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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 January 2004 (08.01.2004)

PCT

(10) International Publication Number
WO 2004/002425 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number:
PCT/US2003/020520

(22) International Filing Date: 27 June 2003 (27.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/392,590 28 June 2002 (28.06.2002) US
10/357,706 4 February 2003 (04.02.2003) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/002425 A2

(54) Title: PROCESS FOR PROMOTING GRAFT ACCEPTANCE BY DEPLETION OF HEMATOPOIETIC STEM CELLS

(57) Abstract: Therapeutic compositions and methods for modulating the immune system for promoting acceptance of a graft and for treating a hematologic malignancy in the absence of myeloablative therapies are disclosed. The described methodology includes the step of depleting and/or inactivating the hematopoietic stem cells of the recipient using a therapeutic composition comprising at least one antibody specific for depleting and/or inactivating hematopoietic stem cells while maintaining mature blood cells, preferably the antibody selectively depletes and/or inactivates primitive hematopoietic stem cells. The therapeutic composition preferably is administered prior to the administration of bone marrow, mobilized peripheral hematopoietic stem cells, or donor leucocytes.

PROCESS FOR PROMOTING GRAFT ACCEPTANCE BY DEPLETION OF HEMATOPOIETIC STEM CELLS

This application claims priority of U.S. Provisional Application Serial No. 60/392,590, filed 28 June 2002, and U.S. Application Serial No.10 /357,706, filed 4 February 2003, the disclosures of which are hereby incorporated by reference in their entirety.

GOVERNMENT RIGHTS

The research leading to the disclosed invention was at least partly funded by the National Institutes of Health, Grant No. 1R43CA096457-1, and therefore the U.S. Government may have rights in this invention.

FIELD OF INVENTION

The present invention relates generally to a method for promoting acceptance in a recipient of a graft in the absence of myeloablative therapies. The method includes the step of using antibodies to deplete and/or inactivate hematopoietic stem cells (HSC), preferably primitive hematopoietic stem cells (PHSC), and most preferably to selectively deplete and/or inactive at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells and subsequently administering bone marrow

cells (BMC) or mobilized peripheral hematopoietic stem cells (MPHSC), preferably from a donor.

BACKGROUND OF THE INVENTION

The mammalian hematopoietic system includes a heterogeneous array of cells ranging from large numbers of differentiated cells with defined function, such as B cells and T cells, to rare pluripotent stem cells with extensive developmental and proliferative potential. Methods for distinguishing stem cell lineage and developmental potential have been imprecise and have used phenotypic and functional characteristics. The defining feature of a hematopoietic stem cell (HSC) that has been found to be useful is the ability of HSC to repopulate the hematopoietic system of a recipient after transplantation, particularly after whole body irradiation treatment. HSCin bone marrow have been found to be able to continuously regenerate all blood and immune cell lineages. HSC are also demonstrable in the yolk sac and later in the fetal liver.

Bone marrow cell (BMC) transplantation procedures have been utilized in replacement therapies both for treatment of hematologic disorders, such as hematologic cancers, and for modulating the immune system of recipients prior to solid organ transplants. Bone marrow cells and mobilized peripheral stem cells (MPSC) provide sources of primitive hematopoietic stem cells as well as HSC. Both donor bone marrow cells and donor grafts bear major histocompatibility (MHC) markers as well as other cell surface antigens. Such markers permit an organism to determine self from non-self. When a patient's immune system is intact, non-self recognition can lead to rejection.

When a recipient is properly conditioned to receive a donor graft, an active state of unresponsiveness is seen with respect to the lymphoid cells' response to a specific antigen such as an MHC marker or pattern of antigens

as a result of their interactions with that antigen or antigens. Specific tolerance is achieved. The principle of the induction of deletional ("central") tolerance to an allograft has been illustrated in man. Hosts which receive complete allogeneic donor bone marrow transplants accept a renal allograft from the same donor without immunosuppression. However, full allogeneic bone marrow transplantation as currently practiced utilizing extensive myeloablative conditioning is limited in its applicability to patients of a particular age range and medical history. Myeloablative conditioning regimes including high doses of total body irradiation (TBI) are often used in BMC transplantation in conjunction with treatments designed to prevent immunological rejection (e.g., cyclophosphamide). Such conditioning is used for procuring engraftment of transplanted allogeneic donor BMC stem cells while eliminating or reducing GvHD in the recipient. However, these treatments can have undesired side effects, such as toxicity (e.g. nephrotoxicity, hyperlipidemia, bone marrow suppression) and the complications of immunodeficiency (for example, infection and malignancy) on the recipient. These side effects are thought to be due in part to cytokine-induced adverse reactions and can result in damage to the recipient's organ systems. Therefore, less toxic pre- and post-transplant conditioning regimens are highly desirable.

In a number of rodent model systems, and subsequently, in non-human primates, it has been demonstrated that the induction of mixed lymphohematopoietic chimerism in a recipient, by BMC hematopoietic stem cell transplantation from the organ donor, will also result in donor-specific immunologic unresponsiveness to a solid organ allograft (Sykes, United States Patent No. 6,006,752, the disclosure of which is incorporated herein by reference). Mixed chimerism is achieved when a percentage of the donor bone marrow cells populate the lymphoid tissue of the host, also known as the recipient. Mixed chimerism has further advantages in that it can be produced without myeloablative conditioning, thereby preserving a greater level of immunocompetence against non-donor antigens, (e.g., infectious agents),

while still resulting in donor-specific immunologic unresponsiveness. Non-myeloablative conditioning regimens (see, for example, Slavin et al. *Current Opinions of Transplantation*, **4**: 184-188 (1999); Giralt et al. *Blood*, **89**: 4531-453 (1997); Sykes et al. *Lancet*. **353**:1755-1759 (1997); Spitzer et al *Transplantation*. **68**, 4:480-4 (1999); Spitzer et al *Biol Blood Marrow Transplant*. **6**, 3A:309-20 (2000), and Feinstein and Storb. *Curr Opin Oncol*,**13**: 95-100 (2001)) are known. Such non-myeloablative conditioning regimes comprise the use of reduced radiation together with immunosuppressive agents such as cytotoxic drugs (e.g., cyclophosphamide and/or fludarabine) and/or T cell depleting antibodies (e.g., anti-thymocyte globulin, CAMPATH, OKT3 and MEDI-507) to reduce the recipient's ability to reject the BMC. A desire for even milder conditioning regimens and methods of enhancing the reliability of the induction of mixed chimerism still remains

Donor lymphocyte infusion (DLI) has emerged as an effective method of treating patients having multiple myeloma who have relapsed after bone marrow transplantation. DLI is effective in inducing a graft-versus-leukemia (GVL) response in patients with chronic myelogenous leukemia (CML) and may be useful in treating non-Hodgkins lymphoma (Sykes et al., 1999, *Hematology* 405-412; Spitzer, et al., 2000, *Biol. Blood Marrow Transplant*. 6:309-320; Alysea, et al.,2001, *Blood* 98(4):934-939). At least partially due to the difficulties of achieving mixed chimerism and the occurrence of treatment-related complications, hematopoietic cell transplantation has yet to realize its full potential for the treatment of hematologic malignancies. A major obstacle to further advancement of treatment of blood borne cancers is graft-versus-host disease (GvHD) and its treatment through immunosuppression. In an attempt to reduce the need for immunosuppressive drugs, GvHD has been treated by removing T cells from the donor stem cell preparation prior to its infusion into the host (Sehn et al., 1999, *J. Clin. Oncol.* 17(2): 561-568). Unfortunately, such T cell depletion has been associated with increased rates of engraftment failure (Gorner, et al., Bone Marrow Transplant 2002 Apr;29(7):621-4 and leukemic response (Cahn, et al., Hematol Cell Ther 1999 Apr;41(2):31-7). Despite

improvements in pharmacologic GvHD prophylaxis, severe acute and chronic GvHD are still major complications of HLA-matched sibling bone marrow transplantation. Immunosuppressive drugs used for GvHD prophylaxis may also increase the relapse rate for certain types of leukemia.

One attempt to reduce or eliminate the need for immunosuppressive therapy has involved increasing the number of infused donor stem cells. G-CSF was used to mobilize peripheral stem cells (MPSC) in the donor. Infusion of a high dose of MPSC combined with non-myeloablative conditioning resulted in an improved graft acceptance and reduced GvHD. However, procuring sufficient MPSC is difficult to achieve using current clinical practice (Reisner and Martelli, US Patent Number 5,806,529) and such high dose infusions may themselves be harmful to the recipient.

BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a method of promoting tolerance in a recipient to a graft in the absence of myeloablative conditioning. Preferably, an antibody such as a monoclonal antibody that depletes and/or inactivates hematopoietic stem cells (HSC), preferably primitive hematopoietic stem cells (PHSC), and most preferably to selectively deplete and/or inactive at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells is administered to the recipient prior to graft transplantation. PHSC can be identified by the ability to form colonies that survive for at least four weeks and preferably beyond in a cobblestone area forming cell (CAFC) assay In one embodiment, specific monoclonal antibodies are produced for use in the present invention, in particular in rats using human primitive hematopoietic stem cell membranes. In a particular embodiment, such an anti-PHSC antibody is an anti-c-kit (or anti-

CD117) antibody, which is administered to the prospective recipient prior to introduction of donor BMC or MPHSC. In alternative embodiments, the antibody is a monoclonal antibody (MAb) and is chosen from the group comprising an anti-CD135 MAb, an anti-VEGFR2 (KDR) MAb, anti-CD133 (AC133) MAb, an anti-TIE MAb, an anti-TEK MAb, an anti-C1qRp MAb and an anti-CD117 MAb or combinations thereof. Antibodies useful in practicing the invention are not limited to those capable of depleting or inactivating hematopoietic stem cells expressing the CD34 antigen but may include those capable of depleting or inactivating $CD34^-$ cells as well.

The invention also relates to a method of facilitating engraftment of donor mammalian hematopoietic pluripotent stem cells in a mammalian recipient using an antibody capable of depleting or inactivating PHSC of the prospective recipient of donor hematopoietic stem cells. Useful antibodies comprise monoclonal, recombinant, and humanized antibodies capable of depleting and/or inactivating hematopoietic stem cells (HSC), preferably primitive hematopoietic stem cells (PHSC), and most preferably capable of selectively depleting and/or inactiving at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells. Such antibodies are administered to the recipient (or prospective graft recipient) prior to donor stem cell transplantation thereby reducing or eliminating the level of cytotoxic drugs or radiation administered or the requirement for donor T cells in the non-myeloablative protocols that are currently used as standard therapy. Preferably, the need for total body irradiation (TBI) is eliminated.

It is one object of the present invention to facilitate use of a less toxic conditioning regimen for establishing mixed hematopoietic cell chimerism (a) in the treatment of malignant and non-malignant diseases, particularly of the blood; (b) in the promotion of immunological acceptance for cellular, tissue, and/or solid organ transplantation; (c) to prevent or reduce graft-versus-host disease (GvHD); (d) to provide a platform for administering donor-leukocyte infusions

(DLI); (e) in the treatment of enzyme deficiency diseases; and (f) in the treatment of autoimmune diseases.

The present invention also relates to a process for inducing acceptance in a recipient mammal of a first species to a graft from a donor mammal of a second species by introducing, such as by intravenous or intraperitoneal injection into the recipient mammal, at least one antibody that specifically binds to and depletes and/or inactivates hematopoietic stem cells (HSC), preferably primitive hematopoietic stem cells (PHSC), and most preferably to selectively deplete and/or inactive at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells of the recipient; and, preferably subsequently, introducing hematopoietic stem cells of the donor into the recipient mammal, and further, preferably implanting a graft from the donor or from a syngeneic donor in the recipient.

The recipient mammal can be, by way of example, a human. The donor mammal can be, by way of example, a human, a non-human primate, a swine, e.g., a miniature swine, or a sheep. Preferably, when the donor is a miniature swine, the swine is from a highly inbred herd (Sachs et al., USSN 09/378,684, the disclosure of which is hereby incorporated by reference) such that different members of the herd can be donors of BMC and graft(s) to a single recipient.

In some embodiments, the present invention contemplates processes wherein multiple donor hematopoietic stem cell administrations are provided to a recipient. In other preferred embodiments, multiple administrations of donor stem cells are provided prior to the implantation of a graft. Two, three, four, five, or more administrations can be provided. In preferred embodiments, a subsequent administration of donor hematopoietic stem cells is provided: at

least two days, one week, one month, or six months after the previous administration of stem cells.

In preferred embodiments, the methods of the invention contemplate inactivating natural killer cells, preferably graft reactive or xenoreactive, preferably swine reactive, NK cells, of the recipient mammal, and preferably by administering to the recipient mammal at least one antibody capable of binding to and depleting or inactivating natural killer cells of the recipient mammal, especially wherein the administration of antibodies, or other treatment to inactivate natural killer cells, occurs prior to introducing hematopoietic stem cells to the recipient mammal or prior to implanting the graft in the recipient.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows staining intensity profiles for biotinylated pSCF and ACK45 and 3C1 antibodies on P815 cells with or without precoating with either ACK2 or 2B8.

FIGURE 2 is the timeline protocol for *in vivo* treatment with 2B8 MAbs in mice.

FIGURE 3 shows effect of 2B8 antibody or Busulfex treatment of B6 mice on the number of bone marrow CFU-Cs (left panel–individual mice) and CAFCs (right panel – pooled for each treatment group with 95% confidence limits). Also shown is depletion of late-developing CAFCs after treatment with another anti-CD117 MAb (3C1).

FIGURE 4 shows effect of 2B8 antibody or Busulfex treatment of BALB/c mice on the number of bone marrow CFU-Cs (left panel–individual mice) and

CAFCs (right panel – pooled for each treatment group with 95% confidence limits).

FIGURE 5 is the timeline protocol for *in vivo* treatment with 2B8 MAb in mice.

FIGURE 6 shows the number of lineage negative (Lin-) bone marrow cells expressing Sca-1 following treatment with anti-CD117 MAb (2 x 0.05 or 0.125 mg 2B8 at –4 and –2 days or –9 and –7 days).

FIGURE 7 shows the effect of 2B8 antibody treatment of BALB/c mice at two different doses and times on the number of bone marrow CFU-Cs (left panel–individual mice) and CAFCs (right panel – pooled for each treatment group with 95% confidence limits).

FIGURE 8 is the timeline protocol for *in vivo* treatment with 3C1 MAb in mice.

FIGURE 9 shows the number of lineage negative (Lin-) bone marrow cells expressing either Sca-1 or c-kit (ACK45) following treatment with anti-CD117 MAb (2 x 0.125 mg 3C1 at –9 and –7 days).

FIGURE 10 shows the effect of 3C1 antibody treatment of BALB/c mice at a dose of 2 x 0.125 mg on the number of bone marrow CFU-Cs (left panel–individual mice) and CAFCs (right panel – pooled for each treatment group with 95% confidence limits).

FIGURE 11 shows the effect of 2B8 and rabbit complement (C') treatment as compared to untreated and isotype control plus C' and 2B8 alone on the frequency of bone marrow CFU-Cs (left panel) and CAFCs (right panel with 95% confidence limits).

FIGURE 12 shows the staining of Mo7e cells with the anti-human CD117 MAbs Nu c-kit and 104D2 and with bio-pSCF following precoating of the cells with SR-1.

FIGURE 13 illustrates how two groups of anti-human can be defined by their ability to recognize different epitopes on the c-kit molecule and to either allow binding or prevent binding of the c-kit ligand (SCF).

FIGURE 14 shows the expression of c-kit in SP positive and SP negative cells of human bone marrow as determined from staining either with the 104D2 or O.N. 183 anti-CD117 MAbs.

FIGURE 15 shows the effect of different anti-human CD117 MAbs and rabbit complement (C') treatment, with or without addition of rat IgG2b anti-mouse IgG1, on lysis of Mo7e cells.

FIGURE 16 shows the effect of human complement (Hu C') vs. rabbit complement (R C') on Mo7e cell lysis using the SR-1 anti-c-kit antibody. Cells were incubated overnight with either 10% human serum or rabbit complement.

FIGURE 17 shows the effect of SR-1 and rabbit complement (C') treatment of human bone marrow as compared to untreated and isotype control plus C' and SR-1 alone on the frequency of CFU-Cs (left panel) and CAFCs (right panel with 95% confidence limits).

FIGURE 18 shows the effect of SR-1, O.N. 181, O.N. 182 and O.N. 183 with and without rabbit complement on the frequency of colony formation following treatment of human bone marrow.

DEFINITIONS

For convenience, certain terms (and various forms thereof) that are employed in the specification, examples, and claims are collected here.

"Antibody" as used herein includes fragments and derivatives of antibodies Antibody includes, but is not intended to be limited to, monoclonal and recombinant antibodies. They may be mouse, rat, porcine, bovine, ovine or human. Anti-human primitive hematopoietic stem cell (hPHSC) antibodies are chosen for their ability to inactivate or deplete human PHSC. An antibody "derivative" as used herein means a chimeric or humanized antibody, single chain antibody, bispecific antibody or other such antibody which binds to the same epitope as its parent. An antibody "fragment" as used herein means a portion of an antibody, by way of example such portions of antibodies shall include but not be limited to CDR, Fab, or such other portions, which bind to the same epitope or any portion thereof as recognized by the chosen anti-HSC antibody of the present invention.

"Cobblestone Area-Forming Cell (CAFC) Assay", as used herein, refers to the assay method described by Breem et al. (*Leukemia* 1994 Jul; 8(7) 1095-1104) and variations thereof.

"Deplete" or "inactivate", as used herein in reference to cells such as HSC and human primitive hematopoietic stem cells (hPHSC), refers to the ability of an agent such as for example an antibody (eg. Anti-c-kit MAb) to prevent or inhibit the proliferation of hPHSC in a cobblestone area forming cell (CAFC) assay. Preferably, the agent is capable of inhibiting the number of CAFC seen at four weeks and preferably beyond 5, 7, 10 weeks, by at least 50%, preferably 60%, 70%, 80%, 90%, 93%, 95%, most preferably by 98%, as compared to the untreated control group, e.g. if a cell is lysed, it is considered to have been inactivated and/or depleted.

"Discordant species combination", as used herein, refers to two species in which hyperacute rejection occurs when an organ, tissue or cell is grafted from one to the other. Generally, discordant species are from different orders, while non-discordant species are from the same order. For example, rats and mice are non-discordant concordant species. Concordant species combinations do not exhibit hyperacute rejection.

"Donor leukocyte cells" (DLI) typically are leukocytes obtained from one member of a species, a donor, for infusion into a recipient, frequently for the purpose of introducing immunologically reactive cells into the recipient capable of depleting T-cells, especially mature T-cells and/or malignant cells in the recipient. DLI can be cells derived from the recipient that have been genetically engineered to perform the same function as those from a donor.

"Graft", as used herein, refers to a body part, organ, tissue, or cells. Organs such as liver, kidney, heart or lung; body parts, such as bone or skeletal matrix; tissue, such as skin, intestines, endocrine glands; and cells such as pancreatic cells and hematopoietic progenitor stem cells of various types, are all examples of potential grafts.

"Help reducing agent", as used herein, is an agent, such as for example an immunosuppressive drug, which results in the reduction of cytokine release. Examples of help reducing agents are cyclosporine, FK-506, and rapamycin. A help reducing agent must be administered in sufficient dose to give the level of inhibition of cytokine release that will result in tolerance. The help reducing agent should be administered in the absence of treatments that promote cytokine, e.g., IL-2, release. Putative help reducing agents can be prescreened by *in vitro* or *in vivo* tests, e.g., by contacting the putative agent with T cells and determining the ability of the treated T cells to release a cytokine, e.g., IL-2. The inhibition of cytokine release is indicative of the putative agent's efficacy as a help reducing agent. Such prescreened putative agents can then be further tested in a kidney transplant assay. In a

kidney transplant assay a putative help reducing agent is tested for efficacy by administering the putative agent to a recipient monkey and then implanting a kidney from a class II matched class I and minor antigen-mismatched donor monkey into the recipient. Tolerance to the donor kidney (as indicated by acceptance of the graft for at least about three months or longer) is indicative that the putative agent is, at the dosage tested, a help reducing agent.

"Help reduction", as used herein, means the reduction of T cell help by the inhibition of the release of at least one cytokine, e.g. any of IL-2, IL-4, IL-6, gamma interferon, or TNF, from T cells of the recipient at the time of the first exposure to an antigen to which tolerance is desired. The inhibition induced in a recipient's T cell secretion of a cytokine must be sufficient such that the recipient is tolerized to an antigen that is administered during the reduction of help. Although not wishing to be bound by theory, it is believed that the desired level of help reduction is one which substantially eliminates the initial burst of IL-2 which accompanies the first recognition of a foreign antigen but which does not eliminate all mature T cells, which cells may be important in educating and producing tolerance.

