(54) Title: COMPOSITIONS AND METHODS FOR PROVIDING HEMATOPOIETIC FUNCTION

Fig. 1

(57) Abstract: The present invention relates to methods and compositions for providing hematopoietic function to human patients in need thereof, by selecting a pool of expanded human cord blood stem/progenitor cell samples for administration to the patient, wherein the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient; and administering the selected pool of expanded human cord blood stem/progenitor cell samples to the patient. Methods for obtaining the pools of expanded human cord blood stem/progenitor cell samples, banks of frozen pools of expanded human umbilical cord blood stem/progenitor cell samples, and methods for producing such banks are also provided herein.

<u>COMPOSITIONS AND METHODS FOR</u>
<u>PROVIDING HEMATOPOIETIC FUNCTION</u>

5   This application claims benefit of U.S. Provisional Application No. 61/322,575
filed April 9, 2010, which is incorporated by reference herein in its entirety.

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HHS010020080064C awarded by the U.S. Department of Health and Human Services
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10   National Heart, Lung and Blood Institute of the National Institutes of Health, U.S.
Department of Health and Human Services. The U.S. government has certain rights in
the invention.

1.   FIELD OF THE INVENTION
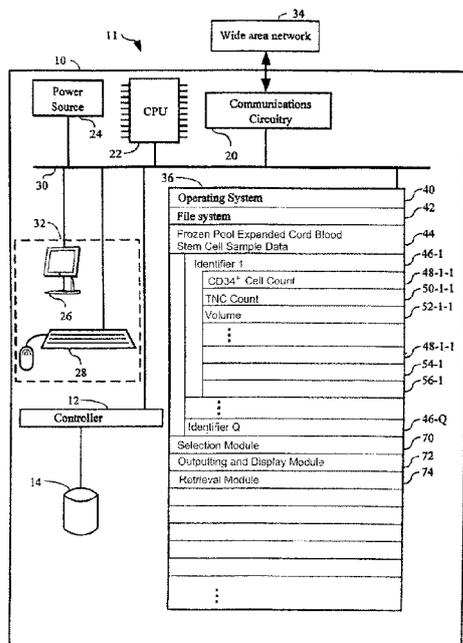
15   The present invention relates to methods and compositions for providing
hematopoietic function to human patients in need thereof, by selecting a pool of
expanded human cord blood stem/progenitor cell samples for administration to the
patient, wherein the samples in the pool collectively do not mismatch the patient at more
than 2 of the HLA antigens or alleles typed in the patient; and administering the selected
20   pool of expanded human cord blood stem/progenitor cell samples to the patient.
Methods for obtaining the pools of expanded human cord blood stem/progenitor cell
samples, banks of frozen pools of expanded human umbilical cord blood
stem/progenitor cell samples, and methods for producing such banks are also provided
herein.

25

2.   BACKGROUND OF THE INVENTION

Prolonged pancytopenia is common following intensive chemotherapy regimens,
myeloablative and reduced intensity regimens for hematopoietic cell transplantation
(HCT), and exposure to acute ionizing radiation. Of particular concern is prolonged
30   neutropenia, which results in a significant risk of infection despite improved
antimicrobial therapy and increases morbidity and mortality. Thus, novel therapies that
can abrogate prolonged pancytopenia/neutropenia following high dose chemotherapy

and/or radiation, and potentially facilitate more rapid hematopoietic recovery, are needed.

Expansion techniques for cord blood stem cells have been described. See, *e.g.*, U.S. Patent No. 7,399,633 B2 to Bernstein *et al.*, and Delaney *et al.*, 2010, Nature Med.,

5   16(2):232-236. Delaney *et al.* reported rapid engraftment after infusion of previously cryopreserved cord blood stem cells which had been selected on the basis of HLA matching, and which had been expanded *ex vivo*.

International Patent Publication No. WO 2006/047569 A2 discloses methods for expanding myeloid progenitor cells that do not typically differentiate into cells of the

10   lymphoid lineage, and which can be MHC-mismatched with respect to the recipient of the cells.

International Patent Publication No. WO 2007/095594 A2 discloses methods for facilitating engraftment of hematopoietic stem cells by administering myeloid progenitor cells in conjunction with the hematopoietic stem cell graft, for example, where the

15   hematopoietic stem cell graft is suboptimal because it has more than one MHC mismatch with respect to the cells of the recipient patient.

U.S. Patent 5,004,681 to Boyse *et al.* discloses the use of human cord blood stem cells for hematopoietic reconstitution.

20   **2.1   HUMAN LEUKOCYTE ANTIGEN**

The human leukocyte antigen system (HLA) is the name of the major histocompatibility complex (MHC) in humans. The superlocus contains a large number of genes related to immune system function in humans. This group of genes resides on chromosome 6, and encodes cell-surface antigen-presenting proteins and many other

25   genes. The HLA genes are the human versions of the MHC genes that are found in most vertebrates (and thus are the most studied of the MHC genes). The proteins encoded by the HLA genes are also known as antigens, as a result of their historic discovery as factors in organ transplantations. The major HLA antigens are essential elements for immune function. Different classes have different functions.

30   HLA class I antigens (HLA-A, HLA-B and HLA-C) are transmembrane proteins that are expressed on the surface of almost all the cells of the body (except for red blood cells and the cells of the central nervous system) and present peptides on the cell surface,

which peptides are produced from digested proteins that are broken down in the proteasomes.

HLA class II antigens (HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR) present antigens from outside of the cell to T-lymphocytes. These

5   particular antigens stimulate T-helper cells to multiply, and these T-helper cells then stimulate antibody-producing B-cells to produce antibodies to that specific antigen. Self-antigens are suppressed by suppressor T-cells.

HLA class III antigens encode components of the complement system.

HLA antigens have other roles. They are important in disease defense. They

10   may be the cause of organ transplant rejections. They may protect against or fail to protect against (if down regulated by an infection) cancers. They may mediate autoimmune disease, e.g., type I diabetes, coeliac disease). Also, in reproduction, HLA may be related to the individual smell of people and may be involved in mate selection.

Diversity of HLA in human population is one aspect of disease defense, and, as a

15   result, the chance of two unrelated individuals having identical HLA molecules on all loci is very low. Thus, in the prior art, there was a need for HLA typing to determine suitable allele matching to avoid rejection of the donor tissue by the recipient or, in the case of hematopoietic stem cell transplants, to avoid the possibility of the donated hematopoietic cells from attacking the recipient. Most tissue typing is done using

20   serological methods with antibodies specific for identified HLA antigens. DNA-based methods for detecting polymorphisms in the HLA antigen-encoding gene are also used for typing HLA alleles. Currently in the clinical setting for cord blood transplants, HLA typing of the donor tissue and the recipient concerns determining six HLA antigens or alleles, usually two each at the loci HLA-A, HLA-B and HLA-DR, or one each at the

25   loci HLA-A, HLA-B and HLA-C and one each at the loci HAL-DRB1, HLA-DQB1 and HLA-DPB1 (see e.g., Kawase et al., 2007, Blood 110:2235-2241). HLA typing can be done (1) by determining the HLA allele, which is done on the DNA sequence level by determining the allele-specific sequences, and/or (2) by determining the HLA antigen serologically, by way of antibodies specific for the HLA-antigen.

30

2.2 HEMATOPOIETIC STEM CELLS

The hematopoietic stem cell is pluripotent and ultimately gives rise to all types of terminally differentiated blood cells. The hematopoietic stem cell can self-renew, or it

5   can differentiate into more committed progenitor cells, which progenitor cells are irreversibly determined to be ancestors of only a few types of blood cell. For instance, the hematopoietic stem cell can differentiate into (i) myeloid progenitor cells, which myeloid progenitor cells ultimately give rise to monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic

10   cells, or (ii) lymphoid progenitor cells, which lymphoid progenitor cells ultimately give rise to T-cells, B-cells, and lymphocyte-like cells called natural killer cells (NK-cells). Once the stem cell differentiates into a myeloid progenitor cell, its progeny cannot give rise to cells of the lymphoid lineage, and, similarly, lymphoid progenitor cells cannot give rise to cells of the myeloid lineage. For a general discussion of hematopoiesis and

15   hematopoietic stem cell differentiation, see Chapter 17, Differentiated Cells and the Maintenance of Tissues, Alberts *et al.*, 1989, Molecular Biology of the Cell, 2nd Ed., Garland Publishing, New York, NY; Chapter 2 of Regenerative Medicine, Department of Health and Human Services, August 2006 (http://stemcells.nih.gov/info/scireport/2006report.htm), and Chapter 5 of Hematopoietic

20   Stem Cells, 2009, Stem Cell Information, Department of Health and Human Services (http://stemcells.nih.gov/info/scireport/chapter5.asp).

