

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 360 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

- 35 -

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 GAACAGGGCC TGGAGTGGAT TGAAGGATT GATCCTGCGA GTGGCGATAC TAAATATGAC 180  
 CCGAAGTTC AGGTCAAGGC CACTATTACA GCGGACACGT CCTCCAACAC AGCCTGGCTG 240  
 CAGCTCAGCA GCCTGACATC TGAGGACACT GCCGTCTACT ACTGTGCAGA CGGAATGTGG 300  
 GTATCAACGG GATATGCTCT GGACTTCTGG GGCCAAGGGA CCACGGTCAC CGTCTCCTCA 360

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 318 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 GGGCAGTCTC CTAAACTGCT GATATATTAT GCATCCAATC GCTACACTGG AGTCCCTGAT 180  
 CGCTTCACTG GCAGTGGATA TGGGACGGAT TTCACTTTCA CCATCAGCAC TGTGCAGGCT 240  
 GAAGACCTGG CAGTTTATTT CTGTCAGCAG GATTATAGCT CTCCGTACAC GTTCGGAGGG 300  
 GGGACCAAGC TGGAGATC 318

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 429 base pairs
  - (B) TYPE: nucleic acid

- 36 -

(C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ix) FEATURE:

(A) NAME/KEY: sig\_peptide  
 (B) LOCATION: 1..57

## (ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 58..429

## (ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 1..429

## (ix) FEATURE:

(A) NAME/KEY: misc\_feature  
 (B) LOCATION: 1  
 (D) OTHER INFORMATION: /note= "pMDR1019 insert: Stage 1  
 heavy chain variable region"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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-19                -15                -10                -5

GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA          96
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
          1                5                10

CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT          144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile
          15                20                25

AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT          192
Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
          30                35                40                45

GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC          240
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp
          50                55                60

CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC          288
Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
          65                70                75

CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC          336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val

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80	85	90	
TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC			384
Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp			
95	100	105	
TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC			429
Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser			
110	115	120	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 143 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly																			
-19				-15															-5
Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg																			
			1				5						10						
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Asn Ile																			
	15						20						25						
Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu																			
	30					35							40						45
Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp																			
				50									55						60
Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn																			
				65															75
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	110																		120

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 386 base pairs

(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ix) FEATURE:  
 (A) NAME/KEY: sig\_peptide  
 (B) LOCATION: 1..57

(ix) FEATURE:  
 (A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 58..384

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..384

(ix) FEATURE:  
 (A) NAME/KEY: misc\_feature  
 (B) LOCATION: 1  
 (D) OTHER INFORMATION: /note= "pBag190 insert: VK1 (DQL)  
 light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly	
-19 -15 -10 -5	
GTT CAC TCC GAC ATC CAG CTG ACC CAG AGC CCA AGC AGC CTG AGC GCC	96
Val His Ser Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala	
1 5 10	
AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAG GCC AGT CAG AGT GTG	144
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val	
15 20 25	
ACT AAT GAT GTA GCT TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG	192
Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys	
30 35 40 45	
CTG CTG ATC TAC TAT GCA TCC AAT CGC TAC ACT GGT GTG CCA AGC AGA	240
Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg	
50 55 60	
TTC AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC	288
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser	
65 70 75	
CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAG GAT TAT AGC	336







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TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC 384  
 Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp  
     95                    100                    105  
 TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC 429  
 Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser  
 110                    115                    120

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 143 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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 Ala His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg  
                     1                    5                    10  
 Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile  
     15                    20                    25  
 Lys Asp Thr Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu  
     30                    35                    40                    45  
 Glu Trp Ile Gly Arg Ile Asp Pro Ala Ser Gly Asp Thr Lys Tyr Asp  
                     50                    55                    60  
 Pro Lys Phe Gln Val Arg Val Thr Met Leu Val Asp Thr Ser Ser Asn  
                     65                    70                    75  
 Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val  
                     80                    85                    90  
 Tyr Tyr Cys Ala Asp Gly Met Trp Val Ser Thr Gly Tyr Ala Leu Asp  
     95                    100                    105  
 Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Glu Ser  
 110                    115                    120

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:



CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAG GAT TAT AGC 336  
 Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser  
 80 85 90  
 TCT CCG TAC ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGT AAG 384  
 Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys  
 95 100 105  
 TG 386

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 128 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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 1 5 10  
 Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val  
 15 20 25  
 Thr Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys  
 30 35 40 45  
 Leu Leu Ile Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg  
 50 55 60  
 Phe Ser Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser  
 65 70 75  
 Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Asp Tyr Ser  
 80 85 90  
 Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Lys  
 95 100 105

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I claim:

1. A method of peripheralizing CD34<sup>+</sup> cells comprising the steps of administering a blocking agent of VLA-4 antigen on the surface of the CD34<sup>+</sup> cells.

2. The method according to claim 1, wherein the blocking agent is selected from the group consisting of anti-VLA-4 or anti-VCAM-1 antibody which may optionally be human, chimeric, single chain, or humanized, or Fab, Fab', F(ab')<sub>2</sub> or F(v) fragments thereof, fibronectin, fibronectin having an alternatively spliced non-type III connecting segment, fibronectin peptides containing the amino acid sequence EILDV or a similar conservatively substituted amino acid sequence that blocks VLA-4-mediated adhesion, soluble VCAM-1, bifunctional VCAM-1/Ig fusion proteins and VCAM-1 peptides.

3. The method according to claim 1, wherein at least a portion of the CD34<sup>+</sup> cells are hematopoietic stem cells.

4. The method of claim 1, further comprising the step of administering a stimulating agent of CD34<sup>+</sup> cell proliferation in vivo.

5. The method according to claim 2, further comprising the step of administering a stimulating agent of CD34<sup>+</sup> cell proliferation in vivo.

6. The method according to claim 3, further comprising the step of administering a stimulating agent of hematopoietic stem cell proliferation in vivo.