"Hematopoietic space", as used herein, refers to a condition created in the bone marrow which promotes engraftment of administered stem cells, such as donor BMC or mobilized peripheral stem cells (MPSC). The most common way of creating hematopoietic space is by whole body irradiation which non-selectively removes dividing cells.

"Hematopoietic space-creating irradiation", as used herein, refers to irradiation directed to the hematopoietic tissue, i.e., to tissue in which stem cells are found, e.g., the bone marrow. It is of sufficient intensity to kill or inactivate a substantial number of hematopoietic cells. It is often given as whole body irradiation.

"Hematopoietic stem cell" as used herein, refers to undifferentiated cells that serve as precursors for multiple cell lineages, including myeloid and lymphoid, and that are demonstrable in bone marrow, mobilized peripheral blood, yolk sac and fetal liver. HSC are pluripotent. Frequently, HSC express the CD34 marker. When CD34 negative, HSC typically express c-kit.

"Immunosuppressive agent capable of inactivating thymic or lymph node T cells", as used herein, is an agent, e.g., a chemical agent, such as for example an antibody or a drug, which when administered at an appropriate dosage, results in the inactivation of thymic or lymph node T cells. Examples of such agents are cyclosporine, FK-506, rapamycin and antigen-presenting cell inhibitors (e.g., co-stimulatory blockade inhibitors). An agent should be administered in a sufficient dose to result in significant inactivation of thymic or lymph node T cells which are not inactivated by administration of an anti-T cell antibody, e.g., an anti-ATG preparation. Putative agents, and useful concentrations thereof, can be prescreened by *in vitro* or *in vivo* tests, e.g., by administering the putative agent to a test animal, removing a sample of thymus or lymph node tissue, and testing for the presence of active T cells in an *in vitro* or *in vivo* assay. Such prescreened putative agents can then be further tested in transplant assays.

"MHC antigen", as used herein, refers to a protein product of one or more MHC genes; the term includes fragments or analogs of products of MHC genes which can evoke an immune response in a recipient organism. Examples of MHC antigens include the products (and fragments or analogs thereof) of the human MHC genes, i.e., the HLA genes. MHC antigens in swine, e.g., miniature swine, include the products (and fragments and analogs thereof of the SLA genes, e.g., the DRB gene.

The term "histocompatibility" refers to the similarities between different individuals of surface antigens on white blood cells and other tissues and organ. The level of histocompatibility describes how well matched or how

antigenically similar a recipient is to a donor. The major histocompatibility antigens are the human leucocyte antigens (HLA) known as HLA-A, HLA-B, and HLA-DR. Each person has two sets of these antigens, one set inherited from each parent. The best kind of match is an identical match wherein all six HLA antigens are the same between recipient and donor. Donors and recipients considered mismatched at one antigen are considered a "5 of 6" match, and so forth. "Allogeneic" refers to the situation wherein most or all of the antigens are matched such as would be the case if one twin acted as a donor for an identical twin, or if a brother and sister acted as donor and recipient. When the donor and the recipient are of different species, especially discordant species, this is referred to as "xenogeneic."

"Miniature swine", as used herein, refers to a wholly or partially inbred miniature swine (See for example USSN 09/378,684 filed 8/20/99, the disclosure of which is hereby incorporated by reference in its entirety).

"Mixed chimerism" as used herein refers to a situation wherein donor HSC co-exist with recipient HSC. This may be determined empirically such as by obtaining a bone marrow sample from the recipient after HSC administration and finding donor stem cells engrafted among the recipient cells. At least 1%, preferably at least 10% and more preferably at least 30, 50 or 75 % of the recipient's engrafted bone marrow comprises donor stem cells. Methods for induction of mixed chimerism are known in the art (Sykes, US Pat No. 6,006,752).

Purified preparations of donor hematopoietic stem cells or preparations including PHSC can be used in the methods of the present invention. The desired hematopoietic stem cells can be separated out of a complex preparation such as from plasma after mobilization of the hematopoietic cells. Alternatively, the complex preparation itself can be administered to a recipient. Hematopoietic stem cells including PHSC can be obtained from fetal, neonatal, immature, or mature animals. Stem cells derived from the

cord blood of the recipient or the donor can be used. See U.S. Pat. No. 5,192,553 and 5,004,681, the disclosure of each of which is hereby incorporated by reference. Although not wishing to be bound by theory, it is believed that the donor primitive hematopoietic stem cells home to a site in the recipient bone marrow.

The term "pharmaceutically acceptable" as used herein means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s).

"Primitive hematopoietic stem cell" (PHSC), as used herein, refers to a cell, such as for example, a bone marrow cell, a fetal liver cell, a spleen cell, a fetal cord blood cell, a muscle stem cell, or a skin stem cell, which is pluripotent capable of developing into all myeloid and lymphoid lineages, and that by virtue of being able to self-renew, can provide long-term hematopoietic reconstitution (LTRC). Such PHSC cells are distinguishable from progenitor hematopoietic stem cells (ProHSC) in that ProHSC: 1) confer 30 day radioprotection or short-term reconstituting cell (STRC) activity when transplanted in vivo; 2) give rise to CFU-s after 12 days when transplanted into lethally irradiated mice; and 3) show little or no proliferative response to single hematopoietic growth factors (HGFs) but proliferate maximally to multiple HGF combinations that always include steel factor (SLF).

The primitive hematopoietic stem cell is distinguishable from its "committed hematopoietic progenitor cell" (CHPC) descendants. Committed progenitor cells generate short-term and transiently repopulating mature cells of blood and lymphoid tissues. The two cell populations can be distinguished from each other experimentally, for example, by performing a Cobblestone-Forming Area Cell Assay (Ploemacher et al. *Blood.* **78**:2527-33 (1991)). The extent of long-term stem cell engraftment is determined by the frequency of cobblestone-area forming cells (CAFC) that appear after 4 to 5 weeks in stromal cell cultures when using human cells or 1-3 weeks when using murine

cells. As such, primitive hematopoietic stem cells can be distinguished from committed hematopoietic progenitor cell populations because the CHPC appear earlier, over a 1 to 3 week period in culture while the PHSC appear at 4 to 5 weeks in culture.

"Promote acceptance of a graft", as used herein, refers to lengthened functional ability of a donor graft in a recipient animal after graft transplantation, especially after the withdrawal or in the absence of continuous immunosuppressant administration. A donor graft is typically rejected within minutes of its introduction into a recipient in the absence of additional treatment of an immunocompetant recipient.

"Selective" or "selectively" or "selected", as used herein refers to a particular population of hematopoietic stem cells capable of survival for at least four weeks or longer in a CAFC assay that are distinguishable by cell surface molecules or patterns thereof from mature blood cells. Subsets of the population may be distinguishable in CAFC assay also based upon the how much longer than 4-5 weeks the members are able to proliferate in CAFC assay.

"Short course of a help reducing agent", as used herein, means a transitory non-chronic course of treatment. The treatment should begin before or at about the time of transplantation of the graft. Alternatively, the treatment can begin before or at about the time of the recipient's first exposure to donor antigens. Optimally, the treatment lasts for a time which is approximately equal to or less than the period required for mature T cells of the recipient species to initiate rejection of an antigen after first being stimulated by the antigen. The duration of the treatment can be extended to a time approximately equal to or less than two, three, four, five, or ten times, the period required for a mature T cell of the recipient species to initiate rejection of an antigen after first being stimulated by the antigen. The duration will usually be at least equal to the time required for mature T cells of the recipient

species to initiate rejection of an antigen after first being stimulated by the antigen. In pigs and monkeys, about 12 days of treatment is sufficient. Experiments with cyclosporin A (CsA; 10 mg/kg) in pigs show that 6 days is not sufficient. Other experiments in monkeys show that IL-2 administered on day 8, 9, or 10 of CsA treatment will result in rejection of the transplanted tissue. Thus, 8, 9, or 10 days of treatment are probably insufficient in pigs. In monkeys, a dose of 10 mg/kg CsA with a blood level of about 500-1,000 ng/ml is sufficient to induce tolerance to class II matched class I and minor antigen-mismatched kidneys. The same blood level, 500-1,000 ng/ml, is sufficient to induce tolerance in pigs. Long-term administration of 5 mg/kg prevents rejection (by long-term immune suppression) but does not result in tolerance.

"Short course of a immunosuppressive agent", as used herein, means a transitory non-chronic course of treatment. The treatment should begin before or at about the time the treatment to induce tolerance is begun, e.g., at about the time, xenogeneic, allogeneic, genetically engineered syngeneic, or genetically engineered autologous stem cells are introduced into the recipient. e.g., the short course can begin on the day of the treatment to induce tolerance is begun, e.g., on the day, xenogeneic, allogeneic, genetically engineered syngeneic, or genetically engineered autologous stem cells are introduced into the recipient or the short course can begin within 1, 2, 4, 6, 8, or 10 days before or after the treatment to induce tolerance is begun, e.g., within 1, 2, 4, 6, 8, or 10 days before or after xenogeneic, allogeneic, genetically engineered syngeneic, or genetically engineered autologous stem cells are introduced into the recipient. The short course can last for: a period equal to or less than about 8-12 days, preferably about 10 days, or a time which is approximately equal to or is less than two, three, four, five, or ten times the 8-12 or 10 day period. Optimally, the short course lasts about 30 days. The dosage should be sufficient to maintain a blood level sufficient to inactivate thymic or lymph node T cells. A dosage of approximately 15 mg/kg/day has been found to be effective in primates.

"Stromal tissue", as used herein, refers to the supporting tissue or matrix of an organ or bone, as distinguished from its functional elements or parenchyma. Hematopoietic stromal tissue may be obtained from a variety of hematopoietic tissues to include, but is not meant to be limited to, fetal or neonatal thymus or liver tissue or a combination thereof or bone marrow or spleen.

"Therapeutically effective amount" as used herein means the amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., treatment, healing, prevention or amelioration of the relevant medical condition, promoting acceptance of a graft or an increase in rate of treatment, healing, prevention or amelioration of such conditions. A therapeutically effective amount can be determined through the use of a Cobblestone Area Forming Cell Assay or by observing primitive hematopoietic stem cell depletion after administration of a therapeutic antibody composition.

"Thymic or lymph node T cell", as used herein, refers to T cells which are resistant to inactivation by traditional methods of T cell inactivation, e.g., inactivation by a single intravenous administration of anti-T cell antibodies, such as for example, an ATG preparation. "Thymic or lymph node thymocytes" refers to thymocytes which are resistant to inactivation by traditional methods of thymocyte inactivation, e.g., inactivation by a single intravenous administration of an ATG preparation.

"Thymic irradiation", as used herein, refers to a treatment in which at least half, and preferably at least 75, 90, or 95% of the administered irradiation is targeted to the thymus. Whole body irradiation, even if the thymus is irradiated in the process of delivering the whole body irradiation, is not considered thymic irradiation.

"Thymic space" as used herein, is a state created by a treatment that facilitates the migration to and/or development in the recipient thymus of donor hematopoietic cells of a type which can delete or inactivate recipient thymocytes that recognize donor antigens. It is believed that the effect is mediated by elimination of recipient cells in the thymus.

"Tolerance" or "acceptance," as used herein, refers to an inhibition of a graft recipient's immune response that would otherwise occur, e.g., in response to the introduction of a non-self MHC antigen into the recipient. Tolerance can involve humoral, cellular, or both humoral and cellular responses. Tolerance, as used herein, refers not only to complete immunologic tolerance to an antigen, but to partial immunologic tolerance, i.e., a degree of tolerance to an antigen which is greater than what would be seen if a method of the invention were not employed. Tolerance, as used herein, refers to a donor antigen-specific inhibition of the immune system as opposed to the broad-spectrum inhibition of the immune system seen with immunosuppressants.

DETAILED DESCRIPTION OF THE INVENTION

CD117 expression in the adult is known to be limited to committed lymphohematopoietic progenitors and primitive hematopoietic stem cells (Kodama et al. *J Exp Med.* **176**:351-361 (1992); Katayama et al. *Blood* **82**:2353-2360 (1993); Matsuzaki et al. *J Exp Med.* **178**:1283-1292 (1993)), mature mast cells, intestinal interstitial cells of Cajal, spermatogonial stem cells and oöcytes (Katayama et al. *Blood* **82**:2353-2360 (1993); Komuro and Zhou, *J Auton Nerv Syst*, **61**:169-174 (1996); Sette et al, *Int J Dev Biol* **44**:599-608 (2000). The expression product of *c-kit* is the tyrosine kinase receptor for stem cell factor (SCF). Its expression has been shown to be more limited and more consistent on pluripotent hematopoietic stem cells,

myeloid progenitor populations, and lymphoid progenitor populations than CD45 or CD34 (Sato et al. *Blood*, **94**:2548-2554 (1999)). While the functional importance of SCF-c-kit interaction on bone marrow progenitors was demonstrated by the administration of an anti-c-*kit* monoclonal antibody (ACK2) to mice, (Ogawa et al. *J Exp Med.* **174**:63-71 (1991); Okada et al. *Blood* **78**:1706-1712 1991)) it has not previously been recognized that administration of an anti-PHSC MAb such as for example anti-*c-kit* MAb prior to donor stem cell transplantation facilitates engraftment.

Radioisotope(^{131}I)-labeled anti-CD45 MAb (a pan leukocyte and mature bllod cell marker) is capable of providing for subsequent engraftment of donor stem cells in syngeneic as well as allogeneic recipients in mice (Matthews et al, *Blood*, **93**:737-745 (1999)), and is the basis for a Phase I clinical trial in patients using ^{131}I-labeled anti-CD45 combined with 120 mg/kg cyclophosphamide (CyP) and 12 Gy, a myeloablative dose of total body irradiation (TBI) (Matthews et al, *Blood*, **94**:1237-1247 (1999)). Utilization of the present invention allows reduction or avoidance of total body irradiation and reduced toxicity is possible. Also,use of antibodies against markers like CD45 is less advantageous because they would deplete a much wider number of cell types, including mature blood cells, thereby leading to excessive immunosuppression and/or toxicity

Pre-clinical evidence in mice has demonstrated that allogeneic donor T cells, in particular, $CD4^+$ cells, are capable of depleting hematopoietic stem cells in the bone marrow of the recipient following sub-lethal radiation of the recipient (Sprent et al. *J Exp Med* **180**:396-317 (1994)) and explains, in part, how donor-type chimerism can be achieved in patients who are at risk of GVHD but that have, nonetheless, been conditioned without the use of agents that are known to ablate stem cells. GVHD might be avoided by (a) *ex vivo* removal of donor T cells or subsets thereof from the graft, or (b) depletion of donor T cells or subsets thereof subsequent to infusion of the stem cell transplant in the recipient.

The inventors have discovered that to achieve long-term donor cell re-population of the recipient hematopoietic system while facilitating donor hematopoietic stem cell engraftment, it is advantageous to deplete hematopoietic stem cells from a prospective graft recipient prior to performing a bone marrow transplant. Use of an agent that selectively depletes and/or inactivates hematopoietic stem cells (HSC), preferably primitive hematopoietic stem cells (PHSC), and most preferably to selectively deplete and/or inactive at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells , would prove extremely useful in the field of long-term stem cell engraftment. Studies performed using a Cobblestone Area Forming Cell Assay, which is a hematopoietic cell subset-distinguishing assay, on bone marrow cells removed from a mice that had been exposed to altered doses and multiple fractionated total body irradiation (TBI), demonstrated that primitive hematopoietic stem cells were better able to survive the regimen as compared to committed progenitor cell populations (Down et al. *Blood.* **86**:122-127 (1990)). Consequently, primitive hematopoietic stem cells may be more capable of inhibiting donor stem cell engraftment than committed progenitors.

Busulfan (1,4-butanediol dimethylsulfonate) has been shown to be more effective than other cytotoxic drugs at depleting primitive hematopoietic stem cells and its use has been shown to facilitate long-term donor cell re-population (Down and Ploemacher. *Exp Hematol.* **21**:913-921 (1993); Down et al. *Br J Cancer* **70**: 611-616 (1994); Westerhof et al. *Cancer Res.* **60**: 5470-5478 (2000) . Further studies (data not shown) have demonstrated that Busulfex® (Orphan Medical, Inc.; Minnetonka, MN) depletes cells bearing the c-kit marker. Treatments or agents that lead to myeloablation may not necessarily deplete primitive hematopoietic stem cells. Examples of chemotherapeutic agents that result in acute myelosuppression without depleting primitive hematopoietic stem cells include cyclophosphamide, melphalan, 5-fluoruracil and thiotepa (Down and Ploemacher. *Exp Hematol*

21: 913-921 (1993); Down et al. *Br J Cancer.* **70**:611-616 (1994); Down et al. *Bone Marrow Transplant.* **21**:327-330 (1998)). Conversely, treatments or agents that lead to depletion of primitive hematopoietic stem cells may not necessarily lead to myeloablation.

The present invention provides improved methods for inducing mixed chimerism and tolerance in a recipient through antibody-mediated removal of HSC, preferably PHSC, and most preferably through selective depletion and/or inactivation of at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells prior to the introduction of donor hematopoietic stem cells such as bone marrow cells or mobilized peripheral stem cells. In another aspect, the invention provides a method for inducing mixed chimerism and tolerance in a recipient by depletion and/or inactivation of the recipient's HSC, preferably PHSC, and most preferably through selective depletion and/or inactivation of at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells and depletion of the recipient's T-cells, prior to administration of donor hematopoietic stem cells. In yet another aspect, the present invention provides a method for inducing mixed chimerism and tolerance by depletion of donor and recipient T cells and depletion and/or inactivation of recipient HSC, preferably PHSC, and most preferably through selective depletion and/or inactivation of at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells.

The present invention finds use in the treatment of malignant and non-malignant diseases, the induction of immunological acceptance for cellular and/or solid organ transplantation, in the prevention or reduction of graft-versus-host disease (GvHD), for providing a platform for administering donor-leukocyte infusions (DLI), and in the treatment of enzyme deficiency diseases and

autoimmune diseases. Suitable combinations with short-term immune modulating agents (e.g., T-cell-depleting antibodies) provide methods for engrafting hematopoietic stem cells from both allogeneic and xenogeneic donors.

In one aspect, the present invention provides methods for improving graft acceptance while reducing GvHD. In another embodiment, the present invention provides improved methods for treating hematological disorders. The methods include the steps of non-myeloablative conditioning of the recipient by reducing or eliminating both the recipient's T cell population and the recipient's HSC, preferably PHSC, and most preferably through selective depletion and/or inactivation of at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells and infusing donor hematopoietic stem cells such as bone marrow cells or mobilized peripheral stem cells into the recipient. In alternative embodiments, a short course of help reducing agent may be administered to the recipient; an organ, tissue or cell graft may be provided; and/or a donor lymphocyte infusion may be administered.

In another aspect of the present invention, hematopoietic stem cells which are pluripotent, are isolated and utilized to raise an antibody or antibodies specific for primitive hematopoietic stem cells. Cell lines producing an antibody are isolated and specific monoclonal antibodies are collected using methods known to those of skill in the art such as for example those described in Harlow, E., et al., (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988)). see example. Each specific antibody is tested for its ability to deplete and/or inactivate hematopoietic stem cells, preferably primitive hematopoietic stem cells, and most preferably selected primitive hematopoietic stem cells from bone marrow utilizing a CAFC assay. Those antibodies capable of depleting and/or inactivating at least about 50%, at least about 60%, preferably at least 70%, more preferably at least 80%,

even more preferably at least 90%, 93%, 95% and most preferably at least about 98% of the pluripotent hematopoietic stem cells from a bone marrow preparation are selected for use. In alternative embodiments, cocktails of monoclonal antibodies are utilized and/or polyclonal antibodies are used. The chosen antibody or antibodies are administered to a recipient in need thereof for inducing mixed chimerism and tolerance.

The methods of the present invention allow utilization of the graft-versus-leukemia (GvL) effects for immunotherapy of blood borne malignancies and engraftment-promoting effects of donor T cells to facilitate formation of mixed chimerism and tolerance in the recipient while minimizing GvHD, especially in HLA-mismatched pairs and in xenogeneic transplantation procedures. Additionally, immunosuppressive therapy can be reduced or substantially eliminated. The methods of the present invention are also useful for exploiting the graft-versus-host reaction obtained when donor hematopoietic cells are administered to a recipient.

In one aspect, the present invention provides a method of promoting graft acceptance in a recipient by depleting and/or inactivating the recipient's HSC, preferably PHSC, and most preferably through selective depletion and/or inactivation of at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while avoiding the use of immunosuppressive regimens by administering to the recipient an antibody, such as a monoclonal antibody, that depletes and/or inactivates pluripotent primitive hematopoietic stem cells preferentially and selectively while not significantly reducing mature blood cell numbers. A useful depleting and/or inactivating antibody is identified by CAFC assay in which cytokines from the same species as the species from which the hematpoietic stem cells were harvested are provided. The antibody, e.g. monoclonal antibody, is capable of eliminating at least about 50%, at least about 60%, preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, 93%, 95% and most preferably

at least about 98% of the hematopoietic stem cells that survive for at least four weeks or longer under the culture conditions provided by the CAFC assay. In a particular embodiment, such antibody is an anti-c-kit (or anti-CD117) antibody. However, the antibodies useful in practicing the invention are not limited to those capable of depleting or inactivating cells with the CD117 antigen but may include those capable of depleting or inactivating CD117 negative ($CD117^-$) cells as well. Preferably, the method includes the additional step of administering to the recipient an agent, such as for example an antibody or combination of antibodies, capable of depleting cells expressing CD4 and CD8 prior to the administration of donor hematopoietic cells.