In vitro and in vivo assays have been developed to characterize hematopoietic stem cells, for example, the spleen colony forming (CFU-S) assay and reconstitution assays in immune-deficient mice. Further, presence or absence of cell surface protein

25   markers defined by monoclonal antibody recognition have been used to recognize and isolate hematopoietic stem cells. Such markers include, but are not limited to, Lin, CD34, CD38, CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, and HLA DR, and combinations thereof. See Chapter 2 of Regenerative Medicine, Department of Health and Human Services, August 2006

30   (http://stemcells.nih.gov/info/scireport/2006report.htm) and the references cited therein.

2.3   NOTCH PATHWAY

Members of the Notch family encode large transmembrane proteins that play central roles in cell-cell interactions and cell-fate decisions during early development in a number of invertebrate systems (Simpson, 1995, Nature 375:736-7; Artavanis-Tsakonis *et al.*, 1995, Science. 268:225-232; Simpson, 1998, Semin. Cell Dev. Biol. 9:581-2; Go *et al.*, 1998, Development. 125:2031-2040; Artavanis-Tsakonas and Simpson, 1991, Trends Genet. 7:403-408). The Notch receptor is part of a highly conserved pathway that enables a variety of cell types to choose between alternative differentiation pathways based on those taken by immediately neighboring cells. This receptor appears to act through an undefined common step that controls the progression of uncommitted cells toward the differentiated state by inhibiting their competence to adopt one of two alternative fates, thereby allowing the cell either to delay differentiation, or in the presence of the appropriate developmental signal, to commit to differentiate along the non-inhibited pathway.

Genetic and molecular studies have led to the identification of a group of genes which define distinct elements of the Notch signaling pathway. While the identification of these various elements has come exclusively from *Drosophila* using genetic tools as the initial guide, subsequent analyses have lead to the identification of homologous proteins in vertebrate species including humans. The molecular relationships between the known Notch pathway elements as well as their subcellular localization are depicted in Artavanis-Tsakonas *et al.*, 1995, Science 268:225-232; Artavanis-Tsakonas *et al.*, 1999, Science 284:770-776; and in Kopan *et al.*, 2009, Cell 137:216-233. Delta and Serrate (or Jagged, the mammalian homolog of Serrate) are extracellular ligands of Notch. The portion of Delta and Serrate ("Serrate" shall be used herein to refer to both *Drosophila* Serrate and its mammalian homolog, Jagged) responsible for binding to Notch is called the DSL domain, which domain is located in the extracellular domain of the protein. Epidermal growth factor-like repeats (ELRs) 11 and 12 in the extracellular domain of Notch are responsible for binding to Delta, Serrate and Jagged. See Artavanis-Tsakonas *et al.*, 1995, Science 268:225-232 and Kopan *et al.*, 2009, Cell 137:216-233.

2.4 NOTCH PATHWAY IN HEMATOPOIESIS

Evidence of Notch-1 mRNA expression in human $CD34^+$ precursors has led to speculation for a role for Notch signaling in hematopoiesis (Milner et al., 1994, Blood 3:2057-62). This is further supported by the demonstration that Notch-1 and -2 proteins are present in hematopoietic precursors, and, in higher amounts, in T cells, B cells, and monocytes, and by the demonstration of Jagged-1 protein in hematopoietic stroma (Ohishi et al., 2000, Blood 95:2847-2854; Varnum-Finney et al., 1998, Blood 91:4084-91; Li et al., 1998, Immunity 8:43-55).

The clearest evidence for a physiologic role of Notch signaling has come from studies of T cell development which showed that activated Notch-1 inhibited B cell maturation but permitted T cell maturation (Pui et al., 1999, Immunity 11:299-308). In contrast, inactivation of Notch-1 or inhibition of Notch-mediated signaling by knocking out HES-1 inhibited T cell development but permitted B cell maturation (Radtke et al., 1999, Immunity 10: 47-58; Tomita et al., 1999, Genes Dev. 13:1203-10). These opposing effects of Notch-1 on B and T cell development raise the possibility that Notch-1 regulates fate decisions by a common lymphoid progenitor cell.

Other studies in transgenic mice have shown that activated Notch-1 affects the proportion of cells assuming a CD4 vs. CD8 phenotype as well as an $\alpha\beta$ vs. $\gamma\delta$ cell-fate (Robey et al., 1996, Cell 87:483-92; Washburn et al., 1997, Cell 88:833-43). Although this may reflect an effect on fate decisions by a common precursor, more recent studies have suggested that these effects may result from an anti-apoptotic effect of Notch-1 that enables the survival of differentiating T cells that would otherwise die (Deftos et al., 1998, Immunity 9:777-86; Jehn et al., 1999, J Immunol. 162:635-8).

Studies have also shown that the differentiation of isolated hematopoietic precursor cells can be inhibited by ligand-induced Notch signaling. Co-culture of murine marrow precursor cells (Lin⁻ $Sca-1^+c-kit^+$) with 3T3 cells expressing human Jagged-1 led to a 2 to 3 fold increase in the formation of primitive precursor cell populations (Varnum-Finney et al., 1998, Blood 91:4084-4991; Jones et al., 1998, Blood 92:1505-11). Incubation of sorted precursors with beads coated with the purified extracellular domain of human Jagged-1 also led to enhanced generation of precursor cells (Varnum-Finney et al., 1998, Blood 91:4084-91).

In a study of human CD34$^+$ cells, expression of the intracellular domain of

Notch-1 or exposure to cells that overexpressed Jagged-2 also led to enhanced

generation of precursor cells and prolonged maintenance of CD34 expression (Carlesso

et al., 1999, Blood 93:838-48). In another study, the effects of Jagged-1-expressing

5   cells on CD34$^+$ cells were influenced by the cytokines present in the cultures; in the

absence of added growth factors, the interaction with cell-bound Jagged-1 led to

maintenance of CD34$^+$ cells in a non-proliferating, undifferentiated state, whereas the

addition of c-kit ligand led to a 2-fold increase in erythroid colony-forming cells

(Walker *et al.*, 1999, Stem Cells 17:162-71).

10   Varnum-Finney *et al.*, 1993, Blood 101:1784-1789 demonstrated that activation

of endogenous Notch receptors in mouse marrow precursor cells by an immobilized

Notch ligand revealed profound effects on the growth and differentiation of the

precurosor cells, and that a multilog increase in the number of precursor cells with short-

term lymphoid and myeloid repopulating ability was observed. Delaney *et al.*, 2005,

15   Blood 106:2693-2699 and Ohishi *et al.*, 2002, J. Clin. Invest. 110:1165-1174

demonstrated that incubation of human cord blood progenitors in the presence of an

immobilized Notch ligand generated an approximate 100-fold increase in the number of

CD34$^+$ cells with enhanced repopulating ability as determined in an immunodeficient

mouse model. See also U.S. Patent No. 7,399,633 B2.

20   Delaney *et al.*, 2010, Nature Med. 16(2):232-236 demonstrated that a population

of CD34$^+$ cells obtained from a frozen cord blood sample, which population had been

cultured in the presence of a Notch ligand (resulting in a greater than 100 fold increase

in the number of CD34$^+$ cells), repopulated immunodeficient mice with markedly

enhanced kinetics and magnitude, and provided more rapid myeloid engraftment in

25   humans in a clinical phase 1 myeloablative cord blood transplant trial.

Citation or identification of any reference in Section 2 or any other section of this

application shall not be construed as an admission that such reference is available as

prior art to the present invention.