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7. The method according to claim 4, wherein the stimulation is mediated by 5-fluorouracil or a cytokine selected from the group consisting of G-CSF, stem cell factor, GM-CSF, M-CSF, T-SCF, SCPF, IL-1, IL-2, IL-3, IL-4, IL-6 and IL-11.

8. The method according to claim 5, wherein the stimulation is mediated by 5-fluorouracil or a cytokine selected from the group consisting of G-CSF, stem cell factor, GM-CSF, M-CSF, T-SCF, SCPF, IL-1, IL-2, IL-3, IL-4, IL-6 and IL-11.

9. The method according to claim 6, wherein the stimulation is mediated by 5-fluorouracil or a cytokine selected from the group consisting of G-CSF, stem cell factor, GM-CSF, M-CSF, T-SCF, SCPF, IL-1, IL-2, IL-3, IL-4, IL-6 or IL-11.

10. The method according to claim 7, wherein the cytokine is G-CSF.

11. The method according to claim 8, wherein the cytokine is G-CSF.

12. The method according to claim 9, wherein the cytokine is G-CSF.

13. The method according to claim 10, wherein the cytokine is administered before administering the blocking agent of VLA-4 antigen on the surface of the CD34<sup>+</sup> cells.

14. The method according to claim 11, wherein the cytokine is administered before the blocking agents.

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15. A method of treating cancer in a patient comprising the steps of:

- (a) peripheralizing CD34<sup>+</sup> cells by administering a blocking agent of VLA-4 antigen on the surface of such cells;
- (b) collecting peripheral blood containing the CD34<sup>+</sup> cells by leukapheresis;
- (c) enriching the CD34<sup>+</sup> cells by immunoadsorption using anti-CD34 antibodies;
- (d) administering chemotherapy and/or radiotherapy to the patient; and
- (e) returning the enriched CD34<sup>+</sup> cells to the patient's circulating blood.

16. The method according to claim 15, further comprising the step of administering a stimulating agent of CD34<sup>+</sup> cell proliferation in vivo prior to leukapheresis.

17. The method according to claim 15, further comprising the step of expanding the enriched CD34<sup>+</sup> cells ex vivo prior to returning the cells to the patient's circulating blood.

18. The method according to claim 16, further comprising the step of expanding the enriched CD34<sup>+</sup> cells ex vivo prior to returning the cells to the patient's circulating blood.

19. A method of treating AIDS in a patient comprising the steps of:

- (a) peripheralizing CD34<sup>+</sup> cells by administering a blocking agent of VLA-4 antigen on the surface of such cells;



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(b) collecting peripheral blood containing the CD34<sup>+</sup> cells by leukapheresis;  
(c) enriching the CD34<sup>+</sup> cells by immunoadsorption using anti-CD34 antibodies;  
(d) administering myeloablative chemotherapy and/or radiotherapy to the patient; and  
(e) returning the enriched CD34<sup>+</sup> cells to the patient's circulating blood.

20. The method according to claim 19, further comprising administering an anti-HIV agent to the patient prior to returning the enriched CD34<sup>+</sup> cells to the patient's circulating blood.

21. The method according to claim 19, further comprising the step of administering a stimulating agent of CD34<sup>+</sup> cell proliferation in vivo prior to leukapheresis.

22. The method according to claim 19, further comprising the step of expanding the enriched CD34<sup>+</sup> cells ex vivo prior to returning the cells to the patient's circulating blood.

23. The method according to claim 20, further comprising the step of administering a stimulating agent of CD34<sup>+</sup> cell proliferation in vivo prior to leukapheresis.

24. The method according to claim 20, further comprising the step of expanding the enriched CD34<sup>+</sup> cells ex vivo prior to returning the cells to the patient's circulating blood.

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25. A method for carrying out gene therapy in a patient having a genetic or acquired disease, the method comprising the steps of:

(a) peripheralizing CD34<sup>+</sup> cells by administering a blocking agent of VLA-4 antigen on the surface of such cells;

(b) collecting peripheral blood containing the CD34<sup>+</sup> cells by leukapheresis;

(c) enriching the CD34<sup>+</sup> cells by immunoadsorption using anti-CD34 antibodies;

(d) transfecting the enriched CD34<sup>+</sup> cells with a retroviral vector, or other suitable vector that expresses a gene that ameliorates the genetic or acquired disease; and

(e) returning the infected cells to the patient's circulating blood.

26. The method according to claim 25, further comprising the step of administering a stimulating agent of CD34<sup>+</sup> cell proliferation in vivo prior to leukapheresis.

27. The method according to claim 25 further comprising the step of administering a stimulating agent of the CD34<sup>+</sup> cell proliferation ex vivo prior to infecting the cells.

28. The method according to claim 26 further comprising the step of administering a stimulating agent of the CD34<sup>+</sup> cell proliferation ex vivo prior to infecting the cells.