In alternative embodiments, the antibody is a monoclonal antibody (MAb) and is chosen from the group comprising an anti-CD135 MAb, an anti-VEGFR2 (KDR) MAb, anti-CD133 (AC133) MAb, an anti-TIE MAb, an anti-TEK MAb, an anti-C1qRp MAb and an anti-CD117 MAb or combinations thereof. Antibodies useful in practicing the invention are not limited to those capable of depleting or inactivating hematopoietic stem cells expressing the CD34 antigen but may include those capable of depleting or inactivating $CD34^-$ cells as well.

C1qRp is the human homologue of the mouse stem cell antigen AA4.1 (Danet et al., *Proc Natl Acad Sci U S. A.* **99**(16):10441-5 (2002)).

The present invention also relates to methods for engrafting donor mammalian hematopoietic stem cells, preferably primitive hematopoietic stem cells, and most preferably selected primitive hematopoietic stem cells in a mammalian recipient which uses anti-hematopoietic-stem-cell antibodies, preferably PHSC antibodies, and most preferably selective PHSC antibodies. In one embodiment, an anti-c-*kit* antibody which is capable of depleting or inactivating sufficient numbers of hematopoietic stem cells (HSC), preferably primitive hematopoietic stem cells (PHSC), and most preferably at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at

least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells prior to donor stem cell transplantation is used. The anti-hematopoietic-stem-cell antibody is administered at a dose and using a schedule capable of reducing or eliminating the level of cytotoxic drugs or radiation administered to a recipient while facilitating induction of tolerance and mixed chimerism and promoting acceptance of a graft.

The present invention also relates to a method for inducing tolerance in a recipient mammal of a first species to a graft from a donor mammal of a second species. The method includes: introducing into the recipient mammal such as by intravenous injection or by intraperitoneal injection or intramuscularly, etc., an antibody that binds to and depletes hematopoietic stem cells (HSC), preferably primitive hematopoietic stem cells (PHSC), and most preferably to selectively deplete and/or inactive at least 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 93%, at least 95%, at least 98% of the PHSC of the recipient while maintaining mature blood cells ; and, preferably thereafter, introducing hematopoietic stem cells of the donor into the recipient mammal. Preferably, the mature T-cells of the recipient are also depleted prior to donor stem cell infusion (for example, BMC or PMSC) while not depleting donor T cells. Most preferably, when an organ transplant or donor lymphocyte infusion is to be provided, mixed chimerism has been induced prior to the transplant or infusion. While not wishing to be bound by theory, antibody depletion of the hematopoietic stem cells especially primitive hematopoietic stem cells, most preferably selected PHSC of the recipient is believed to facilitate the long-term engraftment of the donor hematopoietic stem cells. The donor hematopoietic stem cells are believed to prepare the recipient for subsequent engraftment by inducing tolerance at both the T and B cell levels.

More particularly, the method for inducing tolerance in a recipient mammal of a first species to a graft from a donor mammal of a second species involves the following steps:

(1) Administering an agent that depletes and/or inactivates HSC, preferably PHSC, and most preferably at least about 50%, at least about 60%, preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, 93%, 95 % and most preferably at least about 98% of the primitive hematopoietic stem cells that are capable of surviving for at least four weeks or more under the culture conditions provided by the CAFC assay. In a preferred embodiment the PHSC antibody in a pharmacologically acceptable vehicle and selectively depletes or inactivates the PHSCs of the recipient.

(2) Administering a sufficient number of donor hematopoietic cells to the recipient such that donor stem cells engraft and give rise to mixed chimerism. Preferably the donor hematopoietic cells are administered following one or more of the treatments disclosed herein, e.g., those described below;

(3) Optionally administering immune suppressive antibodies or drugs to the recipient, such as for example, administering an inhibitor of cell proliferation, e.g., deoxyspergualin (DSG), or an anti-metabolite, e.g. brequinar, or an anti-T cell antibody, e.g., one or both of an anti-CD4 or anti-CD8 antibody.

(4) optionally, providing treatments (other than whole body irradiation) which promote engraftment and the formation of mixed chimerism by enhancing the ability of donor cells to compete with host bone marrow cells, such as for example administering stromal cells or administering donor-specific growth factors or cytokines, e.g., administering one or more of SCF, IL-3, or GM-SCF, to the recipient.

(5) optionally creating thymic space in the recipient, such as for example,1) by irradiating the thymus of the recipient, e.g., by administering between 1 and 10, more preferably between 3 and 7, e.g., 7 Gy, of thymic irradiation and/or 2) by administering anti-T cell antibodies to the recipient in a sufficient dose to inactivate recipient thymocytes. Other methods for the creation of thymic space include: the administration of steroids, corticosteroids, brequinar, co-stimulatory

blockade or an immune suppressant chemical or drug, such as for example rapamycin, cyclosporine (available as Sandimmune®, Novartis Pharmaceutical Corp., East Hanover, NJ), or FK506 (available from Fijisawa Healthcare, Inc.). Treatment to create thymic space should be administered, or at least begun, prior to the administration of donor hematopoietic stem cells. An effective treatment should deplete single positive thymocytes to an extent that engraftment and the formation of mixed chimerism is optimized in the absence of the creation of hematopoietic space, e.g., hematopoietic space created by whole body irradiation. In preferred embodiments the recipient's single positive thymocytes are depleted by at least 20, 40, 60, or 80%. Treatments that result in between 10 and 90% depletion are preferred. The length of the treatment will vary with dosage and the effectiveness of the agent but will generally be less than 60, 30, or 15 days. The treatment should last at least 7, and more preferably 10, or 14 days in length. In preferred courses of treatment, e.g., the administration of an immunosuppressive chemical or drug, e.g., cyclosporine, should last between 7 and 30 days. The treatment, e.g., the administration of cyclosporine, should be started at a time such that it is completed prior to the administration of donor stem cells. Administration of the agent should be on a daily basis or as needed to maintain a level of the agent that allows the desired level of depletion in the recipient. A particularly preferred treatment is the administration of an immunosuppressive chemical, e.g., cyclosporine, for more than 7 and less than 30 days. A useful regimen in rodents is 20 mg/kg/day cyclosporine for 14 days ending on the third day before administration of stem cells. The dose and/or timing of administration of the agent should not affect the concentration of donor stem cells administered. For example, if an anti-CD4, anti-CD8 antibody composition is administered to the recipient, the composition should be substantially cleared prior to administration of the donor cells so as not to deplete donor T-cells.

In preferred embodiments mixed chimerism is induced in the recipient and the state of mixed chimerism is formed in the absence of the induction of

hematopoietic space, e.g., in the absence of hematopoietic space created by space creating irradiation, e.g., whole body irradiation.

The number of donor hematopoietic cells administered to the recipient can be increased by either increasing the number of stem cells provided in a particular administration or by providing repeated administrations of donor stem cells.

Repeated stem cell administration can promote engraftment, mixed chimerism, and long-term acceptance in graft recipients. Thus, the invention also includes methods in which multiple hematopoietic stem cell administrations are provided to a recipient. Multiple administrations can substantially reduce or eliminate the need for hematopoietic space-creating irradiation. Administrations can be given prior to, at the time of, or after graft implantation. In preferred embodiments multiple administrations of stem cells are provided prior to the implantation of a graft. Two, three, four, five, or more administrations can be provided. The period between administrations of hematopoietic stem cells can be varied. In preferred embodiments a subsequent administration of hematopoietic stem cell is provided: at least two days, one week, one month, or six months after the previous administration of stem cells; when the recipient begins to show signs of host lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC , e.g. when the donor specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain a level of mixed chimerism sufficient to maintain tolerance to donor antigen.

One or more post graft-implantation-administrations of donor stem cells can also be provided to minimize or eliminate the need for irradiation. Post

graft administration of hematopoietic stem cells can be provided: at least two days, one week, one month, or six months after the previous administration of stem cells; at least two days, one week, one month, six months, or at any time in the life span of the recipient after the implantation of the graft; when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the host-donor-specific antibody response, or by a change in the host-lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor PBMC antigen is equal to or falls below staining with an isotype control which does not bind to PBMC , e.g. when the donor-specific monoclonal stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a graft.

When multiple stem cell administrations are given, one or more of the administrations can include a number of donor hematopoietic cells that is at least 25% to 200% as great as, the number of bone marrow hematopoietic cells found in an adult of the recipient species. Preferred embodiments include use of cytokine mobilization of hematopoietic progenitor cells and leukapheresis.

In a preferred embodiment, the method includes inactivating natural killer cells, preferably graft reactive NK cells, of the recipient mammal. This can be accomplished, e.g., by introducing into the recipient mammal an antibody capable of binding to natural killer cells. The administration of antibodies, or other treatment to inactivate natural killer cells, can be given prior to introducing the hematopoietic stem cells into the recipient mammal or prior to implanting the graft in the recipient. This antibody can be the same or different from an antibody used to inactivate T cells.

In preferred embodiments the method includes inactivating T cells, preferably graft reactive T cells, e.g., by introducing into the recipient mammal an antibody capable of binding to T cells of the recipient mammal, either prior to introducing the hematopoietic stem cells into the recipient or prior to implanting the graft. This antibody can be the same or different from an antibody used to inactivate natural killer cells.

One source of anti-NK antibody is anti-human thymocyte polyclonal anti-serum (ATG). Preferably, a second anti-mature T cell antibody can be administered as well, which lyses T cells as well as NK cells. Lysing T cells is advantageous for both bone marrow and graft survival. Anti-T cell antibodies are present, along with anti-NK antibodies, in anti-thymocyte anti-serum. Repeated doses of antibodies, e.g., anti-NK or anti-T cell antibodies, may be preferable.

In preferred embodiments the recipient does not receive treatments that stimulate the release of a cytokine by mature T cells. For example, the recipient should not receive a substance, e.g., a steroid drug, e.g., prednisone (17,21-dihydroxypregna- 1,4-diene-3,11, 20-trione), at a dosage or concentration which stimulates the release of a cytokine by mature T cells in the recipient. Preferably the recipient is free of such treatment from the time stem cells are first administered until the graft is implanted or until mixed chimerism and tolerance is established.

In preferred embodiments the method includes the administration of an agent such as a drug or other chemical agent, which induces tolerance to unmatched class I and/or minor antigens on the graft which is introduced into the recipient. For example, a short course of help reducing treatment, such as a short course of high-dose cyclosporine, preferably is administered at the time the graft is introduced into the recipient. The duration of the short course of help reducing treatment is approximately equal to or is less than the period required for mature T cells of the recipient species to initiate rejection of an

antigen after first being stimulated by the antigen; in more preferred embodiments, the duration is approximately equal to or is less than two, three, four, five, or ten times, the period required for a mature T cell of the recipient species to initiate rejection of an antigen after first being stimulated by the antigen. These methods are described in detail in U.S. application Serial No. 08/458,720, filed Jun. 1, 1995, which is hereby incorporated by reference, which methods can be combined with the methods described herein.

Other preferred embodiments include treatments to further inactivate recipient T cells, particularly thymic or lymph node thymocytes or T cells. Thymic or lymph node thymocytes or T cells might otherwise inhibit the engraftment or survival of the administered donor cells. Such inactivation can be accomplished by one or more of: 1) irradiating the thymus of the recipient mammal with a dose of radiation sufficient to inactivate thymocytes, e.g., 1-10 Gy, more preferably between 3 and 7, e.g., about 3.5 or 7 Gy of thymic irradiation; 2) administering one or repeated doses of an anti-T cell or anti-thymocyte antibody; or 3) administering to the recipient a short course of an immunosuppressant chemical or drug, as is described herein. Inactivation of thymocytes or T cells most preferably is performed prior to donor hematopoietic stem cell or graft transplantation. In preferred embodiments the method includes diminishing or inhibiting thymocyte or T cell activity, preferably the activity of thymic or lymph node T cells by administering to the recipient a short course of an immunosuppressive agent, e.g., a chemical or drug, e.g., cyclosporine, sufficient to inactivate thymocytes or T cells, preferably thymic or lymph node T cells. The duration of the short course of immunosuppressive agent is: approximately equal to 30 days; approximately equal to or less than 8-12 days, preferably about 10 days; approximately equal to or less than two, three, four, five, or ten times the 8-12 or 10 day period. The short course can begin: before or at about the of time the treatment to induce tolerance is begun, e.g., at about the time stem cells are introduced into the recipient; on the day the treatment to induce tolerance is begun, e.g., on the day stem cells are introduced into the recipient; within 1, 2,

4, 6, 8, 10, or 30 days before or after the treatment to induce tolerance is begun, e.g., within 1, 2, 4, 6, 8, 10, or 30 days before or after stem cells are introduced into the recipient. The short course of an immunosuppressive can be administered in conjunction with an anti-T cell antibody. The short course of an immunosuppressive should be sufficient in concentration and duration to inactivate T cells, e.g., thymic or lymph node T cells, which would not be inactivated by antibody-based inactivation of T cells, e.g., inactivation by intravenous administrations of ATG antibody, or similar, preparations.

Other embodiments include (optionally): the step of, prior to hematopoietic stem cell transplantation, creating hematopoietic space, e.g., by irradiating the recipient mammal with low-dose, e.g., less than 4, preferably less than 3, more preferably less than 2 or 1 Gy, whole body irradiation to deplete or partially deplete the bone marrow of the recipient. As is discussed herein this treatment may be reduced or entirely eliminated.

In preferred embodiments the method includes the step of introducing into the recipient a donor graft such as for example a heart, pancreas, liver, or kidney in addition to administration of hematopoietic cells. This process can be used in conjunction with any of the other methods disclosed herein.

The methods of the present invention also provide for the treatment of patients having a hematological disorder such as for example a hematological malignancy e.g., leukemia or relapsed multiple myeloma.

The methods of the present invention also provide for treatment of a patient, especially a human patient, having non-neoplastic disorders such as for example sickle cell anemia or thalassemia.

The present invention provides a method for treating a hematological disorder in a patient in need thereof comprising: 1) administering a myeloreductive non-myeloablative treatment (for example such as described

in US Patent Nos. 5,876,708 and 6,006,752, the disclosures of which are incorporated herein by reference) in an amount that mixed chimerism is induced in the recipient; 2) depleting the recipient of primitive hematopoietic stem cells by administering the antibody of the present invention in a therapeutic composition; and 3) introducing into the recipient hematopoietic cells from an allogeneic donor to form chimeric bone marrow in the recipient.

In preferred embodiments each of the recited steps is a separate discrete admir.istration or agent.

In preferred embodiments, the donor stem cells are provided as allogeneic bone marrow, mobilized peripheral blood cells, or cord blood cells. The donor stem cells, in some instances, can be expanded ex vivo for transplantation.

In the methods described herein, the donor and the recipient can be the same species (allogeneic) or each can be of a different species (xenogeneic) e.g non-human primate, swine. In allogeneic applications of the methods disclosed herein, the donor is both a source of the hematopoietic cells (e.g BMC or MPSC) and of the graft (e.g. organ, tissue or cells e.g. leukocytes). In xenogeneic methods preferably, the donor is a mammal such as for example an inbred miniature swine (US Patent Applic. No. 09/378,684 filed 8/20/99, the disclosure of which is incorporated herein by reference). In xenogeneic methods, the donor hematopoietic stem cells and the graft can be from the same mammal or can be from a different mammal of the same species as the HSC donor which are MHC matched or highly inbred, eg. the first donor can be from the same herd as the second donor wherein the herd members are inbred at MHC loci or locus.

In preferred embodiments of the present invention when an anti-T cell antibody treatment is administered to the recipient, donor hematopoietic stem cell administration is given after the agent utilized to deplete or inactivate

recipient T-cells has been substantially cleared from the circulatory system. Most preferably, both administered anti-T-cell antibodies and anti-PHSC antibodies have been cleared from the circulatory system of the recipient.

In certain embodiments, the myeloreductive treatment includes treating the recipient, prior to introduction of the donor stem cells, with a cytoreductive agent selected from one or more of alkylating agents (e.g., nitrogen mustards [such as mechloretamine], cyclophosphamide, elphalan and chlorambucil), alkyl sulphonates (e.g., busulphan), nitrosoureas (e.g., carmustine, lomustine, semustine and streptozocine), triazenes (e.g., dacarbazine), antimetabolites (e.g., folic acid analogs such as methotrexate), pyrimidine analogs (e.g. fluorouracil and cytarabine), purine analogs (e.g., fludarabine, idarubicin, cytosine arabinoside, mercaptopurine and thioguanine), vinca alkaloids (e.g., vinblastine, vincristine and vendesine), epipodophyllotoxins (e.g., etoposide and teniposide), antibiotics (e.g., actinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin and mitomycin), dibromomannitol, deoxyspergualine (DSG), dimethyl myleran and thiotepa.

Preferred myeloreductive non-myeloablative agents are alkylating agents, e.g., cyclophosphamide, or fludarabine or similar substances, however, hematopoietic space creating antibodies or drugs, e.g., inhibitors of cell proliferation, e.g., DSG, or an anti-metabolite, e.g. brequinar, or an anti-T cell antibody, e.g., one or both of an anti-CD4 or anti-CD8 antibody can be used
as a myeloreductive non-myeloablative agent.

Antibodies suitable for inactivating T cells include anti-T cell antibodies (including humanized versions thereof) for example an ATG preparation, OKT3, BTI-322® (US Patent No. 5,730,979 the disclosure of which is hereby incorporated by reference).

Antibodies suitable for depleting and/or inactivating hematopoietic stem cells in the recipient include antibodies directed against isolated progenitor cells (for example antibody A3C6E2, exemplified in US Patent No. 5,808,002) and/or antibodies directed against primitive hematopoietic cells and/or antibodies directed against one or more known markers of primitive hematopoietic cells such as for example: CD117, (for example antibody SR-1 exemplified in US Patent Nos. 5,489,516 and 5,919,911; antibody to AC133 antigen exemplified in US Patent No. 5,843,633; antibody to MG1 exemplified in US Patent No. 6,034,348); and antibody to UT-7 exemplified in US Patent No. 6,323,321), the disclosures of each of which are hereby incorporated by reference. Additionally, antibodies for depleting and/or inactivating endothelial cells which produce factors such as for example granulocyte-, granulocyte-macrophage-, and macrophage colony-stimulating factors, which stimulate and support primitive hematopoietic stem cells may be administered either alone or in combination with the above mentioned antibodies,for example, an antibody that inactivates and/or depletes the endothelial specific tyrosine kinase (TEK); an antibody specific for FLT3 (US Patent Nos. 5,777,084; 5,808,002; 6,156,882); an antibody to Rse receptor tyrosine protein kinase (US Patent Nos. 5,709,858 and 5,955,291) an antibody to FLT4 rPTK (US Patent No. 6,107, 046); an antibody to TIE-2 (US Patent No. 6,166,185) and an antibody to VEGFR2 (US Patent No. 6,342,219), the disclosures of the cited patents are hereby incorporated by reference, can be used alone or in combination.

Such antibodies can be combined in a pharmaceutically acceptable carrier to form a therapeutic composition and administered to a patient in need thereof at a therapeutically effective dose. Antibodies to hematopoietic stem cells can for example be administered intravenously or intraperitoneally or intramuscularly. The characteristics of the carrier will depend on the route of administration. Such a composition may also contain (in addition to at least one anti-HSC antibody and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art.

In other embodiments, the immunosuppressant regimen includes treatment with one or more of a macrolide immunosuppressant, azathioprine, steroids (e.g., prednisone, methyl prednisolone), or sub-lethal nonmyleoablative irradiation of lymphocyte-containing tissue.

Treatments which deplete or inactivate recipient T cells may do so by directly or indirectly depleting, inactivating, or reducing recipient T cell activity. Indirect methods of altering T cell activity include reducing the number of cells capable of forming T cells such as for example primitive hematopoietic stem cells, and/or reducing levels of factors, such as for example cytokines, which support and maintain T cells and/or their precursors. Treatments which inhibit recipient primitive hematopoietic stem cells capable to maturing into recipient T cells should be administered prior to donor stem cell administration. T cell activity suppressors (such as for example immunosuppressants) can be administered at any time in the course of the method but should not be administered in such a manner that donor T cell engraftment will be substantially affected when they are administered. Preferably, treatments which selectively deplete or inactivate recipient T cells are provided both before and after the administration of donor hematopoietic stem cells. Treatment prior to the administration of donor hematopoietic stem cells is believed most desirable in that it will condition the recipient for the receipt of the donor hematopoietic stem cells.