30   **3.   SUMMARY OF THE INVENTION**

There exists a need in the art for an "off-the-shelf" product for rapid

hematopoietic reconstitution, that could be stockpiled long term after manufacture. The

present invention fulfills such a need. The present invention provides methods for
providing hematopoietic function to a human patient in need thereof, comprising
administering a pool of expanded human cord blood stem/progenitor cell samples to the
patient, wherein the samples in the pool collectively do not mismatch the patient at more

5   than 2 of the HLA antigens or alleles typed in the patient. The present invention also
provides methods for providing hematopoietic function to a human patient in need
thereof, comprising administering a pool of expanded human cord blood stem cell
samples, or an aliquot thereof, to the patient, wherein the pool comprises two or more
different expanded human cord blood stem cell samples, each different sample in the

10   pool being derived from the umbilical cord blood and/or placental blood of a different
human at birth, wherein the samples in the pool collectively do not mismatch the patient
at more than 2 of the HLA antigens or alleles typed in the patient. In a specific
embodiment, the two or more samples in the pool mismatch the patient at 1 or 2 of the
HLA antigens or alleles typed in the patient and typed in the samples. In another

15   specific embodiment, the two or more samples in the pool mismatch the patient at 2 of
the HLA antigens or alleles typed in the patient.

     The present invention also provides methods for providing hematopoietic
function to a human patient in need thereof, comprising (a) selecting a pool of expanded
human cord blood stem cell samples for administration to the patient from a plurality of

20   pools of expanded human cord blood stem cell samples, wherein the pool comprises two
or more different expanded human cord blood stem cell samples, each different sample
in the pool being derived from the umbilical cord blood and/or placental blood of a
different human at birth, wherein the samples in the pool collectively do not mismatch
the patient at more than 2 of the HLA antigens or alleles typed in the patient; and (b)

25   administering the selected pool, or an aliquot thereof, to the patient. In a specific
embodiment, the selecting further comprises rejecting pools of samples containing
samples having more than 2 HLA antigen or allele mismatches with the patient of the
HLA antigens or alleles typed in the patient. In another specific embodiment, the
selecting further comprises accepting pools of samples containing samples having 1 or 2

30   HLA antigen or allele mismatches with the patient of the HLA antigens or alleles typed
in the patient. In another specific embodiment, the selecting is from among at least 50
frozen expanded human cord blood stem cell pools of samples. In yet another
embodiment, the expanded human cord blood stem cell sample administered to the

patient contains at least 75 million viable CD34$^+$ cells, preferably at least 250 million viable CD34$^+$ cells.

In one embodiment, each different expanded human cord blood stem cell sample of the present invention has been subjected to an expansion technique that has been

5   shown to result in an at least 50-fold increase in hematopoietic stem cells or hematopoietic stem and progenitor cells in an aliquot of a human cord blood stem cell sample subjected to the expansion technique, relative to an aliquot of the human cord blood stem cell sample prior to being subjected to the expansion technique. The hematopoietic stem cells or the hematopoietic stem and progenitor cells can be positive

10   for one or more of the following cell surface markers expressed in increased levels on hematopoietic stem cells or hematopoietic stem and progenitor cells, relative to other types of hematopoietic cells: CD34, CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, and HLA DR and/or negative for Lin and/or CD38 cell surface markers. Preferably, the hematopoietic stem cells or hematopoietic stem and progenitor

15   cells are positive for one or more of CD34, CD133 or CD90 cell surface markers. Preferably, each different expanded human cord blood stem cell sample of the present invention has been subjected to an expansion technique that has been shown (i) to result in an at least 50-fold increase in CD34$^+$ cells in an aliquot of a human cord blood stem cell sample subjected to the expansion technique, relative to an aliquot of the human

20   cord blood stem cell sample prior to being subjected to the expansion technique; or (ii) to increase the number of SCID repopulating cells in a human cord blood stem cell sample subject to the expansion technique, relative to the human cord blood cell stem cell sample prior to being subject to the expansion technique.

In particular embodiments, the pool of expanded human cord blood stem cell

25   samples is frozen and thawed prior to administering to the patient. In one embodiment, the samples in the pool are all derived from umbilical cord blood and/or placental blood of individuals of the same race, *e.g.*, African-American, Caucasian, Asian, Hispanic, Native-American, Australian Aboriginal, Inuit, Pacific Islander, or are all derived from umbilical cord blood and/or placental blood of individuals of the same ethnicity, *e.g.*,

30   Irish, Italian, Indian, Japanese, Chinese, Russian, etc.

In yet another embodiment, the method of providing hematopoietic function comprises, prior to said administering, a step of expanding *ex vivo* isolated human cord blood stem cell, or stem and progenitor cell samples, each sample obtained from the

umbilical cord blood and/or placental blood of one or more humans at birth and pooling the expanded samples. Preferably, the expanding step comprises contacting the human cord blood stem cell, or stem and progenitor cell samples, with an agonist of Notch function. The agonist can be a Delta protein or a Serrate protein, or a fragment of a
5   Delta protein or Serrate protein, which fragment is able to bind a Notch protein.

In a particular embodiment of the present invention, a method for providing hematopoietic function to a human patient in need thereof is provided, which method comprises (a) pooling at least two umbilical cord blood and/or placental blood samples, wherein each sample is obtained at birth of a different human to produce pooled cord
10   blood; (b) enriching for hematopoietic stem cells or hematopoietic stem and progenitor cells from pooled cord blood to produce a population enriched in hematopoietic stem cells or hematopoietic stem and progenitor cells; (c) expanding *ex vivo* the population enriched in hematopoietic stem cells or hematopoietic stem and progenitor cells to produce an expanded stem cell sample; and (d) administering the expanded stem cell
15   sample, or an aliquot thereof, to a human patient in need of hematopoietic function, wherein the expanded stem cell sample does not mismatch at more than 2 of the HLA antigens or alleles typed in the patient. In a preferred embodiment, the expanded cells are $CD34^+$ cells. In another preferred embodiment, the expanded cells are $CD133^+$ cells. In another preferred embodiment, the expanded cells are $CD90^+$ cells. In yet another
20   embodiment, the expanded cells are positive for one or more of the following cell surface markers expressed in increased levels on hematopoietic stem cells or hematopoietic stem and progenitor cells, relative to other types of hematopoietic cells: CD34, CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, and HLA DR and/or negative for Lin and/or CD38 cell surface markers. Preferably, the
25   expanded cells are positive for one or more of CD34, CD133 or CD90 cell surface markers. This method can further comprise the steps of freezing and thawing the expanded cell sample after step (c) and before step (d). In certain embodiments, the patient suffers from pancytopenia or neutropenia, wherein the pancytopenia or neutropenia is caused by an intensive chemotherapy regimen, a myeloablative regimen
30   for hematopoietic cell transplantation, or exposure to acute ionizing radiation.

In another embodiment, the present invention provides a method of producing a bank of frozen, expanded human cord blood stem cells comprising the following steps in the order stated: (a) expanding, *ex vivo*, human cord blood stem cells present in a

population enriched for hematopoietic stem cells or hematopoietic stem and progenitor cells obtained from a pool of umbilical cord blood and/or placental blood, which pool is obtained from two or more different humans at birth, to produce an expanded human cord blood stem cell sample; (b) freezing the expanded human cord blood stem cell

5   sample to produce a frozen expanded human cord blood stem cell sample; (c) storing the frozen expanded human cord blood stem cell sample; and (d) repeating steps (a)-(c) at least 50 times to produce a bank of at least 50 stored, frozen expanded human cord blood stem cell samples. In specific embodiments, steps (a)-(c) are repeated at least 5, 10, 20, 25, 50, 75, 100, 200, 250, 500, 750, 1000, 1500, 2,000, 3,000, 5,000, 7,500, 10,000,

10   25,000, 50,000 or 100,000 times to produce the corresponding number of stored, frozen, expanded human cord blood stem cell samples. In one embodiment, the method further comprises a step of assigning each frozen expanded human cord blood stem cell sample an identifier that distinguishes the frozen expanded human cord blood stem cell sample from other frozen expanded stem cell samples. In another embodiment, the method

15   further comprises a step of storing the identifier in one or more computer databases, wherein said stored identifier is associated with information on the physical location where the frozen expanded human cord blood stem cell sample is stored in said bank. The present invention is also directed to a blood bank comprising at least 50 units of frozen pools of expanded human cord blood stem cell samples, wherein each pool

20   comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the different samples in each pool collectively do not mismatch at more than 2 of the HLA antigens or alleles typed in each samples in each pool. In specific embodiments, the blood bank comprises at least 5, 10,

25   20, 25, 50, 75, 100, 200, 250, 500, 750, 1000, 1500, 2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000 units of frozen pools of expanded human cord blood stem cells.