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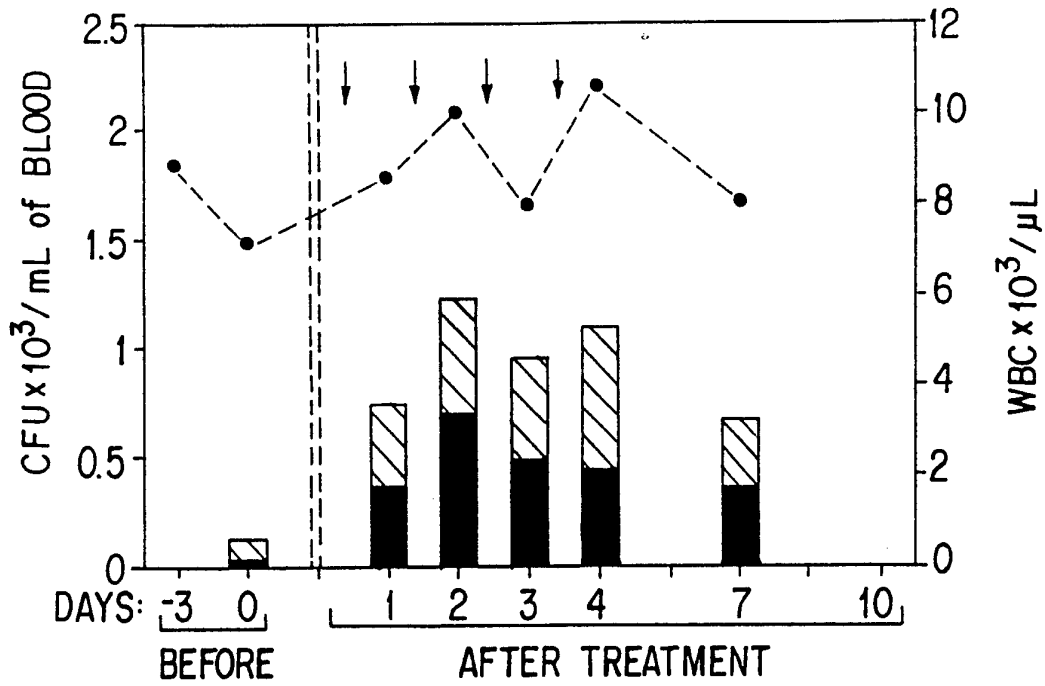


FIG. 1A

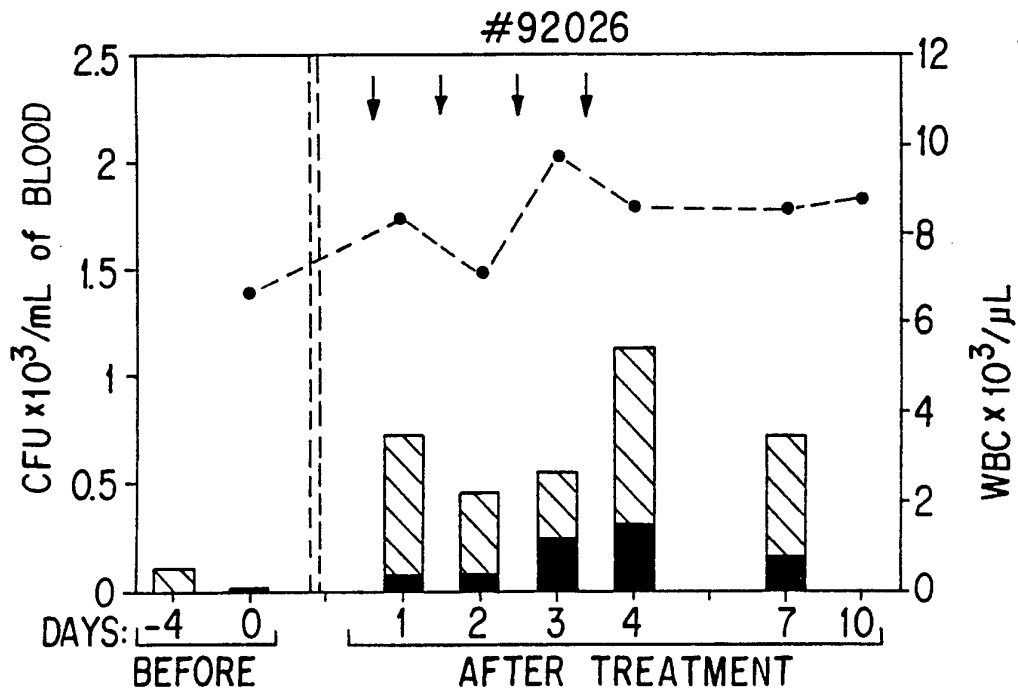


FIG. 1B

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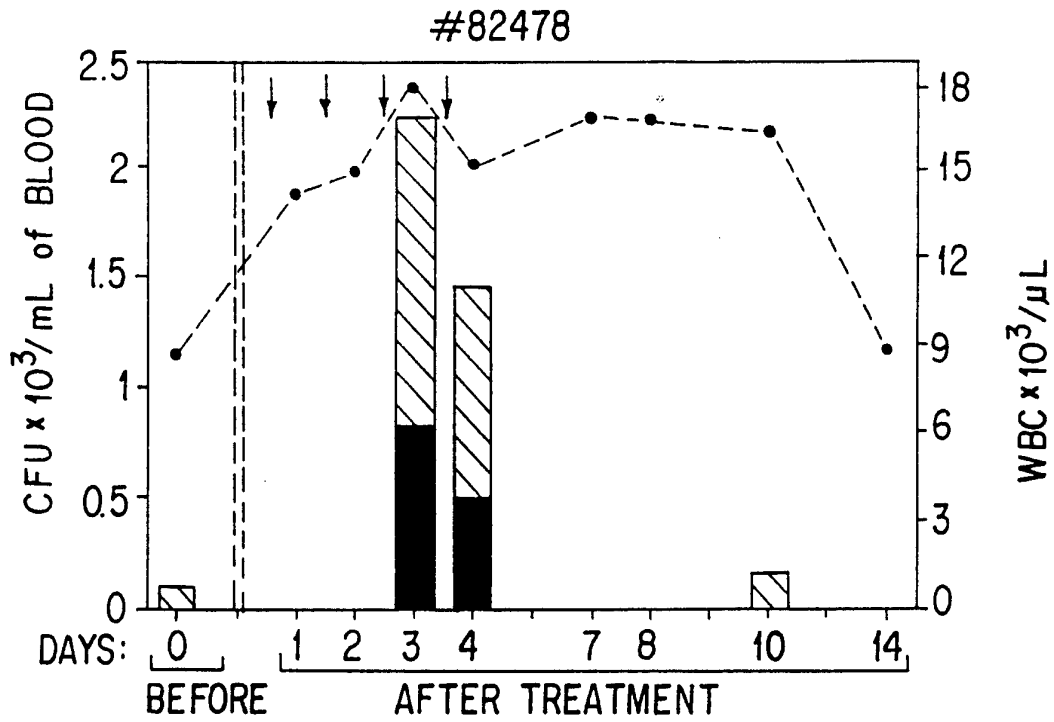