For best results, treatments to inhibit T cell activity, e.g., anti-T-cell antibodies, cyclosporine or antibodies capable of depleting and/or inactivating recipient hematopoietic stem cells (PHSC) preferably PHSC, and most preferably selected PHSC, can be administered repeatedly prior to donor bone marrow transplantation, for example such treatment can be administered one, two, three, or more times prior to donor bone marrow transplantation. Typically, an anti-HSC treatment agent, for example the administration of antibodies, will be given to the patient about 1, 2, 3, 4, or 5

days prior to stem cell transplantation depending upon the rate of clearance of the agent from the recipient's circulation and upon the level of depletion desired. In preferred embodiments, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 93%, at least 95%, at least 98% depletion and/or inactivation of PHSC is achieved. It may be desirable to repeat pre-stem cell administrations every until the patient shows excess antibodies in the serum and about 80, 90, or 99% depletion of peripheral T cells and then to perform the donor HSC transplantation. Treatments can also be administered one, two, three, or more times after donor hematopoietic stem cell transplantation. Typically, a post-stem cell transplant treatment will be given about 1, 2, 3, 4, or 5 days after bone marrow transplantation.

In one embodiment, two or more T-cell inhibiting modalities or treatments can be combined. In particularly preferred embodiments, an antibody, e.g., an anti-T-cell antibody, an agent capable of selectively depleting and/or inactivating HSC, for example an antibody of the present invention alone or in combination with a chemical agent such as Bulsulfex®, an immunosuppressant e.g., cyclosporine, and thymic irradiation, are all administered to the recipient. The immunosuppressant can be administered once, or more than once, but the administrations should be short term and not chronic or long term administration. In general, this will mean the treatment is administered for not more than 30, 45, 60, 90, or 120 days, and in many treatments this means administration on 1, 2, 3, 4, 5, or fewer days.

Cyclosporine and similar agents will generally be administered for not more than 30, 45, 60, 90, or 120 days. PHSC depleting agents such as antibodies will generally be administered for 1, 2, 3, 4, 5, or fewer days prior to donor stem cell infusion.

In preferred embodiments of the method for treating hematological disorders, donor leukocytes are administered to a patient after achievement of mixed chimerism has been confirmed. While not wishing to be bound by

theory, the donor leukocyte infusion (DLI) is believed to provide additional GVL activity--donor leukocytes are believed to further reduce the number of cancer cells in the patient. The need for or appropriateness of donor leukocyte administration can be evidenced by incomplete tumor regression. Donor leukocyte administration should be delayed for at least 10, 20, 30, 35 or 60 days after the administration of any myeloreductive, non-myeloablative treatment. Initial trials showed a delay of about 35 days to be suitable. The donor leukocyte infusion is delayed to avoid introduction of relatively large numbers of donor immune cells into the patient during the period when induced pro-inflammatory conditions exist. Delay allows the DLI recipient to recover from conditioning and to be less susceptible to GVHD as a result of the DLI, especially when mismatched donor tissue is used. While not wishing to be bound by theory, it is thought that the donor leukocyte infusion converts the mixed chimeric state of the recipient to one which is fully chimeric while limiting the graft cell mediated immune attack to the hematopoietic compartment, thereby minimizing GVHD and maximizing GVL effects.

In preferred embodiments, the method includes creating thymic space in the recipient utilizing an agent other than thymic irradiation. However, thymic space can be created by irradiating the thymus of the recipient, for example, by administering between 100 and 1,000, more preferably between 300 and 700, e.g., 700 rads, of thymic irradiation. Most preferably, thymic space is created by administering anti-T cell antibodies in sufficient dose to inactivate thymocytes. Other agents for the creation of thymic space include: the administration of brequinar, or an immune suppressant chemical or drug such as for example rapamycin, cyclosporine, or FK506. An effective agent or combination of agents should deplete single positive thymocytes to an extent that engraftment and the formation of mixed chimerism is optimized. In preferred embodiments the recipient's single positive thymocytes are depleted by at least 20, 40, 60, or 80%. Treatments which result in between 10 and 90% depletion are preferred.

In preferred embodiments the recipient does not receive additional treatments which stimulate the release of a cytokine by mature T cells. For example, the recipient should not receive a substance, e.g., a steroid drug, e.g., Prednisone (17, 21-dihydroxypregna-1, 4diene-3, 11, 20-trione), at a dosage or concentration which stimulates the release of a cytokine by mature T cells in the recipient. Preferably, the subject is free of such treatment from the time stem cells are first administered until mixed chimerism is established and/or donor leukocytes are administered.

The administration of an agent, for example 15-deoxyspergualin, mycophenolate mofetil (MMF), brequinar sodium, or a similar agent, which inhibits the production, levels, or activity of anti-donor antibodies in the recipient may be utilized, especially in cases of increasing mismatch such as for example xenogeneic transplantation.

In preferred embodiments, particularly xenogeneic methods, the method includes: inhibiting natural killer cells of the recipient preferably prior to introducing donor tissue into the subject, for example by introducing into the recipient a drug such as for example deoxyspergualin (DSG) (Bristol-Myers-Sqibbs), an antibody such as anti-IgM or ATG which is capable of binding to and depleting or inactivating natural killer cells of the subject or for example by depleting natural antibodies from the blood of the recipient. Depletion from the blood can also be achieved, by way of example, by contacting the recipient's blood with an epitope which absorbs preformed anti-donor antibody. The epitope can be coupled to an insoluble substrate and provided for example as an affinity column. For example, an alpha (1-3) galactose linkage epitope-affinity matrix, e.g., matrix bound linear B type VI carbohydrate, can be used to deplete natural antibodies when a swine xenograft is provided to a human. Additionally, natural antibody depletion can be achieved by hemoperfusing an organ, e.g., a liver or a kidney, obtained from a mammal of the donor species. (In organ hemoperfusion antibodies in the blood bind to antigens on the cell surfaces of the organ and are thus

removed from the blood.) One or more of, DSG (or similar drugs), anti-IgM antibodies, and hemoperfusion, can be used to deplete or otherwise inactivate natural antibodies in the recipient according to the methods of the invention.

The methods of the present invention can also include the management of GVHD responses post-transplantation by administration of immunosuppressants, or by use of engineered stem cells which give rise to small molecule ablatable T cells or other hematopoietic cells. See, for example, U.S. Pat. No. 5,834,266.

The invention disclosed herein permits improved hematopoietic stem cell (HSC) engraftment in patients without the need to administer life threatening levels of ablative therapy, greatly improving the survival and cure rates of numerous hematopoietic diseases that do not require high-dose ablation which currently rely on the transplantation of HSC. Thus, diseases and conditions treatable by the methods of the present invention include: congenital B- and T-lymphocyte disorders, such as predominantly antibody defects, X-linked agammaglobulinemia, common variable immunodeficiency, immunodeficiency with thymoma, selective IgA deficiency, X-linked immunodeficiency with hyper-IgM antibody deficiency with normal immunoglobulins, subclass deficiency, poor response to polysaccharide antigens, or X-linked lymphoproliferative syndrome; or a combined immunodeficiency-primary defect in cellular immunity, such as severe combined immunodeficiency, autosomal recessive and X-linked, adenosine deaminase deficiency, defective expression of histocompatibility antigens, deficiency of T cell receptors, Omen's syndrome, cellular immunodeficiency with immunoglobulins (Nezelof's syndrome), purine nucleoside phosphorylase deficiency, or an immune deficiency associated with other defects, such as Wiskoff-Aldrich syndrome, ataxia telangiectasia, cartilage-hair hypoplasia, hyperimmunoglobulin E syndrome, or chronic mucocutaneous candidiasis. Also treatable are disorders of phagocytic function, such as disorders of production and consumption, abnormal production, Kostmann's syndrome, Schwachman's syndrome, cyclic neutropenia, Primary

B- and T-lymphocyte disorders, X-linked hyper-IgM, X-linked agammaglobulinemia, ataxia telangiectasia, cartilage-hair hypoplasia, IgA deficiency; disorders of migration and chemotaxis, general defects in leukocyte mobility, non-specific disorders, such as Kartogener's syndrome, lazy leukocyte syndrome, hyper-IgE syndrome, Chediak-Higaschi syndrome; or disorders of intracellular killing, such as chronic granulomatous disease, myeloperoxidase deficiency, gluthathione reductase and peroxidase deficiency, glucose-o-phosphate dehydrogenase deficiency; or a deficiency of leukocyte function antigen 1 (LFA-1).

The invention also finds utility in bone marrow transplantation for such hematologic disorders as marrow aplasia, Fanconi's aplasia, Diamond-Blackfan syndrome, hemoglobinopathies, α-thalassemia major, sickle cell anemia, neutrophil disorders, congenital neutropenia, chronic granulomatous disease, Chediak-Higashi syndrome, Osteopetrosis; immune deficiency disorders, such as severe combined immunodeficiency disease, ADA-deficient SCID, reticular dysgenesis, bare lymphocyte syndrome, PNP deficiency, LFA-1 deficiency, ataxis telangiectasia, or Wiskoff-Aldrich syndrome; metabolic disorders, such as mucopolysaccharidoses, Hurler's syndrome, Hunter's syndrome, Sanfilippo's syndrome, leukodystrophies, metachromatic leukodystrophy, adrenoleucodystrophy, sphingolipidoses, Neimann-Pick syndrome, Gaucher's disease; or in the setting of HIV infection, red cell membrane disorders (such as hereditary spherocytosis, etc.), G-6-PD deficiency, paroxysmal nocturnal hemoglobinuria, myelodysplastic syndrome, or aplastic anemia, for example. Furthermore, the discovery has useful implications for improving the efficiency of gene therapies using HSC, especially in those contexts where less than 100% HSC replacement is required for effectiveness.

The invention will now further be described by the following non-limiting examples and it should be kept in mind that other and different embodiments of the invention will no doubt suggest themselves to those of skill in the relevant art.

EXAMPLE 1

Development Of An Anti-Human Hematopoietic Stem Cell Monoclonal Antibody and Assessment of its Biological Activity in Vitro

The technique for producing rat monoclonal antibodies is well known in the art (H. Bazin (Ed), Rat Hybridomas and Rat Monoclonal Antibodies, CRC Press, Inc., Boca Raton, Fla. 1990; EP 0 380 018 B1). Production of rat monoclonal antibodies that bind to a human antigen specific for human primitive hematopoietic stem cells comprises:

(1) Immunizing a rat or rat immunocompetent cells in vitro with an immunogenic amount of a human cell preparation, such as a membrane preparation, obtained from cells functionally and phenotypically identified as primitive hematopoietic stem cells;

(2) Fusing immunized cells from the rat or immunized rat immunocompetent cells with immunocytoma cells;

(3) selecting hybridoma cells that produce antibody that binds to a protein demonstrable as specific for primitive hematopoietic stem cells, such as for example a human CD117 antigen epitope, and distinct enough from that bound by murine monoclonals so there is no cross blocking, where the selecting is done by testing the monoclonal antibody produced in a CAFC assay such as described in Breems ,et al. (*Leukemia*. 1994 Jul;8(7):1095-104) ;

(4) culturing the selected hybridoma cells obtained by fusion; and

(5) recovering and purifying the antibody.

Isolating of the desired antibody from ascitic fluid by immunoaffinity chromatography takes advantage of the allotypic difference existing between the immunoglobulins of the rat receiving the producing hybridoma and the MAb secreted by the latter (Bazin, H., Cormont F. and DeClercq, L.. J. Immunol. Method., 1984, 71:9). Additional steps are performed to define the

specificity of the antibodies produced, again using techniques well known to those of skill in the art.

Wistar, Sprague-Dawley, Lewis and Louvain rats are among the rat varieties suitable for use as the source of antibody-producing cells for fusion.

The immunogen can be derived from any hematopoietic stem cell or any primitive hematopoietic cell line, such as for example the cell line produced by the method described in US Pat. No 6,280,718, the disclosure of which is hereby incorporated by reference. Human origin of these cells is preferred. Preferably, human bone marrow cells which can be phenotypically and functionally demonstrated to be primitive hematopoietic stem cells will be used to obtain a protein preparation, such as a membrane protein preparation, for use as the antigen. In an example of a particular antigen, CD117 would be obtained from the human acute megakaryocytic cell line designated Mo7e that is known to express CD117 (US Patent Nos. 5,489,516 and 5,919,911; Ashman et al. *J Cell Physiol* 1994; 158:545-54; Broudy et al. *Blood* 1992; 79:338-46) obtainable through the American Type Culture Collection (ATCC)).

Screening for Human Primitive Hematopoietic Stem Cell Specific Monoclonal Antibodies by CAFC Assay

Hybridomas expressing a single monoclonal antibody will be isolated. The MAb collected will be used as the test antibody.

The human bone marrow cells will be resuspended at 10^6 cells/ml in Iscove's Modified Dulbecco's Medium (IMDM; JHR Biosciences Inc; Kansas) supplemented with 10% fetal bovine serum (FBS), gentamycin, 50 μM β-mercaptoethanol and 0, 1, 5 and 10 μg/ml of an isotype control antibody or the test antibody. A CAFC assay will be performed as above as described by Breems ,et al. utilizing human cytokines and a time point for colony formation of about four weeks. Hydridomas producing antibodies resulting in at least a 50%

depletion of colonies as compared to controls will be further characterized for use.

Determination of Complement Mediated Lysis

The human bone cell suspensions will be processed as follows:

i. incubate at 37°C for 18 hours with or without addition of 200 ng/ml human stem cell factor (hSCF);

ii. incubate at 4°C for 1 hour to coat cells with the antibody that is isolated as described above, wash and resuspend in media containing rabbit complement and then incubate at 37°C for a further hour;

iii. incubate at 4°C for 1 hour to coat cells with the antibody, washe, co-culture with human peripheral blood monocytes and then incubate at 37°C for 18 hours.

Following these treatments the cells will be plated in methyl cellulose cultures (see below) to provide a rapid screening for the initial dose range for efficacy and provide some insight as to whether the antibodies alone are inhibitory or whether they require complement or antibody dependent cellular cytotoxicity (ADCC) for complete action.

EXAMPLE 2

Inducing Mixed Lymphohematopoietic Chimerism And Graft-Vs-Lymphoma In Adult Human Recipients Following Non-Myeloablative Therapy And HLA-Mismatched Stem Cell Transplantation

Methods are described for treating patients using the therapeutic composition(s) of the present invention to include treating patients with blood cancers, such as non-Hodgkin's lymphomas. A series of procedures and treatments including pre-transplant administration of antibodies, and thymic irradiation followed by infusion of mobilized peripheral stem cells and

treatment with a short course of an immunosuppressive agent such as cyclosporine (CsA) are provided.

Patients with chemo- and radio-resistant non-Hodgkin's lymphomas (NHL) have a very poor prognosis. HLA-identical allogeneic or autologous bone marrow transplantation has led to durable remissions in only 0-23% of patients. However, animal studies have shown that MHC-disparate bone marrow transplants can mediate anti-tumor effects that greatly exceed those achieved with MHC-matched BMT. The potential of HLA-mismatched bone marrow transplantation as immunotherapy for hematologic malignancies has not yet been exploited, largely due to the high incidence of intractable GVHD and the potentially lethal failure of marrow engraftment associated with standard ablative conditioning regimens.

Previous studies in rodents have shown that mixed hematopoietic chimeras produced across MHC barriers are resistant to the development of GvHD, even when lymphohematopoietic GvH reactions are intentionally induced that convert mixed chimeras to fully allogeneic chimeras (Pelot et al. 1999. *Biol Blood Marrow Transplant.* 5:133-143). Murine mixed chimeras produced with a non-myeloablative conditioning regimen of T cell-depleting MAbs, cyclophosphamide (CP) and thymic irradiation (TI) can be converted into full donor chimeras without developing GvHD when donor lymphocytes are administered 5 weeks post-BMT. The mixed chimerism approach has been adapted for use in humans with hematologic malignancies, using CP for both cytoreduction of malignancy and as an adjunct to host immunosuppression with T cell depletion and TI (Sykes et al 1999. *Lancet.* 353, 9166:1755-9).

The protocol disclosed herein is optimized to further reduce toxicities by using an antibody of the present invention in addition to an anti-T cell antibody and TI, thereby eliminating the need for immunosuppressive agents such as cyclophosphamide and myeloablative whole body irradiation. For

patients not in remission at the time of treatment, it may be advantageous to administer cyclophosphamide as a cytoreductive agent for malignancy.

In accordance with the present invention, patients will undergo non-myeloablative conditioning therapy followed by allogeneic mobilized peripheral hematopoietic stem cell (MPHSC) transplant. Eligibility criteria will include chemotherapy-refractory hematologic malignancy, ECOG performance status of 2 or less, age of 65 years or less, and adequate organ function. A less than three of six HLA antigen-mismatched related donor may be required. Patients and donors will be typed using standard serological techniques for HLA-A and B, and SSOP-or SSP-based analyses for HLA-DR.

Conditioning and transplantation

Administration of anti-stem cell monoclonal antibody (Days –7, -6, and –5)

The first dose of a therapeutic composition of the present invention (TC), such as for example anti-CD117 in a pharmacologically acceptable carrier, (Day –7; 1 mg/kg) should be administered over 30 minutes, and should be preceded 1-4 hours by the administration of intravenous methylprednisolone sodium succinate (8 mg/kg up to a maximum dose of 500 mg) along with diphenhydramine (50 mg p.o.), and acetominophen, (650 mg, p.o.). The second and subsequent doses (Days –6 and –5; 1 mg/kg)) should be 1.0 mg/kg, administered over 2 hours, without steroid pre-medication. Diphenhydramine and acetominophen may be administered prior to the second and subsequent doses, however. Optionally, one or more additional doses can be administered as long as excess antibody has been cleared from the circulation by the time of donor MPHSC infusion.

Administration of anti-T cell monoclonal antibody, (Days –7, -6)

The first dose of anti-T cell antibody, e.g., MEDI-507 (US Pat. No. 5,951,983, incorporated herein by reference) should be on day –7 (0.1 mg/kg) followed on day –6 by a dose of 0.5 mg/kg. The initial dose should be preceded 1-4 hours by the administration of intravenous methylprednisolone

sodium succinate (8 mg/kg up to a maximum dose of 500 mg), along with diphenhydramine(50 mg p.o.), and acetominophen, (650 mg, p.o.). Following pretreatment with methylprednisolone, the therapeutic composition and anti-T cell monoclonal antibodies may be administered concurrently or in succession.

Thymic Irradiation (TI) Day -1

700 cGy of thymic irradiation can be administered in a single dose on Day –1. A field size of approximately 8 cm wide and 10 cm in longitudinal dimension should be used, with the midpoint of the upper edge of the field at the sternal notch. The dose should be calculated at a depth of approximately 6 cm, guided by results of a lateral chest roentgenogram for the approximate location of the thymus. A 10 MV x-ray machine at maximum dose rate should be used. No pre-medication for nausea should be required. The details of field sizes, dose calculation, energy, beam spoiling, and dose rate are recorded on the coronary flow reserve (CRF).

Alternatively, thymic irradiation can be eliminated by increasing the dose of donor mononuclear cells transplanted and/or by administering an antigen-presenting cell inhibitory agent such as a co-stimulatory blocker, e.g. one or more agents that inhibit the CD40-CD40 ligand interaction and/or the CD28-B7 interaction.

Administration of an immunosuppressant, e.g., Cyclosporine

This step is optional. Cyclosporine, either Neoral® or Sandimmune® or equivalent should be administered orally starting on Day-1. The initial dose should be 6 mg/kg given twice with doses administeredapproximately 12 hours apart, followed by a single dose of 4 mg/kg given late in the evening on the day of transplantation (Day 0). Starting on the day after transplantation (Day 1), the dose should be 4 mg/kg given twice a day, and adjusted to provide a trough whole blood concentration of 400-500 ng/mL, as measured by a monoclonal antibody-based assay, or the equivalent if a different assay

or serum rather than whole blood are used, to determine trough cyclosporine concentrations. Cyclosporine should be continued for 35 days, then tapered over 7 days and discontinued on Day 42. If the patient shows signs of GvHD, cyclosporine coverage may be extended.

Administration of Cyclophosphamide (CP)

This step is optional. If CP is used for cytoreduction of the malignancy, it may be given intravenously, 50 mg/kg/d, (with dosing based on actual or ideal body weight, whichever is less) on days –6 through –3. Dexamethasone may be used at a dose of 20 mg/d prior to each dose of CP.