       In another embodiment of the invention, a computer-implemented method for selecting a frozen expanded human cord blood stem cell sample for use in providing

30   hematopoietic function to a human patient in need thereof is provided, which method comprises the following steps performed by a suitably programmed computer: (a) selecting an identifier from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a frozen, stored pool of expanded human cord blood

stem cell samples, wherein each pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, such that the samples in the pool identified by the selected identifier collectively do not mismatch the

5   patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein the selecting is to identify a pool of expanded human cord blood stem cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and (b) outputting or displaying the selected identifier. In specific embodiments, the identifier is outputted or displayed to a user, an internal or

10   external component of a computer, a remote computer, or to storage on a computer readable medium. In another specific embodiment, the outputting or displaying further outputs or displays information on the physical location of each pool of expanded human cord blood stem cell samples identified by the identifier. In yet another embodiment, the computer-implemented method further comprises implementing robotic retrieval of the

15   identified pool of frozen, expanded human cord blood stem cell samples.

In another embodiment of the invention, a computer program product is provided for use in conjunction with a computer system, which computer program product comprises a computer readable storage medium and a computer program mechanism embedded therein, the computer program mechanism comprising: (a) executable

20   instructions for selecting an identifier from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a frozen, stored pool of expanded human cord blood stem cell samples, wherein each pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at

25   birth, wherein the samples in the pool identified by the selected identifier collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein the selecting is to identify a pool of expanded human cord blood stem cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and (b) executable instructions for

30   outputting or displaying the selected identifier. In particular embodiments, the identifier is outputted or displayed to a user, an internal or external component of a computer, a remote computer, or to storage on a computer readable medium.

In yet another embodiment, the present invention provides an apparatus comprising a processor; a memory, coupled to the processor, the memory storing a module, the module comprising (a) executable instructions for selecting an identifier from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a pool of expanded human cord blood stem cell samples, wherein each pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the samples in the pool identified by the selected identifier collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein the selecting is to identify a pool of expanded human cord blood stem cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and (b) executable instructions for outputting or displaying the selected identifier. In particular embodiments, the identifier is outputted or displayed to a user, an internal or external component of a computer, a remote computer, or to storage on a computer readable medium.

In a specific embodiment, in the methods, computer-program products and apparatuses of the invention, the selecting step comprises rejecting identifiers that identify pools of samples that collectively mismatch at more than 2 of the HLA antigens or alleles typed in the patient.

## 4.		DEFINITIONS

Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

As used herein, the term "CB Stem Cells," referred to herein interchangeably as "a CB Stem Cell Sample," refers to a population enriched in hematopoietic stem cells, or enriched in hematopoietic stem and progenitor cells, derived from human umbilical cord blood and/or human placental blood collected at birth. The hematopoietic stem cells, or hematopoietic stem and progenitor cells, can be positive for a specific marker expressed in increased levels on hematopoietic stem cells or hematopoietic stem and progenitor

cells, relative to other types of hematopoietic cells. For example, such markers can be, but are not limited to CD34, CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, HLA DR, or a combination thereof. Also, the hematopoietic stem cells, or hematopoietic stem and progenitor cells, can be negative for an expressed marker,

5   relative to other types of hematopoietic cells. For example, such markers can be, but are not limited to Lin, CD38, or a combination thereof. Preferably, the hematopoietic stem cells, or hematopoietic stem and progenitor cells, are CD34$^+$ cells.

As used herein, "Expanded CB Stem Cells," referred to herein interchangeably as "an Expanded CB Stem Cell Sample," refers to CB Stem Cells that have been

10   subjected to a technique for expanding the cord blood hematopoietic stem cells, or hematopoietic stem and progenitor cells, which technique has been shown to result in (i) an increase in the number of hematopoietic stem cells, or hematopoietic stem and progenitor cells, in an aliquot of the sample thus expanded, or (ii) an increased number of SCID repopulating cells determined by limiting-dilution analysis as shown by

15   enhanced engraftment in NOD/SCID mice infused with an aliquot of the sample thus expanded; relative to that seen with an aliquot of the sample that is not subjected to the expansion technique. In a specific embodiment, the enhanced engraftment in NOD/SCID mice can be detected by detecting an increased percentage of human CD45$^+$ cells in the bone marrow of mice infused with an aliquot of the expanded sample relative

20   to mice infused with an aliquot of the sample prior to expansion, at, *e.g.*, 10 days, 3 weeks or 9 weeks post-infusion (see Delaney et al., 2010, Nature Medicine 16(2):232-236. In a specific embodiment, the expansion technique results in an at least 50-, 75-, 100-, 150-, 200-, 250-, 300-, 350-, 400-, 450-, or 500-fold increase in the number of hematopoietic stem cells or hematopoietic stem and progenitor cells, in an aliquot of the

25   sample expanded, and preferably is a 100-200 fold increase.

5.   **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 illustrates an exemplary embodiment of a computer system useful for implementing the methods of the present invention.

30   Figures 2a-2b are graphs showing SCID repopulating frequency with cord blood cells cultured with Delta1 generates a significant increase in the SRC frequency and improved overall engraftment. Sublethally irradiated NOD/SCID mice were

transplanted with non-cultured CD34$^+$ cord blood cells or the progeny of CD34$^+$ cells cultured for 17 days on Delta1 or control human IgG1 in 5 independent experiments. Human engraftment (CD45%) was measured at 3 (marrow aspiration from knee joint) and 9 (sacrificed and marrow harvested from bilateral femurs and tibiae) weeks. (Fig.

5   2a) Limiting dilution transplants were carried out as described above to calculate the SRC frequency, shown as the SRC frequency per 10^6 starting cells. Results are the mean ± SEM. *P values shown represent Delta1 cultured cells compared to control cultured or non-cultured cells. (Fig. 2b) Human engraftment as measured by total CD45%, lymphoid subset CD19$^+$/CD45$^+$ cells (black) and myeloid subset of CD33$^+$/CD45$^+$

10   double positive cells.

Figure 3 is a graph showing that rapid early engraftment is dependent upon culture with Delta1. CD34$^+$ cord blood progenitors were cultured with Delta1 and compared to non-cultured cells for NOD/SCID repopulating ability. Human engraftment (CD45%) in the marrow was assessed 10 days and 3 weeks after infusion of

15   the cells. Results shown are the mean CD45% ± sem and is representative of one of two experiments where early engraftment was assessed.

Figures 4a-4c show that cryopreservation of *ex vivo* expanded cord blood progenitors does not impair *in vivo* repopulating ability. Overall human engraftment as measured by human CD45 in the marrow of recipient mice is shown on the y axis. The

20   solid lines represent the mean level of human engraftment. (Fig. 4a) Cells infused immediately post culture compared with harvested cells that were cryopreserved prior to infusion. Results shown are at 4 weeks post infusion. (Fig. 4b) *Ex vivo* expanded and cryopreserved progenitor cells were thawed and infused. The figure represents the combined results of two experiments. (Fig. 4c) The expanded progeny derived from

25   expansion of CD34$^+$ progenitors obtained from a single cord blood unit were divided into equal groups and cryopreserved per standard practice. Three methods of thawing prior to infusion were then compared for *in vivo* repopulating ability: thaw and wash, thaw and dilute (albumin/dextran dilution), thaw and direct infusion.

Figure 5 shows a comparison of engraftment of Delta1$^{ext-IgG}$ cultured cells in

30   congenic and allogeneic hematopoietic stem cells transplants (HCTs). LSK cells were cultured on Delta1$^{ext-IgG}$ for 4 weeks as described in Dallas *et al.*, 2007 Blood 109:3579-3587. In the congenic HCT, lethally irradiated (1000 cGY) C57 (H-2d) mice received 10^5 C57 whole BM + 10^6 Delta1$^{ext-IgG}$ -cultured cells. In the allogeneic HCT, lethally

irradiated (1000 cGy) BALB.c (H-2d) mice received 10^5 BALB.c whole BM + 10^6 Delta1$^{ext\text{-}IgG}$ -cultured cells. Blood was analyzed by FACS analysis at 2, 3, 5, and 7 weeks after HCT. N=5-7.