FIG. 1C

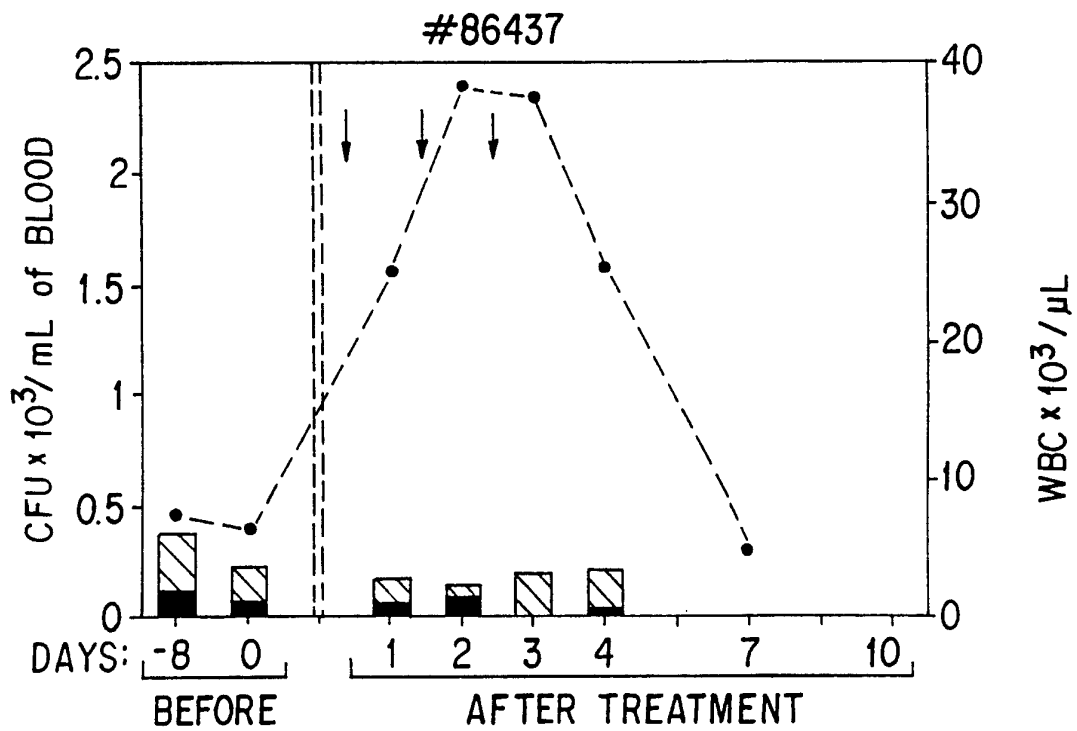


FIG. 2

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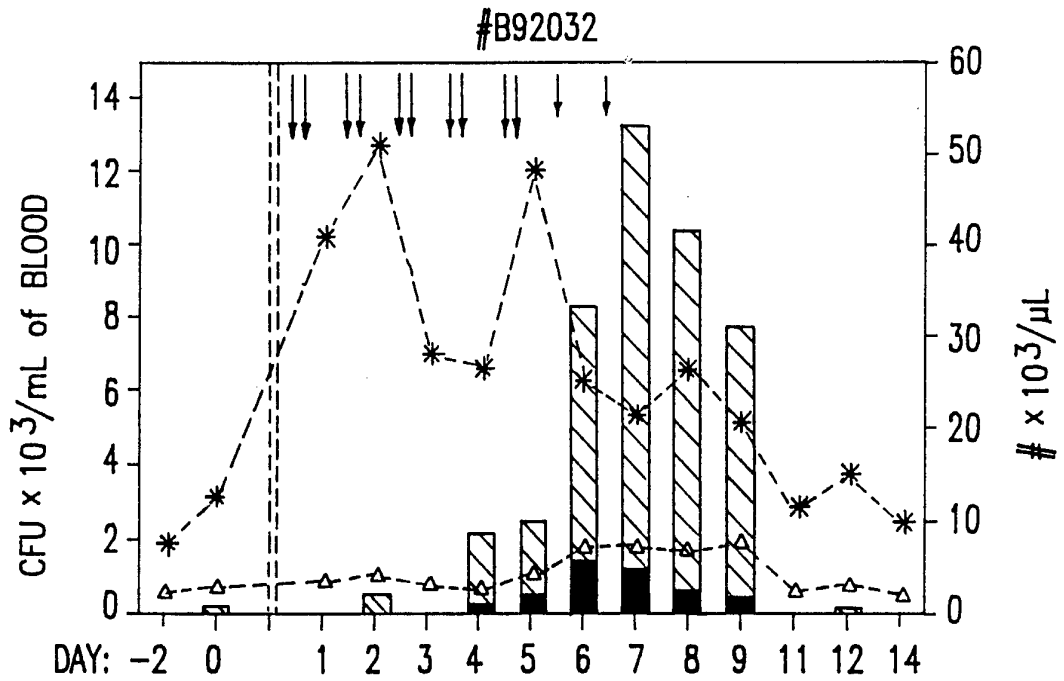