Preparation of Donor Stem Cells

Hematopoietic stem cell donors will undergo treatment with recombinant G-CSF (Filgrastim; Amgen Corp; Thousand Oaks, CA) 5-10 micrograms/kg/day subcutaneously for 4-7 days to mobilize hematopoietic progenitor cells into the peripheral blood where they may be collected by a 3-4 blood volume (15-18 l) leukapheresis using standard techniques. Cytokine mobilization should be monitored, and the day of leukapheresis may be determined by the $CD34^+$ cell count and total white blood cell count in peripheral blood. The leukapheresis product may then be further processed to enrich for the hematopoietic stem cells, e.g. by using a system such as the Isolex® 300i or the Eligix™ T-cell-HDM device. If necessary, the cells may be cryopreserved until needed for infusion into the recipient at the time of transplantation. The total WBC count of the donor should not exceed 70 x $10^3/mm^3$. If this level is reached, the dose of Filgrastim should be reduced and the WBC count followed daily until it falls below this value. Every effort should be made to collect an adequate cell product from these patients, and preferably a high-dose (>5 x 10^6 $CD34^+$ cells/kg).

At least two weeks prior to Filgrastem, a G-CSF treatment, donors may be leukapheresed and aliquots of the product can be cryopreserved for

potential administration of donor leukocyte infusions to increase levels of chimerism and enhance the donor vs. tumor therapeutic effect.

Alternatively, unmobilized bone marrow may be used in place of mobilized peripheral blood (MPHSC). Donor bone marrow may be procured under anesthesia by standard techniques. A target number of 3 x 10^8/kg nucleated cells may be sought. In the case of minor ABO-incompatibility, plasma may be removed from donor marrow prior to transplantation. In the case of major ABO-incompatibility, red blood cells are depleted from the donor marrow using a CS-3000 Blood Cell Separator (Baxter-Fenwal, Round Lake, IL).

Donor Lymphocyte Infusion (DLI)

DLI can be administered to qualified patients, preferably on day 35, and subsequently as deemed potentially beneficial. DLI is used as an anti-tumor immunotherapy and can be administered to patients who do not exhibit symptoms of GvHD. It is a preferred treatment for those patients demonstrating mixed chimerism (<90% donor cells) and for those patients testing positive for disease. DLI can be administered as a single dose of 1 x 10^7 T cells/kg in donor peripheral blood or it may be given as a series of graded doses starting as low as 5 x 10^6 T cells/kg and increasing up to 5 x 10^7 T cells/kg. Patients with confirmed GvHD should not be given DLI. For those patients who become full chimeras after the initial transplant and who do not have GvHD, they may be administered DLI at the discretion of the physician; however, advisement is that the potential benefits may be outweighed by the risks of developing GvHD.

Patient Monitoring

Analysis of Chimerism

Flow cytometry (FCM) is used for lineage analysis of white blood cells and for HLA-typing of donor and recipient. Additionally, minisatellite variable

number of tandem repeats (VNTR) or short tandem repeat (STR) markers may also be used to distinguish donor and host. Donor lineage analysis is performed on a weekly basis for the first month, on a monthly basis through month 3, and, thereafter, on a quarterly basis up to one year and at interim points as deemed useful such as following DLI administration.

Engraftment and Establishment of Mixed Chimerism

Patients are monitored for leukopenia and thrombocytopenia during the first month post-transplant. Leukocyte engraftment is confirmed by an ANC>500/mm^3 and platelet recovery to >20,000/mm^3. Patients with delayed recovery will receive platelet transfusions as needed.

Graft-vs-Host Disease (GvHD)

Patients are observed for fever, skin rash, and elevated liver enzymes suggestive of GvHD. Biopsy confirms suspected GvHD. Unresolved or progressive GvHD is treated with a standard course of corticosteroids.

Assessment of Disease Status

Disease is staged at 100 days and at one year, and at interim times as deemed appropriate.

Other Embodiments

The methods described herein for inducing tolerance to, or promoting the acceptance of, an allogeneic antigen or allogeneic graft can be used where, as between the donor and recipient, there is a match or any degree of mismatch at MHC loci or other loci which influence graft rejection. Preferably, there is a mismatch at least at one MHC locus or at least at one other locus that mediates recognition and rejection, e.g., a minor antigen locus. With respect to class I and class II MHC loci, the donor and recipient can be: matched at class I and mismatched at class II; mismatched at class I and matched at class II; mismatched at class I and mismatched at class II; matched at class I, matched at class II. In any of these combinations other

loci which control recognition and rejection, e.g., minor antigen loci, can be matched or mismatched. As stated above, it is preferable that there is mismatch at least at one locus. Mismatched at MHC class I means mismatched for one or more MHC class I loci, e.g., in the case of humans, mismatched at one or more of HLA-A, HLA-B, or HLA-C. Mismatched at MHC class II means mismatched at one or more MHC class II loci, e.g., in the case of humans, mismatched at one or more of a DPα, a DPβ, a DQα, a DQβ, a DRα or a DR.

The methods described herein for inducing tolerance to an allogeneic antigen or allogeneic graft can be used where, as between the donor and recipient, there is any degree of reactivity in a mixed lymphocyte assay, e.g., wherein there is no, low, intermediate, or high mixed lymphocyte reactivity between the donor and the recipient. In preferred embodiments mixed lymphocyte reactivity is used to define mismatch for class II, and the invention includes methods for performing allogeneic grafts between individuals with any degree of mismatch at class II as defined by a mixed lymphocyte assay. Serological tests can be used to determine mismatch at class I or II loci and the invention includes methods for performing allogeneic grafts between individuals with any degree of mismatch at class I and or II as measured with serological methods. In a preferred embodiment the invention features methods for performing allogeneic grafts between individuals which, as determined by serological and or mixed lymphocyte reactivity assay, are mismatched at both class I and class II.

The methods of the invention are particularly useful for replacing a tissue or organ afflicted with a neoplastic disorder, particularly a disorder which is resistant to normal modes of therapy, e.g., chemotherapy or radiation therapy. The methods of the invention are also particularly useful in replacing tissue or organ with a patient who is believed to be, based on past experience or current expectations, unreliable with respect to the careful self-administration of chronic immunosuppressive regimens which would

otherwise be required following the transplantation of a mismatched mammalian organ or tissue. Methods of the invention can be used for inducing tolerance to a graft, e.g., an allograft, e.g., an allograft from a donor which is mismatched at one or more class I loci, at one or more class II loci, or at one or more loci at each of class I and class II. In preferred embodiments: the graft includes tissue from the kidney, liver, heart, lung, thymus, pancreas e.g. islet cells, digestive tract or gut, e.g., tissue from the stomach, or bowel tissue, e.g., small intestine, large intestine, or colon; the graft replaces a portion of the recipient's digestive system e.g., all or part of any of the digestive tract or gut, e.g., the stomach, bowel, e.g., small intestine, large intestine, or colon.

In any of the methods described herein, particularly primate or clinical methods, it is preferable initially to form mixed chimerism as opposed to entirely replacing the recipient's stem cells with donor cells.

Blockers of the CD40 ligand-CD40 or CD28-B7 interactions (or both) can be administered repeatedly. E.g., blockers can be administered one, two, three or more times prior to donor bone marrow transplantation. Typically, a pre-bone marrow transplantation dose is given to the patient about 5 days prior to bone marrow transplantation. Additional, earlier doses 6, 7, or 8 days prior to bone marrow transplantation can also be given. It may be desirable to administer a first treatment, then to repeat pre-bone marrow administrations every 1-5 days. A blocker can also be administered one, two, three, or more times after donor bone marrow transplantation. Typically, a post-bone marrow transplant treatment is given about 2-14 days after bone marrow transplantation. The post-bone marrow administration can be repeated as many times as needed. If more than one administration is given, the administrations can be spaced about 1 week apart. Additional doses can be given if the patient appears to undergo early or unwanted T cell recovery. Preferably a blocker is administered at least once (and preferably two, three, or more times) prior to donor bone marrow transplantation and at lease once

(and preferably two, three, or more times) after donor bone marrow transplantation.

In accordance with the foregoing, any of the methods disclosed herein that comprise administering hematopoietic stem cells can further include multiple administrations of stem cells, such as a first and a second administration of stem cells are provided prior to the implantation of a graft; a first administration of donor stem cells is provided at the time of implantation of the graft. Ir. other preferred embodiments: a first administration of donor stem cells is provided prior to or at the time of implantation of a graft and a second administration of stem cells is provided subsequent to the implantation of a graft. The period between administrations of hematopoietic stem cells can be varied. In preferred embodiments a subsequent administration of hematopoietic stem cell is provided: at least two days, one week, one month, or six months after the previous administration of stem cells; at least two days, one week, one month, or six months after graft implantation.

The methods of the invention may further include the step of administering a second or subsequent dose of hematopoietic stem cells: when the recipient begins to show signs of rejection, e.g., as evidenced by a decline in function of the grafted organ, by a change in the recipient anti-donor antibody response, or by a change in the recipient lymphocyte response to donor antigen; when the level of chimerism decreases; when the level of chimerism falls below a predetermined value; when the level of chimerism reaches or falls below a level where staining with a monoclonal antibody specific for a donor MPHSC antigen is equal to or falls below staining with an isotype control which does not bind to MPHSC, e.g. when the recipient anti-donor specific antibody tag stains less than 1-2% of the cells; or generally, as is needed to maintain tolerance or otherwise prolong the acceptance of a graft. Patients can also be evaluated for the efficacy of further administrations of hematopoietic stem cells.

EXAMPLE 3

Competitive Staining And C-Kit Ligand Blocking Of Different Rat Anti-Mouse CD117 MAbs On P815 Mastocytoma Cells

Method

Five different rat antibodies were available for epitope mapping on the c-kit antigen of mouse cells: ACK2 (purified in-house from hybridoma supernatent), ACK4 (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada), ACK45 (BD Pharmingen), 2B8 (e-Bioscience, San Diego, USA) and 3C1 (Cedarlane Laboratories Ltd.). The mouse mastocytoma cell line P815 (obtained from ATCC No. TIB-64) was used to assess whether pre-coating the cells with a particular antibody has the ability to prevent binding of the biotinylated porcine stem cell factor (pSCF, BioTransplant Inc.) or other antibodies. From this, antibodies were categorized in terms of their recognition of different epitopes on the c-kit molecule. ACK2 and ACK45 appeared to share the same or a similar epitope at the ligand binding site as both ACK2 and ACK45 prevented staining with pSCF and ACK2 competed effectively with ACK45. On the other hand, the antibodies 2B8, 3C1 and ACK2 did not interfere with SCF binding. 2B8 and ACK2 appeared to recognize the same epitope as 2B8 coating prevented ACK2 staining. 3C1 recognized a rather distinct epitope as it did not compete with any of the antibodies tested. Pre-coating with ACK2 did produce lower staining with 3C1 to indicate some steric hindrance and suggesting that the epitope may be relatively close to the ligand binding site.

Results

Figure 1 shows staining intensity profiles for biotinylated pSCF and ACK45 and 3C1 antibodies on P815 cells with or without precoating with either ACK2 or 2B8. Complete blocking of the ligand and ACK45 and partial blocking of 3C1 was shown with ACK2 while no blocking was seen after 2B8.

All the current results are summarized in Table 3.1.

Table 3.1 – Epitope mapping for different rat anti-mouse CD117 MAbs by staining on P815 cells

		Blocking					
Precoating Antibody	**Isotype**	**pSCF (ligand)**	**ACK2**	**ACK45**	**ACK4**	**2B8**	**3C1**
ACK2	rat IgG2b, κ-1a	+		+	-	-	-/+
ACK45	rat IgG2b, κ	+	+		-	ND	ND
ACK4	rat IgG2a	-	ND	ND		ND	ND
2B8	rat IgG2b, κ	-	ND	-	+		-
3C1	rat IgG2b, κ	-	ND	-	-	-	

Two anti-c-kit MAbs from clones 2B8 and 3C1 that recognized a distinctly different epitope on the c-kit molecule and did not interfere with the binding of the c-kit ligand (see Table 3.1) were used for in vivo treatment of mice.

EXAMPLE 4

In Vivo Treatment With Anti-CD117 MAb (clone 2B8) In Mice

Method

Groups of C57BL/6J (B6) or BALB/c male mice received intraperitoneal injections of 2B8 at different dose levels and at different times in relation to recipient bone marrow harvesting (for estimation of bone marrow CFU-C and CAFC content, see below) or bone marrow transplant (for estimation of donor type chimerism). This treatment was compared with a non-myeloablative dose of busulfan (Busulfex® , Orphan Medical, Inc., Minnetonka, MN) that is known to provide for maintained mixed chimerism in both syngeneic and allogeneic BMT recipients (Adams et al. 2001; Andersson et al. 2003). This antibody was given according to the following doses and timing with respect to marrow harvest or BMT:

1. Saline alone.
2. Anti-CD117 (2B8) at doses of 0.5 mg per mouse at –4 and –2 days prior to BM harvest.
3. Anti-CD117 (2B8) at doses of 0.125 mg per mouse at –4 and –2 days prior to BM harvest.
4. Busulfex® (BX) at doses of 10 mg/kg at –3 and –2 days prior to BM harvest.

Figure 2 is the schematic representation of the experimental protocol of Example 4.

Colony-Forming Unit in Culture (CFU-C) Assay

Cells were plated in 35 mm Nalge/NUNC suspension dishes in 1ml complete methyl cellulose medium containing rmSCF, rmIL-3, rhIL-6 and rh erythropoietin (MethoCult GF M3434, Stem Cell Technologies Vancouver, BC, Canada). Typically, 5,000-20,000 cells were plated in 2 dishes. Colonies containing at least 50 cells were counted at day 7 after plating and CFU-C numbers per femur estimated from CFU frequency and femoral cellularity.

Cobblestone-Area Forming Cell (CAFC) Assay

The harvested BMCs were pooled for each experimental group and plated in MyeloCult M5300 (Stem Cell Technologies, Vancouver, BC, Canada) supplemented with 10^{-6} M hydrocortisone in limiting dilution on confluent layers of the murine stromal cell line 721C5 (obtained from Dr. Rob Ploemacher, Erasmus University, Rotterdam, The Netherlands) according to Ploemacher et al. (1989 and 1991). The number of CAFCs per femur and the 95% confidence intervals were calculated using a GW basic computer program according to the method devised by Fazekas de St. Groth (1982).

Results

Treatment of B6 mice with either 2B8 or Busulfex had relatively little effect on CFU-Cs or CAFC subsets appearing over the first 3 weeks in culture

(Figure 3). Nevertheless, this anti-CD117 antibody administered at low or high doses was capable of selectively reducing the CAFCs developing at beyond 4 weeks and in this respect appears more effective than Busulfex. High dose 2B8 and Busulfex appeared to be more effective at depleting progenitor populations in the BALB/c mouse strain but the low dose 2B8 was particularly effective at selective depletion of the primitive CAFC subsets and was again superior to Busulfex (Figure 4).

EXAMPLE 5

In Vivo Treatment With Anti-CD117 MAb (clone 2B8) at Lower Dose and Longer Treatment Schedule In Mice

Method

As in example 4, but the anti-CD117 antibody was given according to the following doses and timing with respect to marrow harvest:

1. Saline alone.
2. Anti-CD117 (2B8) at doses of 0.05 mg per mouse at –4 and –2 days prior to BM harvest.
3. Anti-CD117 (2B8) at doses of 0.05 mg per mouse at –9 and –7 days prior to BM harvest.
4. Anti-CD117 (2B8) at doses of 0.125 mg per mouse at –4 and –2 days prior to BM harvest.
5. Anti-CD117 (2B8) at doses of 0.125 mg per mouse at –9 and –7 days prior to BM harvest.

Figure 5 is the schematic representation of the experimental protocol of Example 4.

Results

Analysis of lineage negative bone marrow cells bearing the hematopoietic cell marker Sca-1showed no clear effect of any of the anti-CD117 MAb treatments using 2B8 (Figure 6) while a moderate depression in CFU-C and a more significant effect on all CAFC subsets were observed (Figure 7).

EXAMPLE 6

In Vivo Treatment With Anti-CD117 MAb (clone 3C1) In Mice

Method

A group of four BALB/c male mice received intraperitoneal injections of 3C1 at doses of 0.125 mp per mouse at –9 and –7 days prior to bone marrow harvesting for estimation of bone marrow CFU-C and CAFC content, according to the method in Example 4.

Figure 8 is the schematic representation of the experimental protocol of Example 6.

Results

Analysis of lineage negative bone marrow cells bearing the hematopoietic cell markers Sca-1 and c-kit (using the non-competing ACK45 MAb) showed no statistically significant ($p>0.05$ by Mann-Whitney U-test) between the untreated control and 3C1 treated mice (Figure 9).

3C1 also had no effect on the femoral content of committed progenitors as determined by CFU-Cs and CAFCs developing at 7 days but this antibody was particularly selective at significantly depleting the late CAFC subsets leaving a 20% surviving fraction after 5 weeks in culture (Figure 10).

EXAMPLE 7

In Vitro Treatment With Anti-CD117 MAb (clone 2B8)

Method

Harvested femoral bone marrow cells from a BALB/c male mouse were resuspended at 10^7 cells/ml in IMDM supplemented with 10% FBS, incubated at 4°C for 1 hour to coat cells with 10 μg/ml of either control rat IgG2b or 2B8 antibody, washed and resuspended in media containing 10% rabbit complement serum (Batch # 02119, Pelfreeze, Clinical Systems, Milwaukee, WI) and incubated at 37°C for 4 hours.

Following treatment the marrow cells were washed and cultured for estimation of CFU-C and CAFC subset frequencies (based on pretreatment cell numbers) as described for in vivo treatment above.

Results

Figure 11 shows that 2B8 plus complement had very little effect on CFU-Cs or CAFCs forming at day 7 while a progressive loss of CAFCs were seen with time in culture giving about 20% survival for the most primitive CAFC day 35 subset. These results are similar to the selective depletion of long-term repopulating stem cells following in vivo treatment with the same antibody and lend support to the notion that this effect is complement mediated.

EXAMPLE 8

Competitive staining and c-kit ligand blocking of different mouse anti-human CD117 MAbs on Mo7e leukemia cells

Method

The ability of the various anti-CD117 antibodies to interfere with one another and to prevent the binding of the c-kit ligand was tested by first precoating Mo7e cells with one antibody and then exposing the cells to one other antibody or to biotinylated porcine recombinant stem cell factor (bio-pSCF, BioTransplant Inc.).

Results

Table 8.1 lists how all of the13 different anti-CD117 antibodies can either block or allow staining by bio-pSCF. The anti-murine CD117 MAbs 2B8 and 3C1 were also tested on Mo7e cells but failed to show cross-reactive staining (Figure 12).

Figure 13 illustrates how two classes of antibodies interact with the c-kit antigen. These results support the previous epitope mapping studies in which SR-1 identifies an epitope independent of those bound by two other anti-CD117 MAbs, YB5.B8 and 17F11, while the latter two antibodies bound to distinct but interacting isotypes (Ashman et al. 1994). In contrast to YB5.B8 and 17F11, SR-1 potently blocked the binding of the ligand stem cell factor (SCF) to c-kit on both HEL-DR and Mo7e human leukemia cell lines (Broudy et al. 1992; Ashman et al. 1994). Furthermore, it exhibits growth inhibitory effects on normal bone marrow CFU-Cs and in a long-term Dexter-type marrow culture system (Papayannopoulou et al. 1991; Broudy et al. 1992; Liesveld et al. 1995).

Table 8.1 – Biological properties of 13 different mouse anti-human CD117 MAbs on Mo7e cells

Anti-Human CD117 MAb	Source	Isotype	Ligand Blocker	Mo7e Cell Lysis
104D2	Caltag	mouse IgG1	No	No
K44.2	BioSource	mouse IgG1	Yes	ND
K45	BioSource	mouse IgG2a	No	ND
K69	BioSource	mouse IgG1	No	No
Nu c-kit	Biotrend	mouse IgG1	Yes	ND
57A5	CalBiochem	mouse IgG1	No	No
SR-1	In-house from HB	mouse IgG2a	Yes	Yes
M9909235	Fitzgerald Industries	mouse IgG1	No	No
1.BB.178	U.S. Biological	mouse IgG1	No	No
1.BB.176	U.S. Biological	mouse IgG1	Yes	No
O.N. 181	U.S. Biological	mouse IgG1	No	No
O.N. 182	U.S. Biological	mouse IgG1	Yes	No
O.N. 183	U.S. Biological	mouse IgG2a	No	Yes

EXAMPLE 9

c-kit expression on side population (SP) cells of human bone marrow

Method

The SP cells in the marrow are a rare population defined by the ability to exclude the Hoechst 33342 dye from two fluorescence emission characteristics and is a phenotype commonly regarded to be associated with primitive stem cells in both mouse and human bone marrow (Goodell et al. 1996; Goodell et al. 1997). It is thus of interest to evaluate whether the anti-CD117 MAbs also stain these cells consistent with expression on the stem cell population.

Stocks of vertebral bone marrow have been obtained from The National Disease Research Interchange (Philadelphia, PA) and are stored in liquid nitrogen. Thawed and filtered human bone marrow was resuspended at 10^6 cells per ml in pre-warmed HBSS+ (Hanks Balanced Salt Solution (HBSS, Gibco) containing 2% Fetal Calf Serum and 10 mM HEPES buffer Gibco)) and Hoechst 33342 (Bis-Benzimide, Sigma) was added to a final concentration of 5 µg/ml. The cells were then incubated for 120 minutes at 37°C and then resuspended in cold HBSS+. The cells were then maintained on ice to prohibit leakage of the Hoechst dye from the cells and stained with 104D2-PE anti-CD117 MAb or O.N. 182 anti-CD117 MAb using goat anti-mouse Ig-PE as a secondary. Non-viable cells were discarded based on 7-AAD (BD PharMingen) exclusion and flow cytometric analysis performed using a Becton Dickinson FACSVantage SE with CellQuest™ software.