Figure 6 shows engraftment of Delta1$^{ext\text{-}IgG}$ -cultured cells in HLA-mismatched

5   recipients. LSK cells were cultured for 4 weeks as described in Ohisi *et al.*, 2002, J. Clin. Invest. 110:1165-1174 and Dallas *et al.*, 2007 Blood 109:3579-3587. Lethally irradiated BALB.c (H-2d, CD45.2) recipients received 10^6 Ly5.1 (H-2b, CD45.1) Delta1$^{ext\text{-}tIgG}$-cultured LSK cells along with 10^3 BALB.c (H-2d, CD45.2) LSK cells or 10^3 Ly5.1 (H-2b ,CD45.1) LSK cells + 10^3 BALB.c (H-2d, CD45.2). Mice were

10 sacrificed at day 3 and 7; bone marrow engraftment was determined by FAC analysis (n=5).

Figure 7 is a schematic drawing of the experimental protocol for expansion of stem and progenitor cells and infusion of the expanded cells into irradiated mice, in order to compare engraftment of the expanded stem and progenitor cells with non-

15 expanded stem and progenitor cells.

Figures 8a-8b graphically show the engraftment of mismatched expanded stem and progenitor cells as detected in bone marrow and in peripheral blood of lethally irradiated mice.

Figures 9a-9b show the overall survival of mice exposed to 7.5 Gy or 8 Gy of

20 radiation after infusion with expanded stem and progenitor cells that were previously cryopreserved, as compared to a control saline group.

Figure 10 depicts the overall survival of mice irradiated at 8.5 Gy after infusion of expanded stem and progenitor cells (cultured with a Delta derivative) as compared to infusion of non-expanded cord blood stem and progenitor cells (IgG cultured).

25 Figures 11a-11b show that donor engraftment of expanded murine stem and progenitor cells (DXI) is enhanced with an increasing dose of radiation.

Figure 12 shows clinical grade culture of cord blood progenitors with Delta1$^{ext\text{-}IgG}$ results in significant *in vitro* expansion of CD34$^+$ cells and more rapid neutrophil recovery in a myeloablative double CBT setting. CD34$^+$ cord blood progenitor cells

30 were enriched and placed into culture with Delta1$^{ext\text{-}IgG}$. The individual and median times (solid line) to absolute neutrophil counts (ANC) of ≥500/µl for patients receiving double unit cord blood transplants with two non-manipulated units ("conventional")

versus with one *ex vivo* expanded unit and one non-manipulated unit ("expanded") is presented.

Figure 13 is a flow chart demonstrating an exemplary procedure for enriching a population of CD34^{+} cells, and expanding the enriched population.

5   Figure 14 is a flow chart setting forth a plan for induction therapy for patients with AML.

Figure 15 is a chart setting forth the characteristics of the patients treated, and infused cell count and neutrophil recovery time.

Figure 16 is a chart depicting expanded cord blood stem and progenitor cell

10   engraftment expressed as a percentage of donor cells at day 7 post-infusion of the expanded cord blood stem and progenitor cell sample.

Figure 17 is a flow chart setting forth a protocol for treating a hematologic malignancy, such as AML, by administering a cord blood transplant and an expanded cord blood stem and progenitor cell sample.

15   Figure 18 shows the time required post-transplant to achieve an absolute neutrophil count (ANC) of greater than or equal to 100 per µl.

Figure 19 shows the time required post-transplant to achieve an absolute neutrophil count (ANC) of greater than or equal to 500 per µl.

Figure 20 is a chart depicting the results of a peripheral blood cell DNA

20   chimerism analysis at day 7 post-infusion (QNS, quantity not sufficient).

6.   **DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides a method for providing hematopoietic function to a human patient in need thereof by administering a pool of expanded human cord blood

25   stem cell samples to the patient, wherein the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient. In one embodiment, the expanded human cord blood stem cells can differentiate into cells of the myeloid lineage. In another embodiment, the expanded human cord blood stem cells can differentiate into cells of the lymphoid lineage.

The ideal therapeutic product for treatment of chemotherapy or radiation induced pancytopenia is one that, when infused, would give rise to rapid hematopoietic reconstitution, especially of granulocytes, and also facilitate autologous recovery of hematopoiesis. Moreover, in order to be delivered in an expedited fashion, it is essential

5   that the therapeutic product be developed as on "off-the-shelf" product that could be stockpiled long term after generation (manufacture). Hypothetically, while marrow or mobilized peripheral blood stem cells could provide a transient population of blood cells that could be infused to help mitigate pancytopenia that results from high dose chemotherapy/radiation, these would require matching at the 6 HLA antigen or alleles

10   currently typed for cord blood transplants for use, and procurement of these cells would not be easy or amenable to stockpiling. Similarly, the collection and use of granulocytes for transfusion as treatment for infection occurring in the setting of prolonged neutropenia is not promising. Current evidence indicates relatively little or no effect of granulocyte transfusions, possibly due to a limited lifespan (hours) of the cells infused

15   and absence of *in vivo* generation of additional cells (not a renewable source of cells).

Prior to the present invention, it was not appreciated that Expanded CB Stem Cells could provide hematopoietic benefit to a human patient with only limited HLA matching, since it was believed that the detrimental effect of graft versus host disease (GVHD) would destroy the potential therapeutic benefit. The present invention takes

20   advantage of the prompt hematopoietic benefit provided by the Expanded CB Stem Cells to provide a benefit to a human patient where the Expanded CB Stem Cells and the patient are mismatched at no more than 2 HLA antigens or alleles. While not being bound by any mechanism, it is believed that the Expanded CB Stem Cells can provide therapeutic benefit in a limited mismatch setting because the rapidity of engraftment

25   provided by these cells allows for a beneficial effect on hematopoietic function before GVHD can develop and obviate such effect. Also, the increased hematopoietic cell numbers (including stem and progenitor cells) provided by the expansion methods described herein are believed to overcome, at least temporarily, host resistance to foreign cells. Additionally, other cell types generated in the expanded population, such

30   as dendritic cell or natural killer (NK) cell precursors, is believed to prevent rejection of the infused cells by the host. Thus, provision of hematopoietic function can be achieved even in a limited mismatched setting, and administration to a patient can be therapeutic

regardless of whether the patient and the expanded cord blood stem cell sample are mismatched at no more than 2 of the HLA antigens or alleles typed.

Frequent infections are a common complication of induction chemotherapy and salvage regimens used in the treatment of hematopoietic malignancies, and in fact are a

5   leading cause of treatment failure. The chemotherapeutic agents also can be profoundly immunosuppressive and/or highly myelosuppressive, which can lead to periods of prolonged neutropenia. Infusion of the Expanded CB Stem Cells of the invention can provide a therapeutic benefit in overcoming these challenges by abrogating neutropenia, preventing infectious complications, and facilitating host hematopoietic recovery post-

10   chemotherapy.

Moreover, since according to the present invention, only limited matching of HLA-type is necessary for therapeutic use of the Expanded CB Stem Cells, it is now practical to store frozen Expanded CB Stem Cells, or pools of Expanded CB Stem Cells, since the present invention teaches that useful amounts can practically be stored. In the

15   prior art, since it was expected that HLA matching to the recipient would generally be necessary to find a useful sample of Expanded CB Stem Cells for therapeutic use, an unattainably large number of different Expanded CB Stem Cell samples had to be stored to make it feasible generally to find a match for a patient, the large numbers making it impractical to store expanded samples, due to the even larger amount of storage space

20   needed to store expanded units. In contrast, and in accordance with the present invention, no HLA matching is required, and thus, the generation of a "bank" of CB Stem Cells which have been expanded and then cryopreserved, useful for the general human population to use in stem cell transplantation, is feasible, since any Expanded CB Stem Cell sample in the bank could feasibly be used with any recipient in a therapeutic

25   method of the invention.