FIG.3A

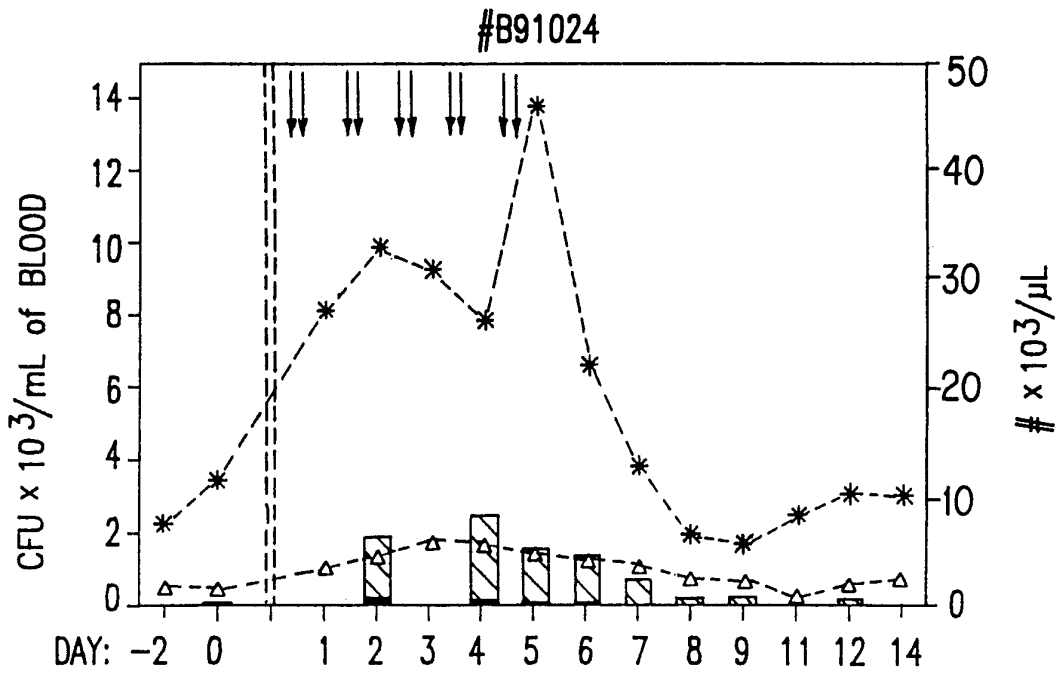


FIG.3B

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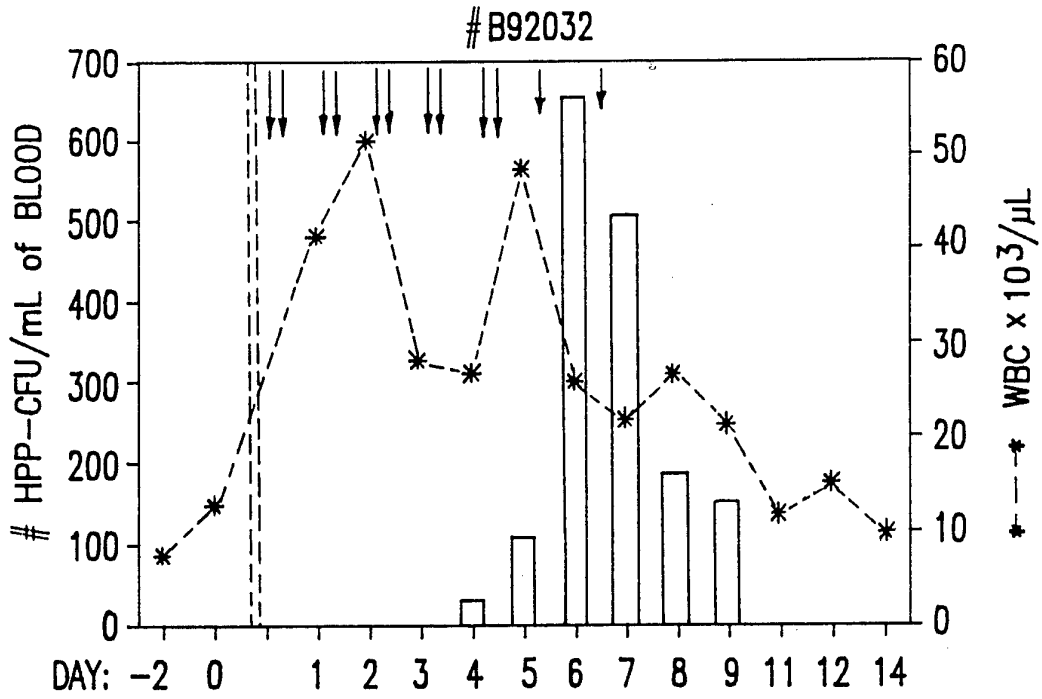