Results

As shown in Figure 14, a proportion of both SP positive as well as SP negative cells stain with anti-CD117 MAbs: about 17% and 27% of the SP cells were c-kit positive according to the 104D2 and O.N. 183 staining respectively. Whether this subpopulation of SP cells also contains the primitive stem cell subset is currently unknown and would warrant sorting of these cells and evaluation of long-term proliferative potential in the CAFC assay in vitro or of long-term engraftment in in vivo in transplanted NOD/SCID mice. These cells may also be valuable in generating hybridomas from rats preimmunized with intrasplenic injections of highly enriched stem cells. Such a strategy may allow us to generate our own stem cell-depleting antibodies for commercialization and with the possibility that we may identify other useful, perhaps unique, target antigens.

EXAMPLE 10

Complement-mediated lysis of Mo7e cells

Methods

The different anti-human CD117 MAbs were screened for their ability to lyse Mo7e cells via rabbit complement. Mo7e cells, an acute megakaryoblastic leukemia cell line (c-kit$^+$), were incubated at 1 x 10^7 cells/ml with either mouse isotype controls or the anti-c-kit antibodies [10 μg/ml] at 4° C to allow for antibody binding. Following a wash to remove unbound antibody, the cells were resuspended in RPMI+2% FBS supplemented with either 10% or 20% rabbit complement (Pelfreeze). The cells were treated with complement for 4 hours at 37° C to allow for lysis. Following the complement treatment step the cells were pelleted and supernatants from each sample was assayed for LDH release as a measure of cell lysis using the Cytotox 96 kit (Promega). The Campath 1G antibody (rat IgG2b) was used as a positive control in these assays.

Results

Of the 10 antibodies tested, only two showed evidence of complement-mediated lysis of the Mo7e line (See Figure 15). Both SR-1 and O.N. 183 are mouse IgG2a isotypes. Addition of a rat (IgG2b) anti-mouse IgG1 secondary antibody had no effect on the ability of these antibodies to mediate complement lysis of the Mo7e target cells.

While the mouse IgG2a isotype anti-CD117 antibodies could mediate lysis of Mo7e cells in conjunction with rabbit complement, the addition of 10% human serum (Male AB serum, N.A.B.I., Miami, FL) was not effective in the lysis of these targets, even following a 24 hour incubation (Figure 16).

EXAMPLE 11

Complement-mediated lysis of human bone marrow progenitor subsets

Method

Selected anti-human CD117 MAbs were also assessed for their ability to deplete various hematopoietic cell subsets present in human bone marrow. Stocks of vertebral bone marrow have been obtained from The National Disease Research Interchange (Philadelphia, PA) and are stored in liquid nitrogen. The cells were thawed and resuspended at 10^6 cells/ml in IMDM supplemented with 10% FBS, gentamycin, 50 μM β-mercaptoethanol and 10 μg/ml of an isotype control antibody or the test anti-CD117 antibody. The cell suspensions were incubated at 4°C for 1 hour to coat cells with the antibody, washed and resuspended in media containing 10% rabbit complement and incubated at 37°C for a further four hours.

Functional CFU-C and CAFC Assays

The functional assays consisted of evaluating the growth potential of short-term repopulating progenitors in the CFU-C assay and short- and long-term repopulating subsets in the CAFC assay. For the CFU-C assay, treated cells were plated in 1 ml complete methyl cellulose medium containing rhSCF, rhIL-3, rhGM-CSF, rh G-CSF, rhIL-6 and rh erythropoietin (MethoCult GF$^+$ M4435, Stem Cell Technologies Vancouver, BC, Canada) and the number of colonies with mono- (BFU-E and CFU-E), bi- (CFU-GM) and multipotential (CFU-GEMM or CFU-MIX) characteristics determined after 14 days of growth. The CAFC assay was based on the method of Breems et al. (1994) whereby test marrow cells were overlaid over a series of dilutions on 721C5 stromal cell layers in MyeloCult H5100 medium (Stem Cell Technologies Vancouver, BC, Canada) supplemented with 10^{-6} M hydrocortisone, 10 ng/ml rhIL-3 and 20 ng/ml rhG-CSF and the frequency of cobblestones at 2, 4, 6 and 8 weeks

in culture determined from limiting dilution analysis. Of particular importance to these experiments is the effect of anti-c-kit MAbs on the late-appearing CAFCs at 6 weeks and beyond as these correspond to the most primitive cells that need to be depleted in the recipient before achieving long-term engraftment of donor stem cells in the transplant setting.

A comparison of four different anti-human c-kit monoclonal antibodies was made using the CFU assay. Two mouse IgG1 (O.N. 181 and O.N. 182) and two mouse IgG2a (SR-1 and O.N. 183) antibodies were used to treat human bone marrow cells with and without rabbit complement as described above. SR-1 and O.N. 182 both block the binding of SCF to c-kit.

Results

Figure 17A shows that there was little effect of treatment with the SR-1 antibody plus rabbit complement on any of the CFU subsets. While there was no significant effect of this antibody on the CAFC frequency at the earliest time point of 2 weeks, subsequent CAFC subsets were significantly depleted out to 10 weeks in long-term culture (Figure 17B).

As for the comparison of two mouse IgG1 (O.N. 181 and O.N. 182) and two mouse IgG2a (SR-1 and O.N. 183), Figure 18 shows that none of the antibodies, either alone or in conjunction with complement had any effect on colony formation.

Summary

Examples 3 to 11 involving a number of different anti-mouse and anti-human c-kit monoclonal antibodies demonstrate that an agent can be developed commercially for application in stem cell transplant conditioning therapy. Initial studies on *in vivo* treatment of mice with antibodies that recognise distinctly different epitopes of the c-kit molecule has shown that

inhibition of binding of the c-kit ligand is a not a prerequisite for these antibodies to deplete hematopoietic stem cells. In the case of administering the inhibitor of SCF binding to c-kit, ACK2, marked depletion of the committed hematopoietic progenitor population was found, as shown in the CFU-C and the early-forming CAFC frequencies in the BALB/c mouse strain. However, analysis of the later-forming CAFC content revealed that the more primitive stem cell subset was refractory to the depleting effects of this antibody. On the other hand, treatment with the 2B8 MAb that is incapable of inhibiting SCF binding had the most promising effect in preferentially depleting the late-forming CAFC subsets. In this respect, the antibody was similar to the stem cell-depleting effect of administering Busulfex which was previously demonstrated to provide for long-term engraftment of donor stem cells. The comparable CAFC depletion results following the in vitro treatment of mouse marrow cells with 2B8 and rabbit complement suggests that complement-mediated cell killing may be partially responsible for the in vivo results.

The ability of anti-human c-kit MAbs to lyse after rabbit complement fixation the c-kit expressing human cell line Mo7e enabled rapid identification of those antibodies capable of depleting cells via complement-mediated cell killing. Among 10 mouse antibodies, two were identified that had efficacy in this respect, clones SR-1 and O.N. 183. As these were the only two that has the IgG2a isotype (the rest are IgG1), it appears that this was the most important factor. SR-1 blocked the ligand while O.N. 183 did not. Thus, this is not a characteristic that predisposes the cells to complement-mediated cell lysis. In contrast to the lysis of Mo7e cells, the normal human bone marrow progenitor population (CFU-Cs) were unaffected by any of the anti-human c-kit MAbs. This is perhaps not surprising given that the anti-mouse CD117 MAb 2B8 also had little effect on the bone marrow CFU-C population. In the one experiment in which the CAFC assay was used to evaluate the range of different hematopoietic subsets following SR-1 treatment and rabbit complement, significant depletion of the more primitive CAFC subsets appearing at 6 to 10 weeks indicates that this antibody can indeed selectively deplete stem cells in a similar fashion to the 2B8, bearing in mind that the

human CAFC assay system has a slower growth kinetics as compared to the murine counterpart (Breems et al. 1994). These examples showed that a monoclonal antibody such as anti-c-kit (anti-CD117) can be specifically directed against hematopoietic cells of in both mouse and human bone marrow and therefore can be included in a BMT conditioning regimen and replace agents such as busulfan or radiation in allowing for permanent engraftment of transplanted stem cells and thereby greatly reducing the toxiciticies associated with existing regimens.

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WHAT IS CLAIMED IS:

1. A therapeutic composition for enhancing engraftment in a recipient of donor stem cells comprising a pharmaceutically acceptable carrier and at least one antibody which deletes hematopoietic stem cells wherein said antibody is present in a therapeutically effective amount.
2. The therapeutic composition of claim 1 wherein said hematopoietic stem cells are primitive hematopoietic stem cells.
3. The therapeutic composition of claim 2 wherein said antibody selectively depletes said primitive hematopoietic stem cells.
4. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 50% of said primitive hematopoietic stem cells.
5. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 60% of said primitive hematopoietic stem cells.
6. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 70% of said primitive hematopoietic stem cells.
7. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 80% of said primitive hematopoietic stem cells.
8. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 90% of said primitive hematopoietic stem cells.
9. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 93% of said primitive hematopoietic stem cells.
10. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 95% of said primitive hematopoietic stem cells.

11. The therapeutic composition of claim 3 wherein said antibody selectively depletes at least 98% of said primitive hematopoietic stem cells.

12. The therapeutic composition of claim 1 wherein said antibody is a monoclonal antibody.

13. The therapeutic composition of claim 2 wherein said antibody is a monoclonal antibody.

14. The therapeutic composition of claim 1 wherein said antibody is a humanized antibody.

15. The therapeutic composition of claim 2 wherein said antibody is a humanized antibody.

16. The therapeutic composition of claim 1 wherein said antibody is a recombinant antibody.

17. The therapeutic composition of claim 2 wherein said antibody is a recombinant antibody.

18. The therapeutic composition of claim 1 wherein the at least one antibody for hematopoietic stem cells is selected from the group consisting of an anti-CD117 MAb, an anti-CD135 MAb, an anti-VEGFR2 (KDR) MAb, an anti-CD133 (AC133) MAb, an anti-TIE MAb, an anti-TEK MAb, an anti-CD117 MAb, an anti-C1qRp MAb and a combination thereof.

19. The therapeutic composition of claim 2 wherein the at least one antibody for primitive hematopoietic stem cells is selected from the group consisting of an anti-CD117MAb, an anti-CD135 MAb, an anti-VEGFR2 (KDR) MAb, an anti-CD133 (AC133) MAb, an anti-TIE MAb, an anti-TEK MAb, an anti-CD117 MAb, an anti-C1qRp MAb and a combination thereof.

20. The therapeutic composition of claim 1 wherein said antibody selectively depletes said hematopoietic stem cells

21. The therapeutic composition of claim 2 wherein said antibody selectively depletes said primitive hematopoietic stem cells

22. The therapeutic composition of claim 21 wherein said antibody selectively depletes said primitive hematopoietic stem cells which express CD34 antigen.

23. The therapeutic composition of claim 21 wherein said antibody selectively depletes said primitive hematopoietic stem cells which are CD34 negative.

24. A therapeutic composition for enhancing engraftment in a recipient of donor stem cells comprising a pharmaceutically acceptable carrier, at least one antibody specific for T cells, and at least one antibody which depletes hematopoietic stem cells wherein each of said antibody specific for T cells and said antibody for hematopoietic stem cells is present in a therapeutically effective amount.

25. The therapeutic composition of claim 24 wherein said hematopoietic stem cells are primitive hematopoietic stem cells.

26. The therapeutic composition of claim 25 wherein said antibody selectively depletes said primitive hematopoietic stem cells.

27. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 50% of said primitive hematopoietic stem cells.

28. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 60% of said primitive hematopoietic stem cells.

29. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 70% of said primitive hematopoietic stem cells.

30. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 80% of said primitive hematopoietic stem cells.

31. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 90% of said primitive hematopoietic stem cells.

32. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 93% of said primitive hematopoietic stem cells.

33. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 95% of said primitive hematopoietic stem cells.

34. The therapeutic composition of claim 26 wherein said antibody selectively depletes at least 98% of said primitive hematopoietic stem cells.

35. The therapeutic composition of claim 24 wherein said antibody is selected group the group consisting of monoclonal antibodies, polyclonal antibodies, antibody fragments and antibody derivatives.

36. The therapeutic composition of claim 25 wherein said antibody is selected group the group consisting of monoclonal antibodies, polyclonal antibodies, antibody fragments and antibody derivatives.

37. The therapeutic composition of claim 35 wherein said antibody is a humanized antibody.

38. The therapeutic composition of claim 36 wherein said antibody is a humanized antibody.

39. The therapeutic composition of claim 35 wherein said antibody is a monoclonal antibody.

40. The therapeutic composition of claim 36 wherein said antibody is a monoclonal antibody.

41. The therapeutic composition of claim 24 wherein the at least one antibody for hematopoietic stem cells is selected from the group consisting of an anti-CD117 MAb, an anti-CD135 MAb, an anti-VEGFR2 (KDR) MAb, an anti-CD133 (AC133) MAb, an anti-TIE MAb, an anti-TEK MAb, an anti-CD117 MAb, an anti-C1qRp MAb and a combination thereof.

42. The therapeutic composition of claim 25 wherein said at least one antibody for primitive hematopoietic stem cells is selected from the group consisting of an anti-CD117 MAb, an anti-CD135 MAb, an

anti-VEGFR2 (KDR) MAb, an anti-CD133 (AC133) MAb, an anti-TIE MAb, an anti-TEK MAb, an anti-CD117 MAb, an anti-C1qRp MAb and a combination thereof.

43. The therapeutic composition of claim 1 wherein said antibody selectively depletes said hematopoietic stem cells

44. The therapeutic composition of claim 2 wherein said antibody selectively depletes said primitive hematopoietic stem cells

45. The therapeutic composition of claim 21 wherein said antibody selectively depletes said primitive hematopoietic stem cells which express CD34 antigen.

46. The therapeutic composition of claim 21 wherein said antibody selectively depletes said primitive hematopoietic stem cells which are CD34 negative.

47. The therapeutic composition of claim 3 wherein the at least one antibody specific for primitive hematopoietic stem cells selectively depletes cells capable of forming colonies detectable after at least about four weeks in a CAFC assay.

48. The therapeutic composition of claim 3 wherein the at least one antibody specific for primitive hematopoietic stem cells selectively depletes cells capable of forming colonies detectable after at least about five weeks in a CAFC assay.

49. The therapeutic composition of claim 3 wherein the at least one antibody specific for primitive hematopoietic stem cells selectively depletes cells capable of forming colonies detectable after at least about ten weeks in a CAFC assay.

50. A method of promoting acceptance of a graft from a donor by a recipient comprising administering to said recipient at least one antibody which depletes hematopoietic stem cells prior to the introduction of the graft from the donor.

51. The method of claim 50 wherein said hematopoietic stem cells are primitive hematopoietic stem cells.

52. The method of claim 51 wherein said primitive hematopoietic stem cells are depleted selectively.

53. The method of claim 52 wherein at least 50% of said primitive hematopoietic stem cells are depleted selectively.

54. The method of claim 52 wherein at least 60% of said primitive hematopoietic stem cells are depleted selectively.

55. The method of claim 52 wherein at least 70% of said primitive hematopoietic stem cells are depleted selectively.

56. The method of claim 52 wherein at least 80% of said primitive hematopoietic stem cells are depleted selectively.

57. The method of claim 52 wherein at least 90% of said primitive hematopoietic stem cells are depleted selectively.

58. The method of claim 52 wherein at least 93% of said primitive hematopoietic stem cells are depleted selectively.

59. The method of claim 52 wherein at least 95% of said primitive hematopoietic stem cells are depleted selectively.

60. The method of claim 52 wherein at least 98% of said primitive hematopoietic stem cells are depleted selectively.

61. The method of claim 52 wherein said antibody does not react with mature blood cells.

62. The method of claim 50 wherein said antibody is administered prior to graft transplantation.

63. The method of claim 50 wherein said antibody is a monoclonal (MAb) antibody.

64. The method of claim 50 wherein said antibody is a recombinant antibody.

65. The method of claim 51 wherein said antibody is an anti-c-kit (anti-CD117) antibody.

66. The method of claim 50 wherein said antibody is selected from the group consisting of an anti-CD135 MAb, an anti-VEGFR2 (KDR) MAb, an anti-CD133 (AC133) MAb, an anti-TIE MAb, an anti-TEK

MAb, an anti-CD117 MAb, an anti-C1qRp MAb and a combination thereof.

67. The method of claim 51 wherein said antibody is one that depletes primitive hematopoietic stem cells expressing the CD34 antigen.

68. The method of claim 51 wherein said antibody depletes $CD34^-$ cells.

69. The method of claim 50 wherein said administering occurs intravenously.

70. The method of claim 50 wherein said graft is a xenograft.

71. The method of claim 70 wherein the donor of said xenograft is a miniature swine.

72. The method of claim 71 wherein the recipient of said xenograft is a human.

73. The method of claim 70 wherein the recipient of said xenograft is a human and the donor of said xenograft is a miniature swine.

74. The method of claim 50 wherein said antibody is administered intramuscularly.

75. The method of claim 50 wherein said graft comprises mammalian hematopoietic stem cells.

76. The method of claim 50 wherein said antibody is a c-kit antibody.

77. The method of claim 50 wherein said method does not involve use of cytotoxic drugs.

78. The method of claim 50 wherein said method does not involve use of radiation.

79. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting for the purpose of treating a malignant disease.

80. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting for the purpose of treating a non-malignant disease.

81. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting to induce mixed chimerism prior to administering a solid organ graft.

82. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting for the purpose of treating graft versus host disease (GvHD).

83. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting for the purpose of preventing graft versus host disease (GvHD).

84. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting for the purpose of supporting donor-leukocyte infusions (DLI).

85. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting or the purpose of treating an enzyme deficiency disease.

86. The method of claim 75 wherein said human hematopoietic stem cells are administered for engrafting for the purpose of treating an autoimmune disease.

87. The method of claim 70 further comprising administering to the recipient of said xenograft an effective amount of hematopoietic stem cells derived from the donor of said xenograft.

88. The method of claim 87 wherein said hematopoietic stem cells are administered intravenously.

89. The method of claim 88 further comprising more than one administering of said hematopoietic stem cells.

90. The method of claim 72 further comprising administering to said recipient an effective amount of an anti-natural killer cell (anti-NK) antibody to inactivate natural killer cells (NK cells) of the recipient.

91. The method of claim 90 wherein the NK cells to be inactivated are graft-reactive NK cells.

92. The method of claim 91 wherein the graft is a xenograft and the NK cells to be inactivated are xenoreactive NK cells.

93. The method of claim 92 wherein the graft donor is swine and the NK cells to be inactivated are swine reactive NK cells.

94. The method of claim 90 wherein the anti-NK antibody is administered prior to introducing said graft.

95. The method of claim 90 wherein the anti-NK antibody is administered prior to said antibody which depletes hematopoietic stem cells.