6.1   COLLECTING CORD BLOOD

Human umbilical cord blood and/or human placental blood are sources of the CB Stem Cells according to the present invention. Such blood can be obtained by any

30   method known in the art. The use of cord or placental blood as a source of Stem Cells provides numerous advantages, including that the cord and placental blood can be obtained easily and without trauma to the donor. See, e.g., U.S. Patent No. 5,004,681

for a discussion of collecting cord and placental blood at the birth of a human. In one embodiment, cord blood collection is performed by the method disclosed in U.S. Patent No. 7,147,626 B2 to Goodman *et al.*

5   Collections should be made under sterile conditions. Immediately upon collection, cord or placental blood should be mixed with an anticoagulent. Such an anticoagulent can be any known in the art, including but not limited to CPD (citrate-phosphate-dextrose), ACD (acid citrate-dextrose), Alsever's solution (Alsever *et al.*, 1941, N. Y. St. J. Med. 41:126), De Gowin's Solution (De Gowin, *et al.*, 1940, J. Am. Med. Ass. 114:850), Edglugate-Mg (Smith, *et al.*, 1959, J. Thorac. Cardiovasc. Surg.

10   38:573), Rous-Turner Solution (Rous and Turner, 1916, J. Exp. Med. 23:219), other glucose mixtures, heparin, ethyl biscoumacetate, etc. See, generally, Hum, 1968, Storage of Blood, Academic Press, New York, pp. 26-160). In one embodiment, ACD can be used.

          The cord blood can preferably be obtained by direct drainage from the cord

15   and/or by needle aspiration from the delivered placenta at the root and at distended veins. See, generally, U.S. Patent No. 5,004,681. Preferably, the collected human cord blood and/or placental blood is free of contamination.

          In certain embodiments, the following tests on the collected blood sample can be performed either routinely, or where clinically indicated:

20   (i) Bacterial culture: To ensure the absence of microbial contamination, established assays can be performed, such as routine hospital cultures for bacteria under aerobic and anaerobic conditions.

          (ii) Diagnostic screening for pathogenic microorganisms: To ensure the absence of specific pathogenic microorganisms, various diagnostic tests can be employed.

25   Diagnostic screening for any of the numerous pathogens transmissible through blood can be done by standard procedures. As one example, the collected blood sample (or a maternal blood sample) can be subjected to diagnostic screening for the presence of Human Immunodeficiency Virus-1 or 2 (HIV-1 or HIV-2). Any of numerous assay systems can be used, based on the detection of virions, viral-encoded proteins, HIV-

30   specific nucleic acids, antibodies to HIV proteins, etc. The collected blood can also be tested for other infectious diseases, including but not limited to human T-Cell

lymphotropic virus I and II (HTLV-I and HTLV-II), Hepatitis B, Hepatitis C, Cytomegalovirus, Syphilis, West Nile Virus.

Preferably, prior to collection of the cord blood, maternal health history is determined in order to identify risks that the cord blood cells might pose in transmitting genetic or infectious diseases, such as cancer, leukemia, immune disorders, neurological disorders, hepatitis or AIDS. The collected cord blood samples can undergo testing for one or more of cell viability, HLA typing, ABO/Rh typing, $CD34^+$ cell count, and total nucleated cell count.

6.2   ENRICHMENT OF CORD BLOOD STEM CELLS

Once the umbilical cord blood and/or placental blood is collected from a single human at birth, the blood is processed to produce an enriched hematopoietic stem cell population, or enriched hematopoietic stem and progenitor cell population, forming a population of CB Stem Cells. The hematopoietic stem cells, or hematopoietic stem and progenitor cells, can be positive for a specific marker expressed in increased levels on the hematopoietic stem cells or hematopoietic stem and progenitor cells, relative to other types of hematopoietic cells. For example, such markers can be, but are not limited to, CD34, CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, HLA DR, or a combination thereof. The hematopoietic stem cells, or hematopoietic stem and progenitor cells, also can be negative for a specific marker, relative to other types of hematopoietic cells. For example, such markers can be, but are not limited to, Lin, CD38, or a combination thereof. Preferably, the hematopoietic stem cells, or hematopoietic stem and progenitor cells, are $CD34^+$ cells. Preferably, the CB Stem Cell population is enriched in $CD34^+$ stem cells or $CD34^+$ stem and progenitor cells (and, thus, T cell depleted). Enrichment thus refers to a process wherein the percentage of hematopoietic stem cells, or hematopoietic stem and progenitor cells in the sample is increased (relative to the percentage in the sample before the enrichment procedure). Purification is one example of enrichment. In certain embodiments, the increase in the number of $CD34^+$ cells (or other suitable antigen-positive cells) as a percentage of cells in the enriched sample, relative to the sample prior to the enrichment procedure, is at least 25-, 50-, 75-, 100-, 150-, 200-, 250-, 300-, 350-fold, and preferably is 100-200 fold. In a preferred embodiment, the $CD34^+$ cells are enriched using a monoclonal antibody to

CD34, which antibody is conjugated to a magnetic bead, and a magnetic cell separation device to separate out the CD34$^+$ cells.

In a preferred embodiment, prior to processing for enrichment, the collected cord and/or placental blood is fresh and has not been previously cryopreserved.

5   Any technique known in the art for cell separation/selection can be used to carry out the enrichment for hematopoietic stem cells, or hematopoietic stem and progenitor cells. For example, methods which rely on differential expression of cell surface markers can be used. For example, cells expressing the cell surface marker CD34 can be positively selected using a monoclonal antibody to CD34, such that cells expressing

10   CD34 are retained, and cells not expressing CD34 are not retained. Moreover, the separation techniques employed should maximize the viability of the cell to be selected. The particular technique employed will depend upon efficiency of separation, cytotoxicity of the methodology, ease and speed of performance, and necessity for sophisticated equipment and/or technical skill.

15   Procedures for separation may include magnetic separation, using antibody-coated magnetic beads, affinity chromatography, cytotoxic agents joined to a monoclonal antibody or used in conjunction with a monoclonal antibody, e.g., complement and cytotoxins, and "panning" with antibody attached to a solid matrix, e.g., plate, or other convenient technique. Techniques providing accurate separation/selection

20   include fluorescence activated cell sorters, which can have varying degrees of sophistication, e.g., a plurality of color channels, low angle and obtuse light scattering detecting channels, impedance channels, etc.

The antibodies may be conjugated with markers, such as magnetic beads, which allow for direct separation, biotin, which can be removed with avidin or streptavidin

25   bound to a support, fluorochromes, which can be used with a fluorescence activated cell sorter, or the like, to allow for ease of separation of the particular cell type. Any technique may be employed which is not unduly detrimental to the viability of the remaining cells.

In a preferred embodiment of the present invention, a fresh cord blood unit is

30   processed to select for, i.e., enrich for, CD34$^+$ cells using anti-CD34 antibodies directly or indirectly conjugated to magnetic particles in connection with a magnetic cell separator, for example, the CliniMACS® Cell Separation System (Miltenyi Biotec,

Bergisch Gladbach, Germany), which employs nano-sized super-paramagnetic particles composed of iron oxide and dextran coupled to specific monoclonal antibodies. The CliniMACS® Cell Separator is a closed sterile system, outfitted with a single-use disposable tubing set. The disposable set can be used for and discarded after processing

5   a single unit of collected cord and/or placental blood to enrich for CD34$^+$ cells. Similarly, CD133$^+$ cells can be enriched using anti-CD133 antibodies. In a specific embodiment, CD34$^+$CD90$^+$ cells are enriched for. Similarly, cells expressing CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD166, HLA DR, or a combination of the foregoing, can be enriched for using antibodies against the antigen.

10   In one embodiment, one or more umbilical cord blood and/or placental blood samples can be pooled prior to enriching for the hematopoietic stem cells, or hematopoietic stem and progenitor cells. In another embodiment, individual CB Stem Cell samples can be pooled after enriching for the hematopoietic stem cells, or hematopoietic stem and progenitor cells. In specific embodiments, the number of

15   umbilical cord blood and/or placental blood samples, or CB Stem Cell samples, that are pooled is 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or 40, or at least any of the foregoing numbers, preferably 20, or no more than 20 or 25, umbilical cord blood and/or placental blood samples, or CB Stem Cell samples, respectively. The umbilical cord blood/placental blood samples or CB Stem Cell samples are pooled such that each

20   sample in the pool does not mismatch the other samples in the pool by more than 2 HLA antigens or alleles typed. In certain embodiments, the samples in the pool are derived from the umbilical cord blood and/or placental blood of individuals of the same race, e.g., African-American, Caucasian, Asian, Hispanic, Native-American, Australian Aboriginal, Inuit, Pacific Islander, or derived from umbilical cord blood and/or placental

25   blood of individuals of the same ethnicity, e.g., Irish, Italian, Indian, Japanese, Chinese, Russian, etc.