FIG.4A

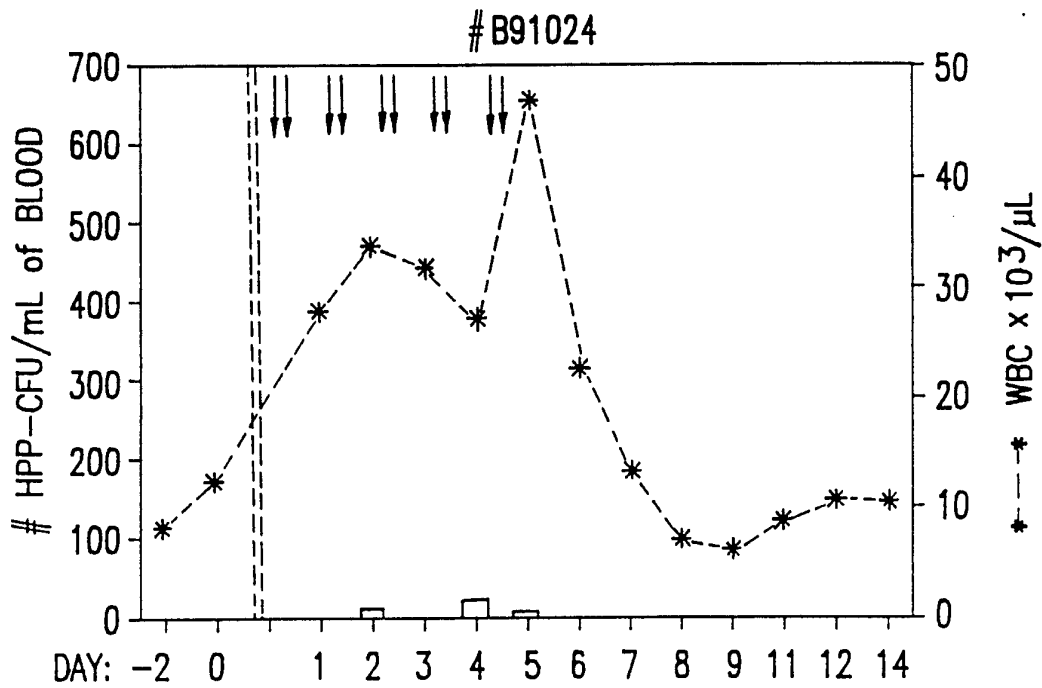


FIG.4B

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GTCAAACAGCAGCTCTGGGGCAGAGCTTGTGAAGCCAGGGCCCTCAGT  
CAAGTTGTCTGCACAGCTTCTGGCTTCAACATTAAGACACCTATATGC  
ACTGGGTGAAGCAGAGGCCCTGAACAGGGCCCTGGAGTGGATTGGAAGGATT  
GATCCTGCGAGTGGCGATACTAATAATATGACCCGGAAGTCCAGGTCAAGGC  
CACTATTACAGCGGACACGTCCTCCACACAGCCTGGCTGCAGCTCAGCA  
GCCTGACATCTGAGGACACTGCCGCTCTACTACTGTGCAGACGGAAATGTGG  
GTATCAACGGGATATGCTCTGGACTTCTGGGGCCAAAGGACCACGGTCAC  
CGTCTCCTCA

FIG. 5A

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AGTATTGTGATGACCCAGACTCCCAAATTCCCTGCTTGTTCAGCAGGAGA  
CAGGGTTACCATAACCTGCAAGGCCAGTCAGAGTGCTACTAATGATGTAG  
CTTGGTACCAACAGAAGCCAGGGCAGTCTCCTAAACTGCTGATATATAT  
GCATCCAATCGCTACACTGGAGTCCCTGATCGCTTCACTGGCAGTGGATA  
TGGGACGGATTTCACTTTCACCATCAGCACTGTGCAGGCTGAAGACCTGG  
CAGTTTATTCTGTCAGCAGGATTATAGCTCTCCGTACACGTTCCGGAGGG  
GGGACCAAGCTGGAGATC

FIG. 5B



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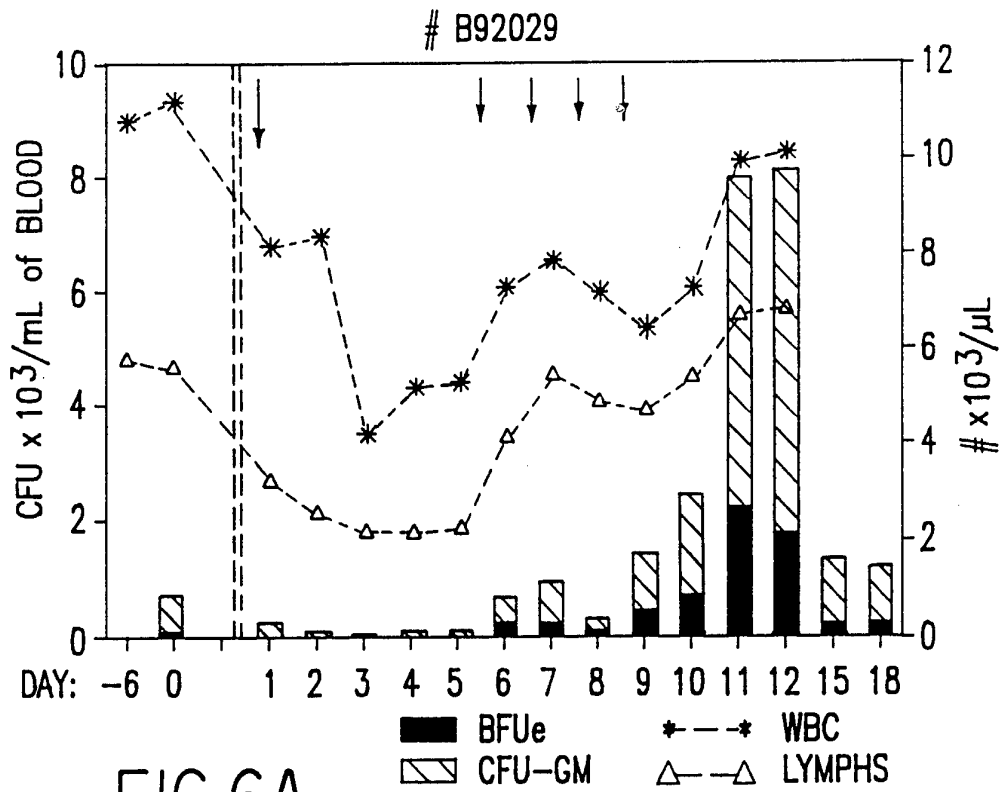