FIGURE 1

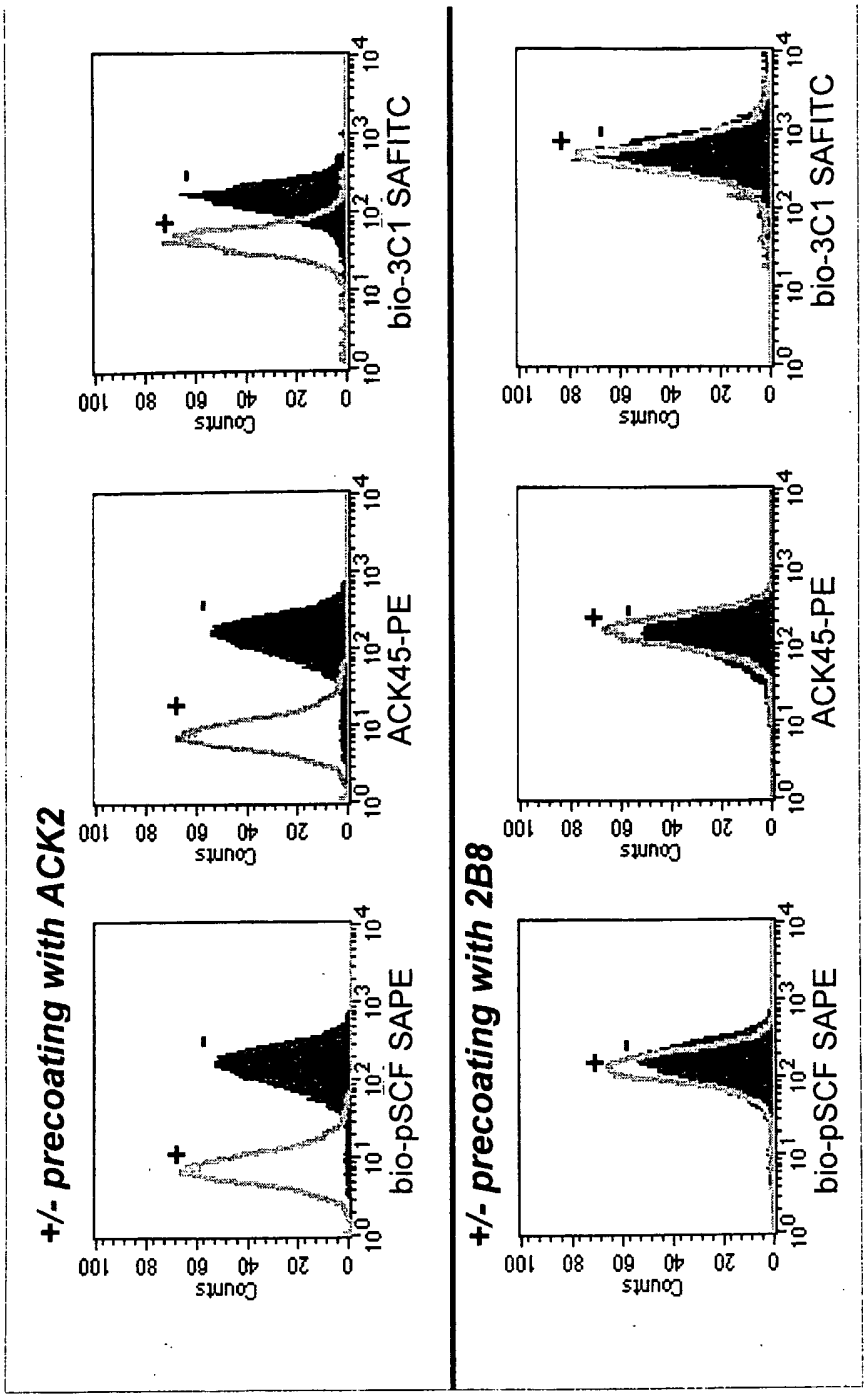

+/- precoating with ACK2
+/- precoating with 2B8
bio-pSCF SAPE
ACK45-PE
bio-3C1 SAFITC
Counts
0
20
40
60
80
100
+
-

FIGURE 2

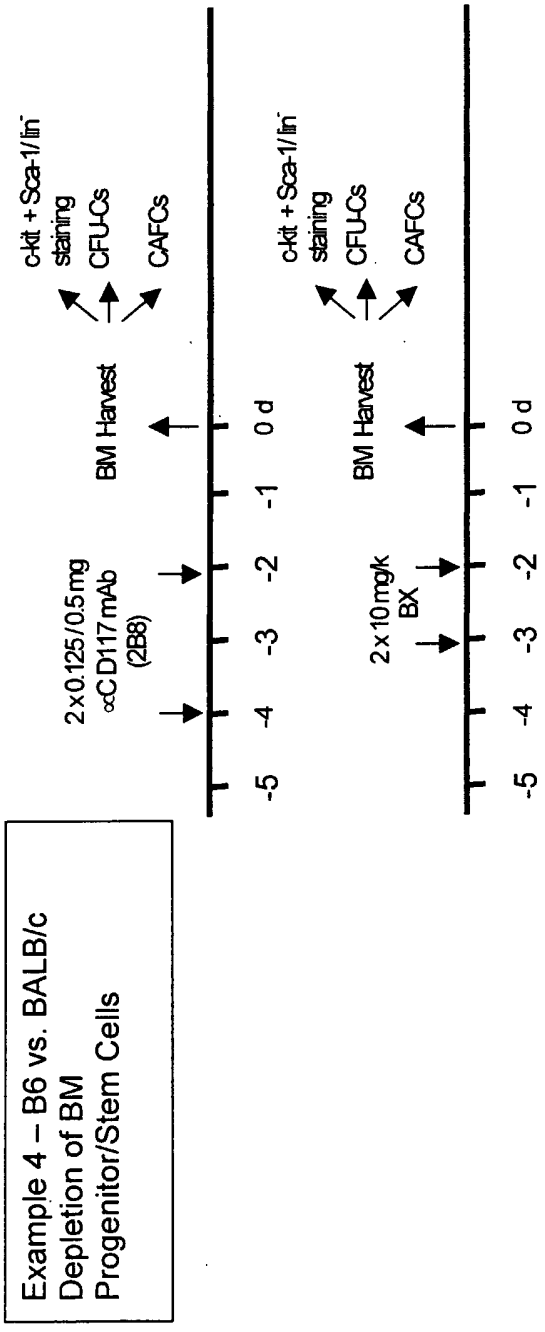

Example 4 – B6 vs. BALB/c
Depletion of BM
Progenitor/Stem Cells
2x0.125/0.5mg
αCD117 mAb
(2B8)
BM Harvest
c-kit + Sca-1/lin-
staining
CFU-Cs
CAFCs
-5 -4 -3 -2 -1 0 d
2x10 mg/k
BX
BM Harvest
c-kit + Sca-1/lin-
staining
CFU-Cs
CAFCs
-5 -4 -3 -2 -1 0 d

FIGURE 3

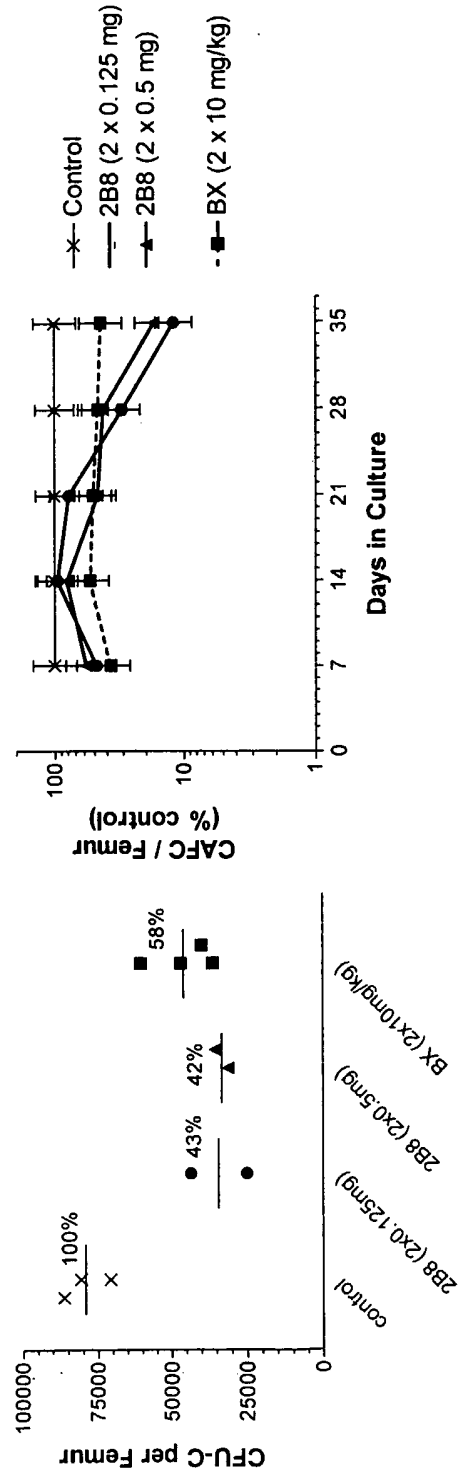

Control
2B8 (2 x 0.125 mg)
2B8 (2 x 0.5 mg)
BX (2 x 10 mg/kg)
CAFC / Femur (% control)
100
10
1
0
7
14
21
28
35
Days in Culture
CFU-C per Femur
100000
75000
50000
25000
0
100%
43%
42%
58%
control
2B8 (2x0.125mg)
2B8 (2x0.5mg)
BX (2x10mg/kg)

FIGURE 4

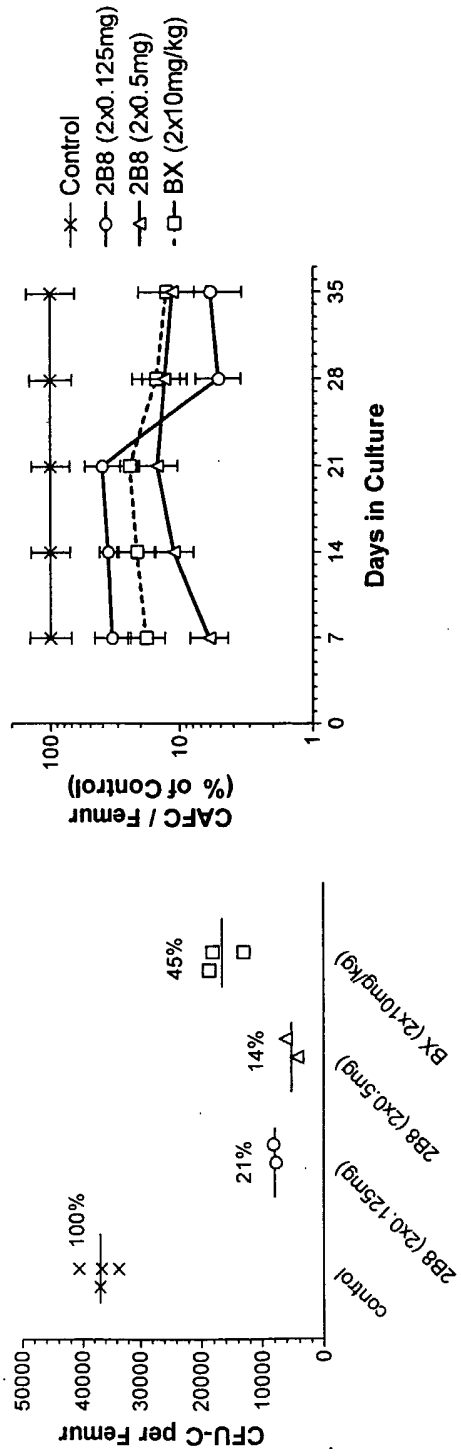

Control
2B8 (2x0.125mg)
2B8 (2x0.5mg)
BX (2x10mg/kg)
CAFC / Femur
(% of Control)
100
10
1
0
7
14
21
28
35
Days in Culture
CFU-C per Femur
50000
40000
30000
20000
10000
0
100%
21%
14%
45%
control
2B8 (2x0.125mg)
2B8 (2x0.5mg)
BX (2x10mg/kg)

FIGURE 5

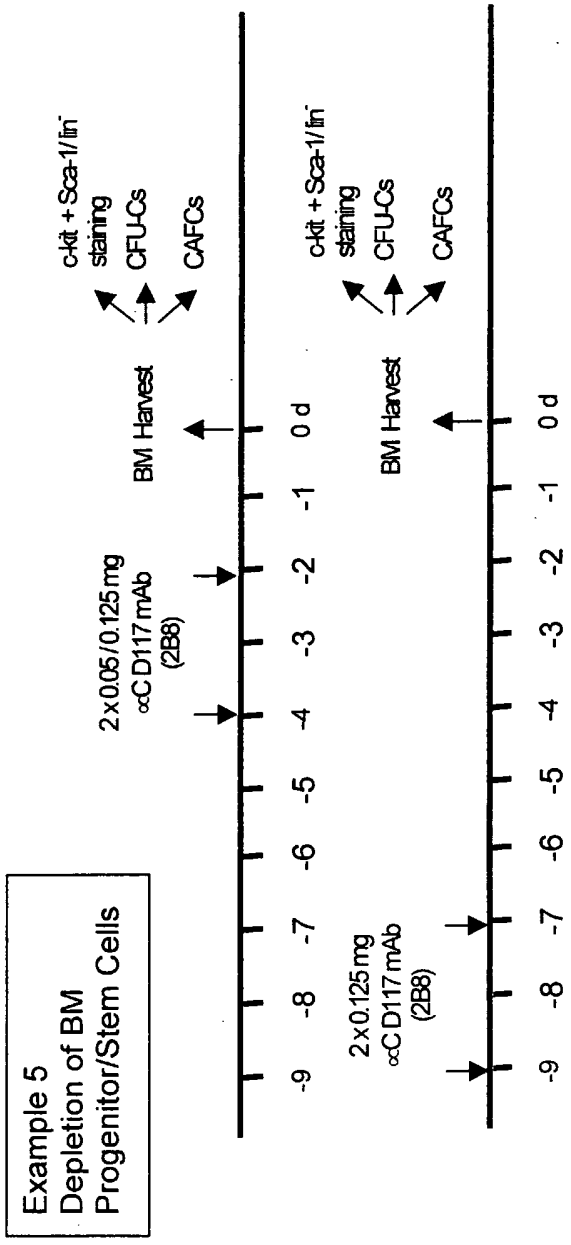

Example 5
Depletion of BM
Progenitor/Stem Cells
2 x 0.05/0.125 mg
αC D117 mAb
(2B8)
BM Harvest
c-kit + Sca-1/lin-
staining
CFU-Cs
CAFCs
2 x 0.125 mg
αC D117 mAb
(2B8)
BM Harvest
c-kit + Sca-1/lin-
staining
CFU-Cs
CAFCs
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 d

FIGURE 6

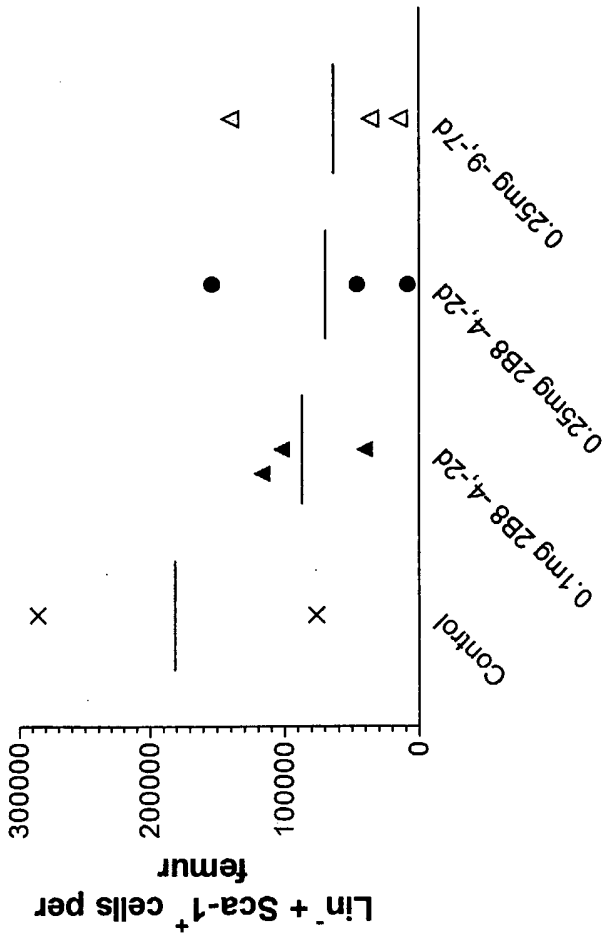

Lin⁻ + Sca-1⁺ cells per femur
300000
200000
100000
0
Control
0.1mg 2B8 4-2d
0.25mg 2B8 4-2d
0.25mg 9-7d

FIGURE 7

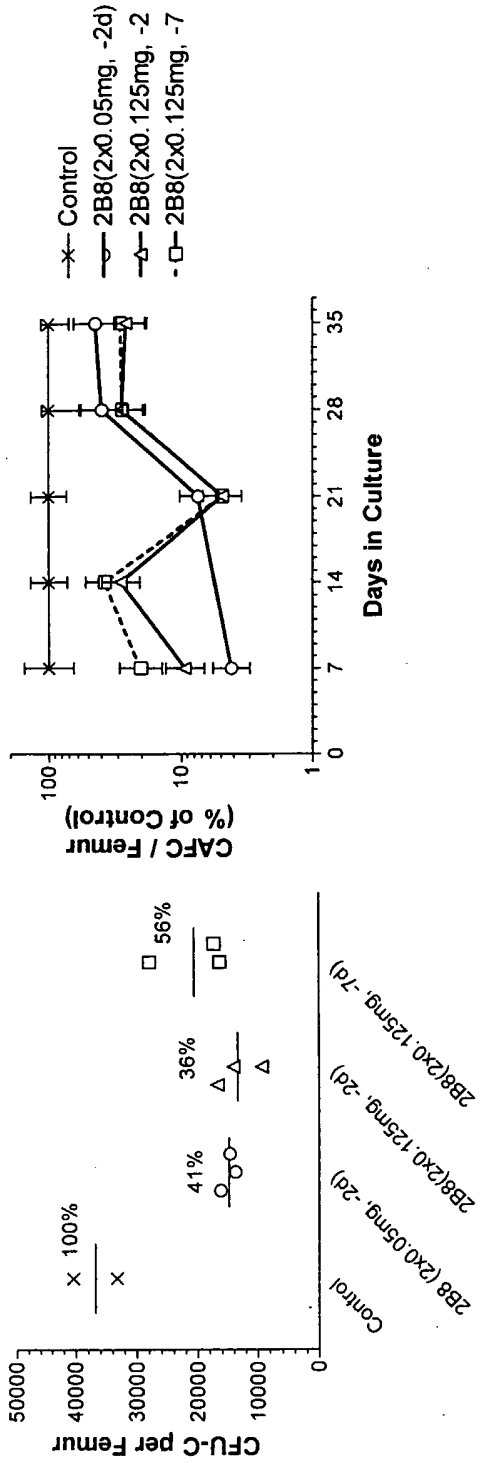

Control
2B8(2x0.05mg, -2d)
2B8(2x0.125mg, -2
2B8(2x0.125mg, -7
CAFC / Femur
(% of Control)
100
10
1
0
7
14
21
28
35
Days in Culture
CFU-C per Femur
50000
40000
30000
20000
10000
0
100%
41%
36%
56%
Control
2B8 (2x0.05mg, -2d)
2B8(2x0.125mg, -2d)
2B8(2x0.125mg, -7d)

FIGURE 8

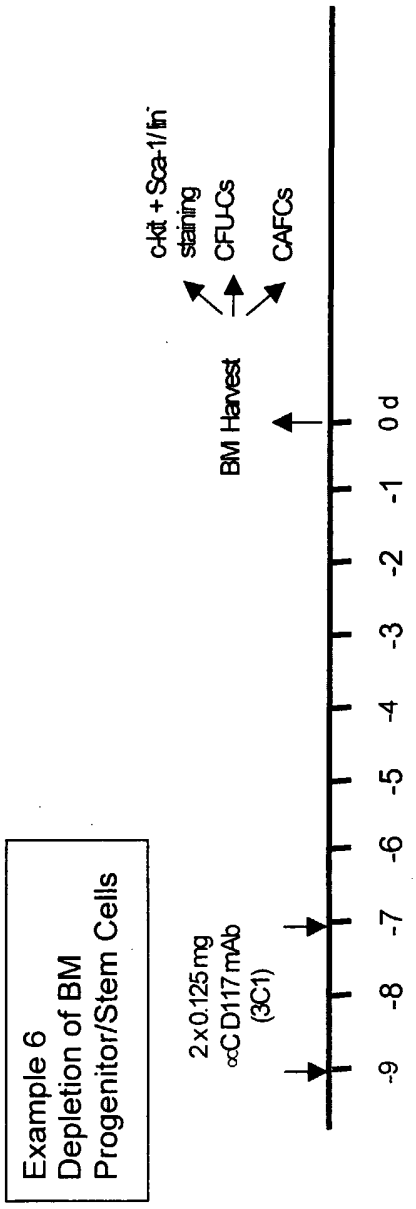

Example 6
Depletion of BM
Progenitor/Stem Cells
2 x 0.125 mg
αCD117 mAb
(3C1)
BM Harvest
c-kit + Sca-1/lin
staining
CFU-Cs
CAFCs
-9
-8
-7
-6
-5
-4
-3
-2
-1
0 d

FIGURE 9

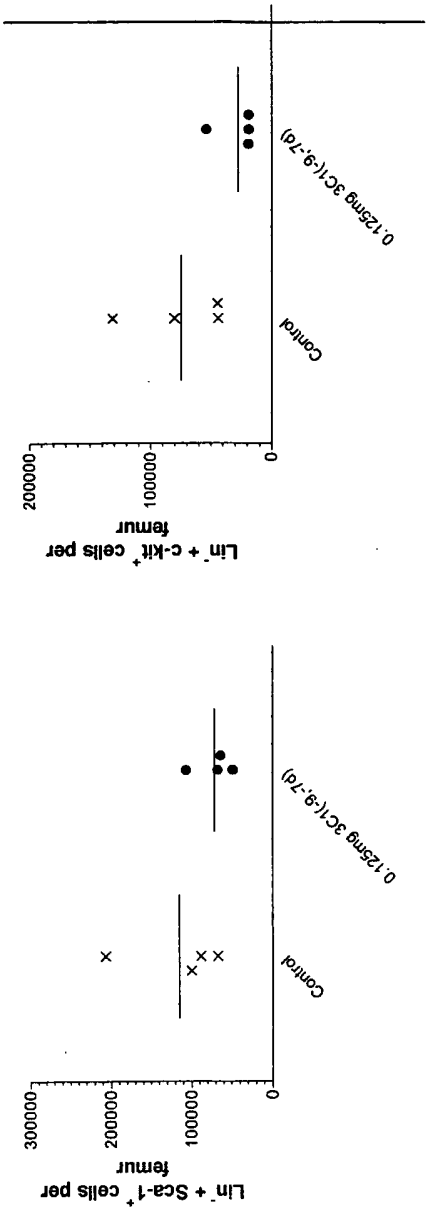

Lin⁻ + Sca-1⁺ cells per femur
300000
200000
100000
0
Control
0.125mg 3CI(-9,-7d)
Lin⁻ + c-kit⁺ cells per femur
200000
100000
0
Control
0.125mg 3CI(-9,-7d)