          Optionally, prior to enrichment for hematopoietic stem cells or hematopoietic stem and progenitor cells, the red blood cells and white blood cells of the cord blood can be separated. Once the separation of the red blood cells and the white blood cells has

30   taken place, the red blood cell fraction can be discarded, and the white blood cell fraction can be processed in the magnetic cell separator as above. Separation of the white and red blood cell fractions can be performed by any method known in the art, including centrifugation techniques. Other separation methods that can be used include

the use of commercially available products FICOLL™ or FICOLL-PAQUE™ or PERCOLL™ (GE Healthcare, Piscataway, New Jersey). FICOLL-PAQUE™ is normally placed at the bottom of a conical tube, and the whole blood is layered above. After being centrifuged, the following layers will be visible in the conical tube, from top

5   to bottom: plasma and other constituents, a layer of mono-nuclear cells called buffy coat containing the peripheral blood mononuclear cells (white blood cells), FICOLL-PAQUE™ , and erythrocytes and granulocytes, which should be present in pellet form. This separation technique allows easy harvest of the peripheral blood mononuclear cells.

Optionally, prior to CD34$^+$ cell selection, an aliquot of the fresh cord blood unit

10   can be checked for total nucleated cell count and/or CD34$^+$ content. In a specific embodiment, after the CD34$^+$ cell selection, both CD34$^+$ ("CB Stem Cells") and CD34- cell fractions are recovered. Optionally, DNA can be extracted from a sample of the CD34- cell fraction for initial HLA typing and future chimerism studies, even though HLA matching to the patient is not done according to the methods of the present

15   invention. The CD34$^+$ enriched stem cell fraction ("CB Stem Cells") can be subsequently processed prior to expansion, for example, the Stem Cells can be suspended in an appropriate cell culture medium for transport or storage. In a preferred embodiment, the cell culture medium consists of STEMSPAN™ Serum Free Expansion Medium (StemCell Technologies, Vancouver, British Columbia) supplemented with 10

20   ng/ml recombinant human Interleukin-3 (rhIL-3), 50 ng/ml recombinant human Interleukin-6 (rhIL-6), 50 ng/ml recombinant human Thrombopoietin (rhTPO), 50 ng/ml recombinant human Flt-3 Ligand (rhFlt-3L), 50 ng/ml and recombinant human stem cell factor (rhSCF).

In a specific embodiment, the umbilical cord blood and/or placental blood

25   sample are red cell depleted, and the number of CD34$^+$ cells in the red cell depleted fraction is calculated. Preferably, the umbilical cord blood and/or placental blood samples containing more than 3.5 million CD34$^+$ cells are enriched by the enrichment methods described above.

30   **6.3   METHODS OF CORD BLOOD STEM CELL EXPANSION**
After the CB Stem Cells have been isolated from human cord blood and/or human placental blood collected from one or more humans at birth according to the

enrichment methods described above or other methods known in the art, the CB Stem Cells are expanded in order to increase the number of hematopoietic stem cells or hematopoietic stem and progenitor cells, *e.g.*, CD34$^+$ cells. Any method known in the art for expanding the number of CB Stem Cells that gives rise to Expanded CB Stem

5   Cell can be used. Preferably, the CB Stem Cells are cultured under cell growth conditions (*e.g.*, promoting mitosis) such that the CB Stem Cells grow and divide (proliferate) to obtain a population of Expanded CB Stem Cells. In one embodiment, individual populations of CB Stem Cells each derived from the umbilical cord blood and/or placental blood of a single human at birth can be pooled, prior to or after the

10   expansion technique. In another embodiment, the sample that is expanded is not a pool of samples. Preferably, the technique used for expansion is one that has been shown to (i) result in an increase in the number of hematopoietic stem cells, or hematopoietic stem and progenitor cells, *e.g.*, CD34$^+$ cells, in the expanded sample relative to the unexpanded CB Stem Cell sample, or (ii) results in an increased number of SCID

15   repopulating cells in the expanded sample determined by limiting-dilution analysis as shown by enhanced engraftment in NOD/SCID mice infused with the expanded sample, relative to that seen with the unexpanded sample, where the unexpanded sample and expanded sample are from different aliquots of the same sample, wherein the expanded sample but not the unexpanded sample is subjected to the expansion technique. In

20   certain embodiments, the technique results in a 50-, 75-, 100-, 150-, 200-, 250-, 300-, 350-, 400-, 450-, or 500-fold increase, preferably a 100-200 fold increase in the number of hematopoietic stem cells or hematopoietic stem and progenitor cells in the expanded sample, relative to the unexpanded CB Stem Cell sample. The hematopoietic stem cells or hematopoietic stem and progenitor cells can be positive for one or more of CD34,

25   CD43, CD45RO, CD45RA, CD59, CD90, CD109, CD117, CD133, CD166, and HLA DR and/or negative for Lin and/or CD38. In a specific embodiment, the enhanced engraftment can be detected by detecting an increased percentage of human CD45$^+$ cells in the bone marrow of mice infused with an aliquot of the expanded sample relative to mice infused with an aliquot of the unexpanded sample at, e.g., 10 days, 3 weeks or 9

30   weeks post-infusion (see Delaney et al., 2010, Nature Medicine 16(2):232-236).

Such expansion techniques include, but are not limited to those described in U.S. Patent No. 7,399,633 B2; Delaney *et al.*, 2010, Nature Medicine 16(2):232-236; Zhang

et al., 2008, Blood 111:3415-3423; and Himburg *et al.*, 2010, Nature Medicine doi:10.1038/nm.2119 (advanced online publication), as well as those described below.

In one embodiment of the invention, the CB Stem Cells are cultured with growth factors, and are exposed to cell growth conditions (*e.g.*, promoting mitosis) such that the

5   Stem Cells proliferate to obtain an Expanded CB Stem Cell population according to the present invention. In a preferred embodiment of the invention, the CB Stem Cells are cultured with an amount of an agonist of Notch function effective to inhibit differentiation, and are exposed to cell growth conditions (*e.g.*, promoting mitosis) such that the CB Stem Cells proliferate to obtain an Expanded CB Stem Cell population

10   according to the present invention. In a more preferred embodiment, the CB Stem Cells are cultured with an amount of an agonist of Notch function effective to inhibit differentiation and in the presence of growth factors, and are exposed to cell growth conditions (*e.g.*, promoting mitosis) such that the CB Stem Cells proliferate to obtain an Expanded CB Stem Cell population according to the present invention. The Expanded

15   CB Stem Cell population so obtained can be frozen and stored for later use, for example, to provide hematopoietic function to an immunodeficient human patient. Optionally, the Notch pathway agonist is inactivated or removed from the Expanded CB Stem Cell population prior to transplantation into the patient (*e.g.*, by separation, dilution).

In specific embodiments, the CB Stem Cells are cultured for 2, 3, 4, 5, 6, 7, 8, 9,

20   10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days or more; or, preferably, the CB Stem Cells are cultured for at least 10 days.

An exemplary culture condition for expanding the CB Stem Cells include is set forth in Section 7.1 *infra*, and comprises culturing the Stem Cells for 17-21 days in the presence of fibronectin fragments and the extracellular domain of a Delta protein fused

25   to the Fc domain of human IgG (Delta1$^{ext-IgG}$) in serum free medium supplemented with the following human growth factors: stem cell factor, Flt-3 receptor ligand, thrombopoietin, interleukin-6 and interleukin-3. Preferably, the foregoing growth factors are present at the following concentrations: 50-300 ng/ml stem cell factor, 50-300 ng/ml Flt-3 receptor ligand, 50-100 ng/ml thrombopoietin, 50-100 ng/ml

30   interleukin-6 and 10 ng/ml interleukin-3. In more specific embodiments, 300 ng/ml stem cell factor, 300 ng/ml of Flt-3 receptor ligand, 100 ng/ml thrombopoietin, 100 ng/ml interleukin-6 and 10 ng/ml interleukin-3, or 50 ng/ml stem cell factor, 50 ng/ml of Flt-3 receptor ligand, 50 ng/ml thrombopoietin, 50 ng/ml interleukin-6 and 10 ng/ml

interleukin-3 are used. Preferably, the Delta1$^{ext-IgG}$ is immobilized on the surface of the cell culture dishes. In a specific embodiment, the cell culture dishes are coated overnight at 4° C (or for a minimum of 2 hours at 37° C) with 2.5 µg/ml Delta1$^{ext-IgG}$ and 5 µg/ml RetroNectin® (a recombinant human fibronectin fragment) in phosphate

5   buffered saline, before adding the CB Stem Cells.