FIG. 6A

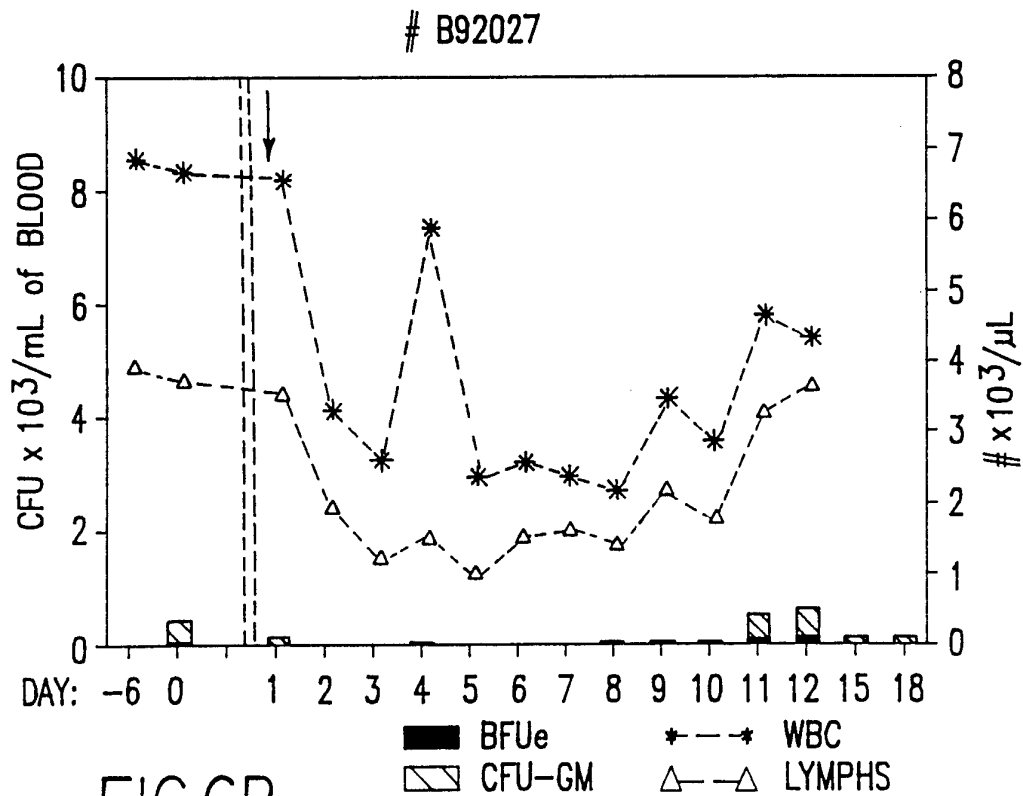


FIG. 6B

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ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT  
GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA  
CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GTG TCT GGC TTC AAC ATT  
AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT  
GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC  
CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC  
CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC  
TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC  
TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC

FIG. 7A

ATG GGT TGG TCC TGC ATC ATC CTG TTC CTG GTT GCT ACC GCT ACC GGT  
GTT CAC TCC GAC ATC CAG CTG ACC CAG AGC CCA AGC CTG AGC GCC  
AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAG GCC AGT CAG AGT GTG  
ACT AAT GAT GTA GCT TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG  
CTG CTG ATC TAC TAT GCA TCC AAT CGC TAC ACT GGT GTG CCA AGC AGA  
TTC AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC  
CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAG GAT TAT AGC  
TCT CCG TAC ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGT AAG  
TG

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FIG. 7B

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ATG GAC TGG ACC TGG AGG GTC TTC TGC TTG CTG GCT GTA GCA CCA GGT  
GCC CAC TCC CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA  
CCT AGC CAG ACC CTG AGC CTG ACC TGC ACC GCG TCT GGC TTC AAC ATT  
AAA GAC ACC TAT ATG CAC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT  
GAG TGG ATT GGA AGG ATT GAT CCT GCG AGT GGC GAT ACT AAA TAT GAC  
CCG AAG TTC CAG GTC AGA GTG ACA ATG CTG GTA GAC ACC AGC AGC AAC  
CAG TTC AGC CTG AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC  
TAT TAT TGT GCA GAC GGA ATG TGG GTA TCA ACG GGA TAT GCT CTG GAC  
TTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GAG TCC

FIG. 8A

ATG GGT TGG TCC TGC ATC ATC CTG TTC CTG GTT GCT ACC GCT ACC GGT  
 GTC CAC TCC AGC ATC GTG ATG ACC CAG AGC CCA AGC CTG AGC GCC  
 AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAG GCC AGT CAG AGT GTG  
 ACT AAT GAT GTA GCT TGG TAC CAG CAG CCA GGT AAG GCT CCA AAG  
 CTG CTG ATC TAC TAT GCA TCC AAT CGC TAC ACT GGT GTG CCA GAT AGA  
 TTC AGC GGT AGC GGT TAT GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC  
 CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAG GAT TAT AGC  
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TG

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FIG. 8B

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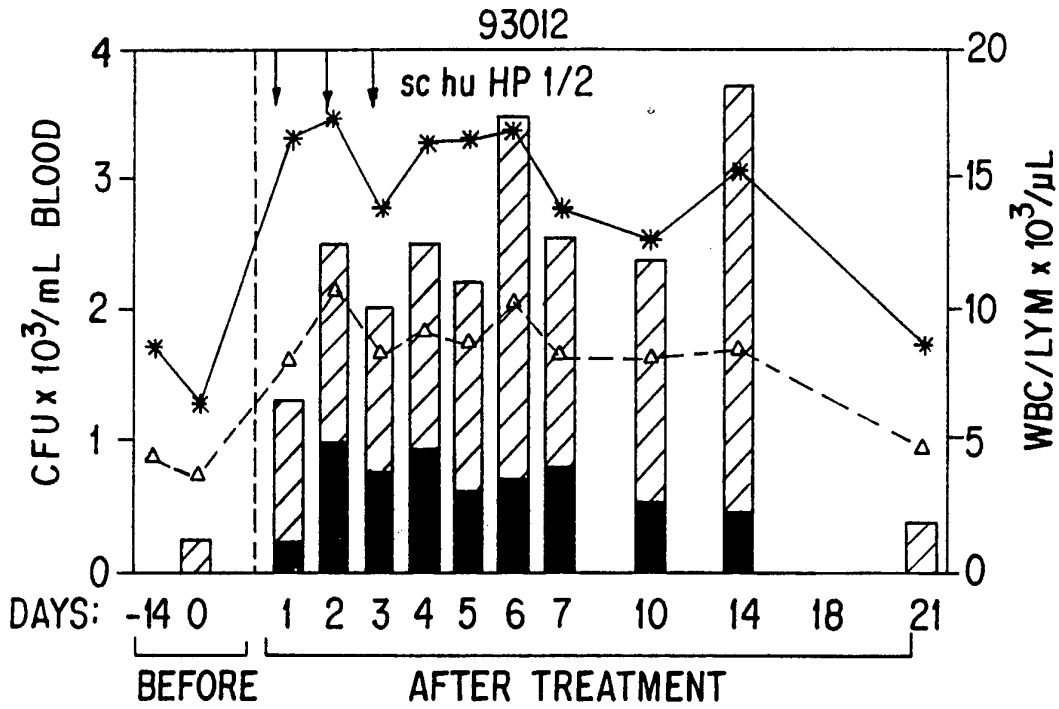