FIGURE 10

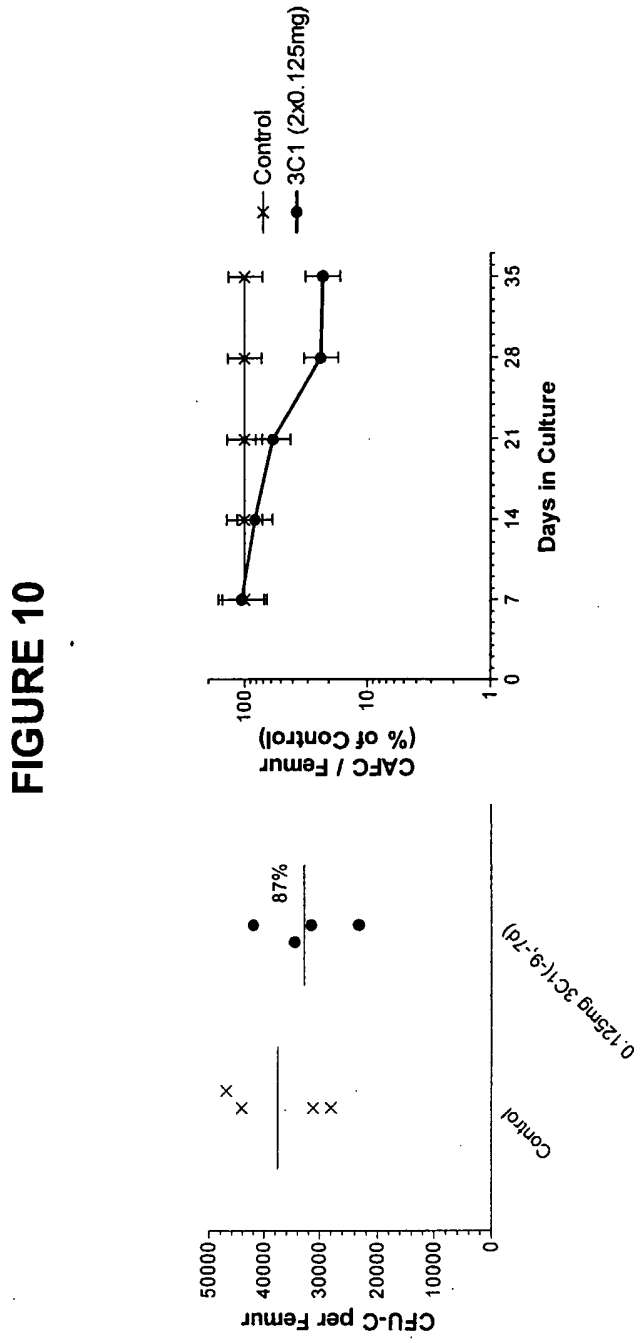

Control
3C1 (2x0.125mg)
CAFC / Femur (% of Control)
100
10
1
0
7
14
21
28
35
Days in Culture
CFU-C per Femur
50000
40000
30000
20000
10000
0
87%
Control
0.125mg 3C1(-9,-7d)

FIGURE 11

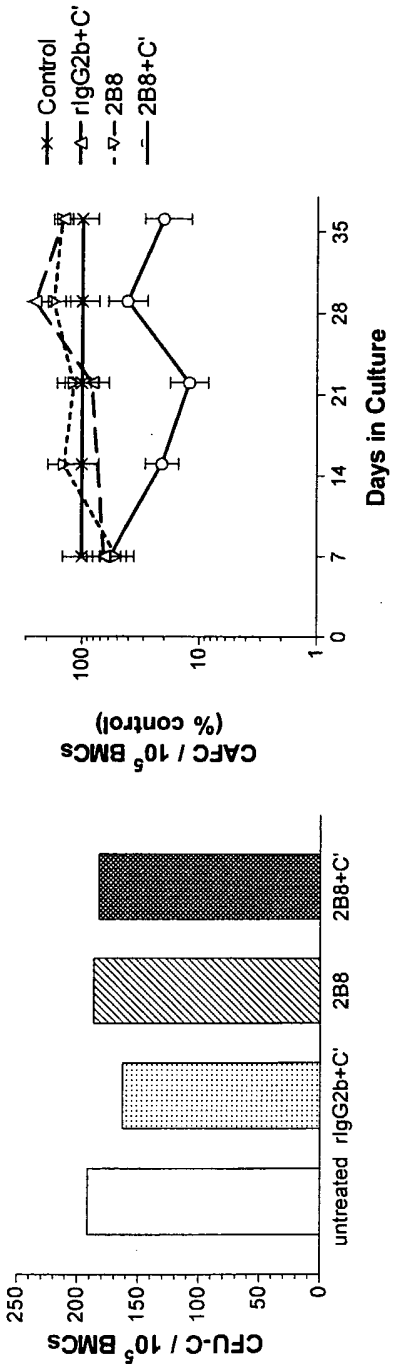

Control
rIgG2b+C'
2B8
2B8+C'
CAFC / 10^5 BMCs (% control)
100
10
1
0
7
14
21
28
35
Days in Culture
CFU-C / 10^5 BMCs
250
200
150
100
50
0
untreated
rIgG2b+C'
2B8
2B8+C'

FIGURE 12

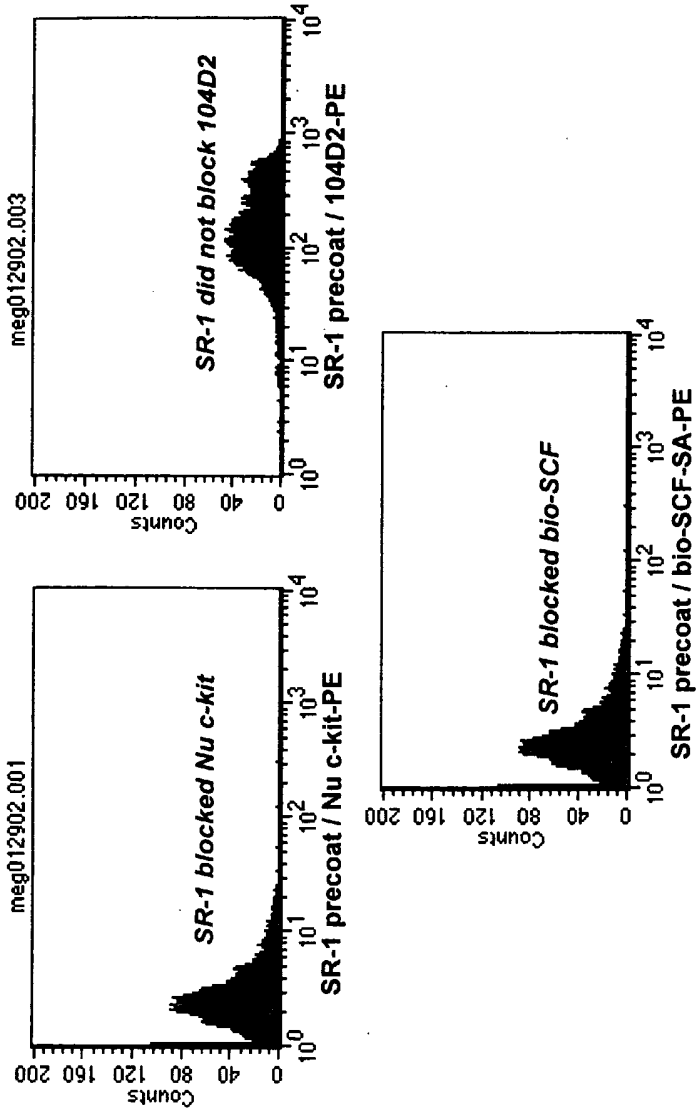

meg012902.003
SR-1 did not block 104D2
SR-1 precoat / 104D2-PE
meg012902.001
SR-1 blocked Nu c-kit
SR-1 precoat / Nu c-kit-PE
SR-1 blocked bio-SCF
SR-1 precoat / bio-SCF-SA-PE
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4

FIGURE 13

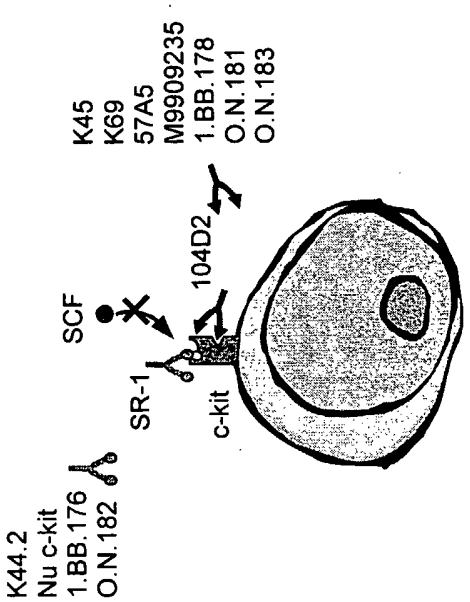

K45
K69
57A5
M9909235
1.BB.178
O.N.181
O.N.183
104D2
SCF
SR-1
c-kit
K44.2
Nu c-kit
1.BB.176
O.N.182

FIGURE 14

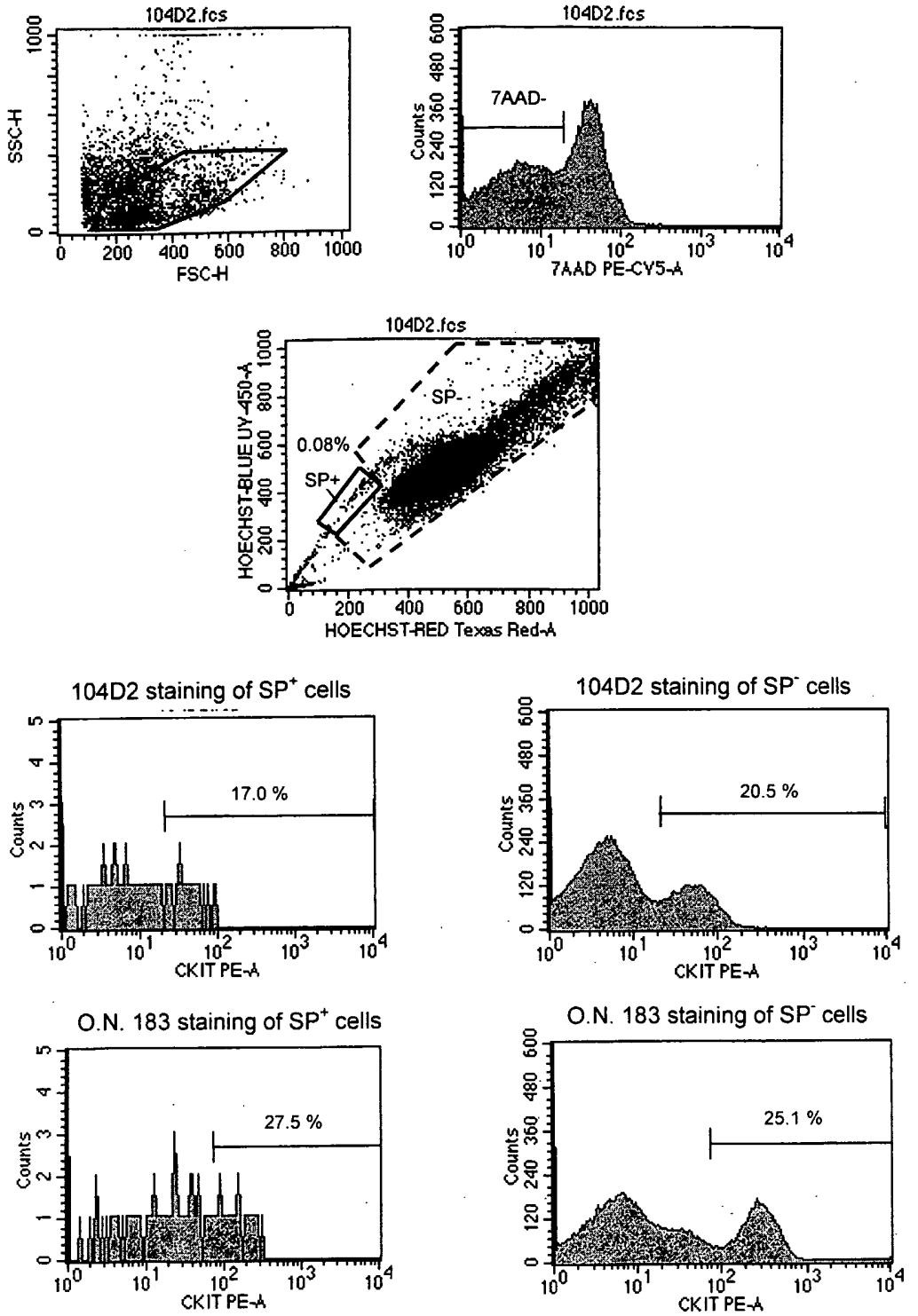
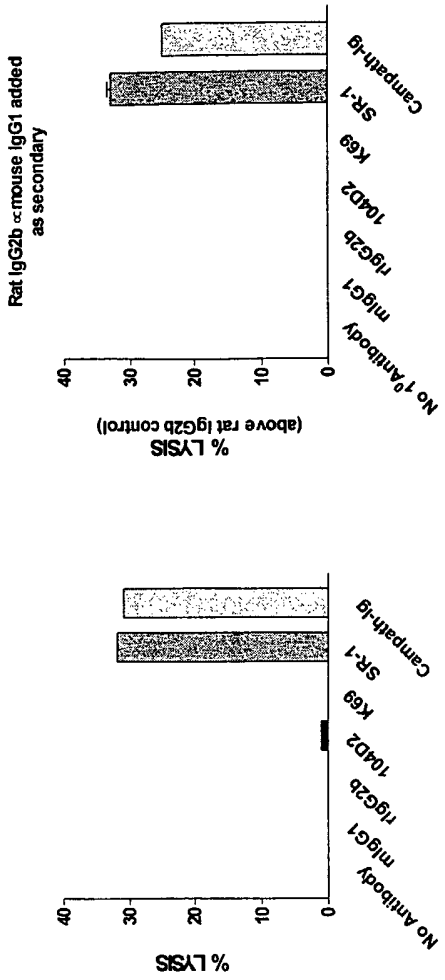

104D2.fcs
SSC-H
FSC-H
104D2.fcs
7AAD-
Counts
7AAD PE-CY5-A
104D2.fcs
SP-
0.08%
SP+
HOECHST-BLUE UV-450-A
HOECHST-RED Texas Red-A
104D2 staining of SP+ cells
17.0 %
Counts
CKIT PE-A
104D2 staining of SP- cells
20.5 %
Counts
CKIT PE-A
O.N. 183 staining of SP+ cells
27.5 %
Counts
CKIT PE-A
O.N. 183 staining of SP- cells
25.1 %
Counts
CKIT PE-A

FIGURE 15


Rat IgG2b α mouse IgG1 added as secondary
% LYSIS
(above rat IgG2b control)
No 1°Antibody
mIgG1
rIgG2b
104D2
K69
SR-1
Campath-Ig
% LYSIS
No Antibody
mIgG1
rIgG2b
104D2
K69
SR-1
Campath-Ig

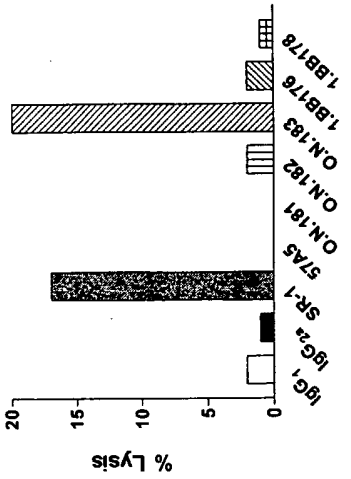

% Lysis
IgG1
IgG2a
SR-1
57A5
O.N.181
O.N.182
O.N.183
1.BB178
1.BB178

FIGURE 16

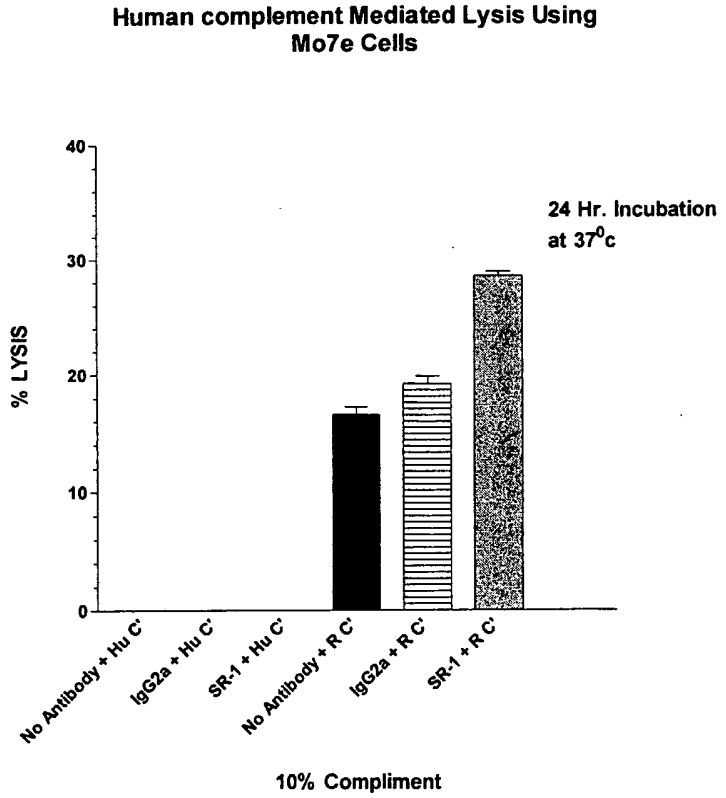

Human complement Mediated Lysis Using Mo7e Cells
24 Hr. Incubation at 37°c
% LYSIS
40
30
20
10
0
No Antibody + Hu C'
IgG2a + Hu C'
SR-1 + Hu C'
No Antibody + R C'
IgG2a + R C'
SR-1 + R C'
10% Compliment

FIGURE 17

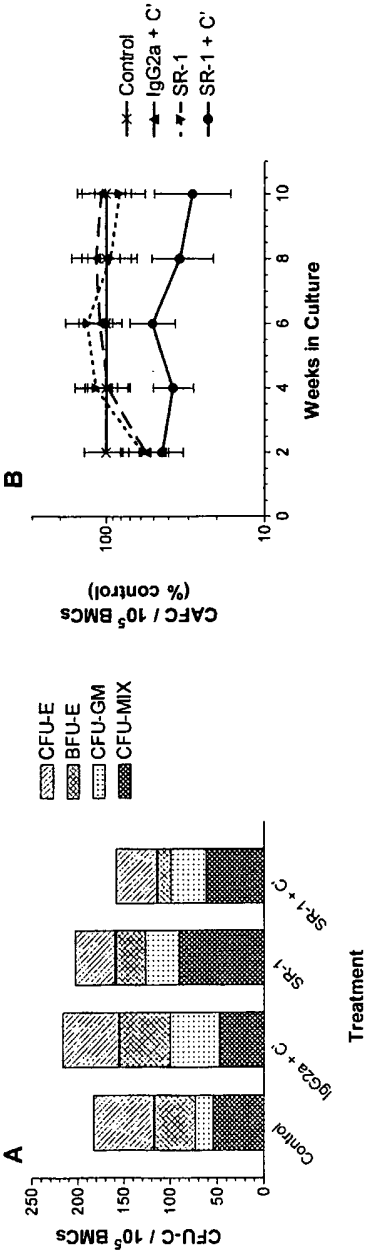

A
CFU-C / 10^5 BMCs
250
200
150
100
50
0
CFU-E
BFU-E
CFU-GM
CFU-MIX
Control
IgG2a + C'
SR-1
SR-1 + C'
Treatment
B
CAFC / 10^5 BMCs (% control)
100
10
0
2
4
6
8
10
Weeks in Culture
Control
IgG2a + C'
SR-1
SR-1 + C'

FIGURE 18
CFU assay using anti-human c-kit mAbs