FIG. 9

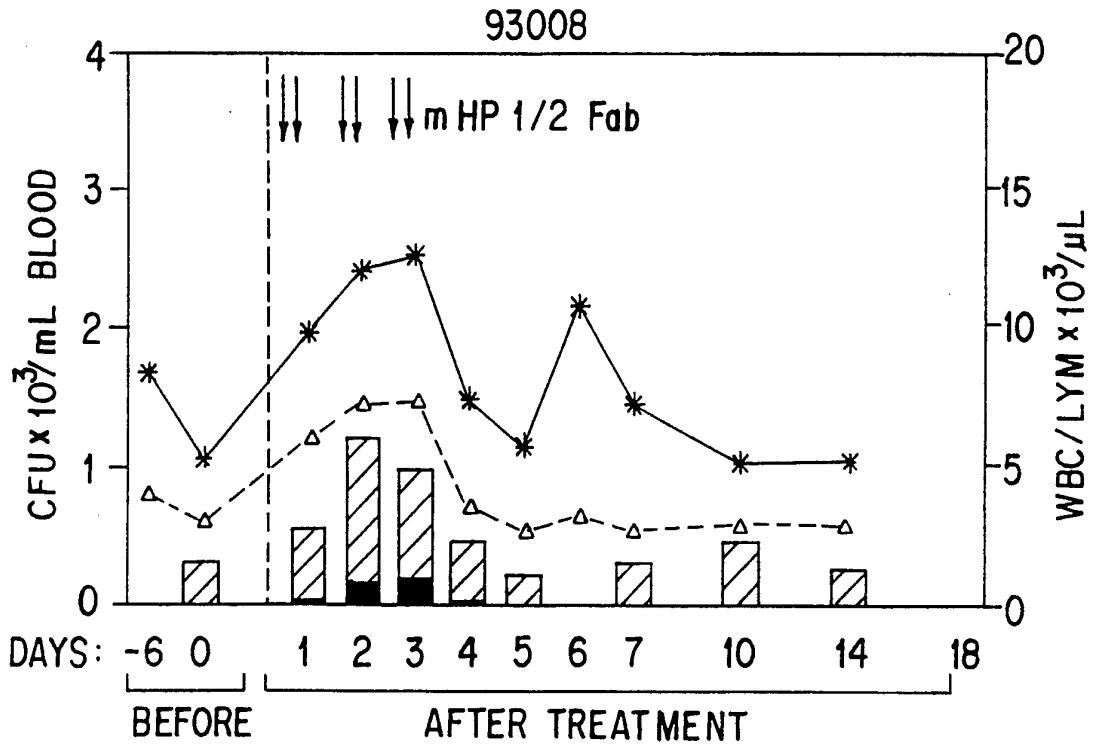


FIG. 10

## INTERNATIONAL SEARCH REPORT

Int ional Application No PCT/US 93/11060
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A. CLASSIFICATION OF SUBJECT MATTER IPC 5 A61K39/395 A61K37/02 A61K31/505 A61K35/28 A61K48/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 5 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BLOOD vol. 80, no. 2 , 15 July 1992 , NEW YORK, USA pages 388 - 395 P. SIMMONS ET AL. 'Vascular cell adhesion molecule-1 expressed by bone marrow stromal cells mediates the binding of hematopoietic progenitor cells.' cited in the application	1-9, 13, 14
Y	see abstract ---	25-28
Y	EP,A,0 455 482 (BECTON DICKINSON AND COMPANY) 6 November 1991 see claims ---	25-28
	-/--	
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents :		
'A' document defining the general state of the art which is not considered to be of particular relevance 'E' earlier document but published on or after the international filing date 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) 'O' document referring to an oral disclosure, use, exhibition or other means 'P' document published prior to the international filing date but later than the priority date claimed		'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. '&' document member of the same patent family
Date of the actual completion of the international search  8 April 1994		Date of mailing of the international search report  15 -04- 1994
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016		Authorized officer  Nooij, F

## INTERNATIONAL SEARCH REPORT

Int ional Application No  
PCT/US 93/11060

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>JOURNAL OF CLINICAL INVESTIGATIONS vol. 90, no. 2 , August 1990 , NEW YORK, USA pages 358 - 367 J. TEIXIDO ET AL. 'Role of beta 1 and beta 2 integrins in the adhesion of human CD34hi stem cells to bone marrow stroma.' cited in the application see the whole document ---</p>	1-9, 13, 14
P,X	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA vol. 90, no. 20 , 15 October 1993 , WASHINGTON DC, USA pages 9374 - 9378 T. PAPAYANNOPOULOU ET AL. 'Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin.' see the whole document ---</p>	1-14
P,X	<p>JOURNAL OF IMMUNOLOGY vol. 149, no. 11 , 1 December 1992 , BALTIMORE MD, USA pages 3759 - 3764 D. RYAN ET A. 'Inhibition of human bone marrow lymphoid progenitor colonies by antibodies to VLA integrins.' see abstract ---</p>	1-3
P,X	<p>BLOOD vol. 81, no. 1 , 1 January 1993 , NEW YORK, USA pages 112 - 121 J. LIESVELD ET AL. 'Expression of integrins and examination of their adhesive function in normal and leukemic hematopoietic cells.' see abstract -----</p>	1-3



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/11060

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0455482	06-11-91	AU-B- 641230	16-09-93
		AU-A- 7429391	07-11-91
		JP-A- 5076354	30-03-93
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