Other exemplary culture condition for expanding the CB Stem Cells of the invention comprises are set forth in Zhang *et al.*, 2008, Blood 111:3415-3423. In a specific embodiment, the CB Stem Cells can be cultured in serum free medium supplemented with heparin, stem cell factor, thrombopoietin, insulin-like growth factor-

10   2 (IGF-2), fibroblast growth factor-1 (FGF-1), and Angptl3 or Angptl5. In a specific embodiment, the medium is supplemented with 10 µg/ml heparin, 10 ng/ml stem cell factor, 20 ng/ml thrombopoietin, 20 ng/ml IGF-2, and 10 ng/ml FGF-1, and 100 ng/ml Angptl3 or Angptl5 and the cells are cultured for 19-23 days. In another specific embodiment, the CB Stem Cells can be expanded by culturing the CB Stem Cells in

15   serum free medium supplemented with 10 µg/ml heparin, 10 ng/ml stem cell factor, 20 ng/ml thrombopoietin, 10 ng/ml FGF-1, and 100 ng/ml Angptl5 for 11-19 days. In another specific embodiment, the CB Stem Cells can be expanded by culturing the CB Stem Cells in serum free medium supplemented with 50 ng/ml stem cell factor, 10 ng/ml thrombopoietin, 50 ng/ml Flt-3 receptor ligand, and 100 ng/ml insulin-like growth factor

20   binding protein-2 (IGFBP2) or 500 ng/ml Angptl5 for 10 days. In yet another embodiment, the CB Stem Cells can be expanded by culturing the CB Stem Cells in serum free medium supplemented with 10 µg/ml heparin, 10 ng/ml stem cell factor, 20 ng/ml thrombopoietin, 10 ng/ml FGF-1, 500 ng/ml Angptl5, and 500 ng/ml IGFBP2 for 11 days. See Zhang *et al.*, 2008, Blood 111:3415-3423.

25   Another exemplary culture condition for expanding the CB Stem Cells of the invention is set forth in Himburg *et al.*, 2010, Nature Medicine doi:10.1038/nm.2119 (advanced online publication). In a specific embodiment, the CB Stem Cells can be cultured in liquid suspension culture supplemented with thrombopoietin, stem cell factor, Flt-3 receptor ligand, and pleiotrophin. In a specific embodiment, the liquid

30   suspension culture is supplemented with 20 ng/ml thrombopoietin, 125 ng/ml stem cell factor, 50 ng/ml Flt-3 receptor ligand, and 10, 100, 500, or 1000 ng/ml pleiotrophin and the CB Stem Cells are cultured for 7 days.

In a preferred embodiment of the invention, after expansion of the CB Stem Cells, the total number of cells and viable CD34$^+$ cells are determined to measure the potency of the sample to provide hematopoietic function. Numerous clinical studies have shown that the total nucleated cell dose and the CD34$^+$ cell dose in stem cell grafts are highly correlated with neutrophil and platelet engraftment as well as the incidence of graft failure and early transplant-related complications (primarily lethal infections) following stem cell transplantation. For example, at day 5-8 post culture initiation during expansion, a sample can be taken for determination of the total viable nucleated cell count. In addition, the total number of CD34$^+$ cells can be determined by multi-parameter flow cytometry, and, thus, the percentage of CD34$^+$ cells in the sample. Preferably, cultures that have not resulted in at least a 10-fold increase in the absolute number of CD34$^+$ cells at this time are discontinued. Similarly, prior to cryopreservation or after thawing, an aliquot of the Expanded CB Stem Cell sample can be taken for determination of total nucleated cells and percentage of viable CD34$^+$ cells in order to calculate the total viable CD34$^+$ cell number in the Expanded CB Stem Cell sample. In a preferred embodiment, those Expanded CB Stem Cell samples containing less than 75 million CD34$^+$ viable cells can be discarded.

In a specific embodiment, total viable CD34$^+$ (or other antigen-positive) cell numbers can be considered the potency assay for release of the final product for therapeutic use. Viability can be determined by any method known in the art, for example, by trypan blue exclusion or 7-AAD exclusion. Preferably, the total nucleated cell count (TNC) and other data are used to calculate the potency of the product . The percentage of viable CD34$^+$ cells can be assessed by flow cytometry and use of a stain that is excluded by viable cells. The percentage of viable CD34$^+$ cells = the number of CD34$^+$ cells that exclude 7-AAD (or other appropriate stain) in an aliquot of the sample divided by the TNC (both viable and non-viable) of the aliquot. Viable CD34$^+$ cells in the sample can be calculated as follows: Viable CD34$^+$ cells = TNC of sample x % viable CD34$^+$ cells in the sample. The proportional increase during enrichment or expansion in viable CD34$^+$ cells can be calculated as follows: Total Viable CD34$^+$ cells Post-culture/Total Viable CD34$^+$ cells Pre-culture. As will be apparent, antigens other than or in addition to CD34 can be used.

6.3.1 Notch Agonists

In a preferred embodiment of the present invention, the CB Stem Cells are expanded by culturing the cells in the presence of an agonist of Notch function and one of more growth factors or cytokines for a given period of time. Culturing the CB Stem Cells can take place under any suitable culture medium/conditions known in the art (see, e.g., Freshney Culture of Animal Cells, Wiley-Liss, Inc., New York, NY (1994)). The time in culture is for a time sufficient to produce an Expanded CB Stem Cell population, as defined herein. For example, the CB Stem Cells can be cultured in a serum-free medium in the presence of an agonist of Notch function and one or more growth factors or cytokines for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days; or, preferably, for at least 10 days. Optionally, at any point during the culturing period, the culture medium can be replaced with fresh medium or fresh medium can be added.

A Notch agonist is an agent that promotes, i.e., causes or increases, activation of Notch pathway function. As used herein, "Notch pathway function" shall mean a function mediated by the Notch signaling (signal transduction) pathway, including but not limited to nuclear translocation of the intracellular domain of Notch, nuclear translocation of RBP-Jκ or its Drosophila homolog Suppressor of Hairless; activation of bHLH genes of the Enhancer of Split complex, e.g., Mastermind; activation of the HES-1 gene or the KBF2 (also called CBF1) gene; inhibition of Drosophila neuroblast segregation; and binding of Notch to Delta, Jagged/Serrate, Fringe, Deltex or RBP-Jκ/Suppressor of Hairless, or homologs or analogs thereof. See generally the review article by Kopan et al., 2009, Cell 137:216-233 for a discussion of the Notch signal transduction pathway and its effects upon activation; see also Jarriault et al., 1998, Mol. Cell. Biol. 18:7423-7431.

Notch activation is carried out by exposing a cell to a Notch agonist. The agonist of Notch can be but is not limited to a soluble molecule, a molecule that is recombinantly expressed on a cell-surface, a molecule on a cell monolayer to which the precursor cells are exposed, or a molecule immobilized on a solid phase. Exemplary Notch agonists are the extracellular binding ligands Delta and Serrate which bind to the extracellular domain of Notch and activate Notch signal transduction, or a fragment of Delta or Serrate that binds to the extracellular domain of Notch and activates Notch signal transduction. Nucleic acid and amino acid sequences of Delta and Serrate have

been isolated from several species, including human, are known in the art, and are disclosed in International Patent Publication Nos. WO 93/12141, WO 96/27610, WO 97/01571, Gray et al., 1999, Am. J. Path. 154:785-794. In a preferred mode of the embodiment, the Notch agonist is an immobilized fragment of a Delta or Serrate protein

5   consisting of the extracellular domain of the protein fused to a myc epitope tag (Delta$^{ext-myc}$ or Serrate$^{ext-myc}$, respectively) or an immobilized fragment of a Delta or Serrate protein consisting of the extracellular domain of the protein fused to the Fc portion of IgG (Delta$^{ext-IgG}$ or Serrate$^{ext-IgG}$, respectively). Notch agonists of the present invention include but are not limited to Notch proteins and analogs and derivatives (including

10   fragments) thereof; proteins that are other elements of the Notch pathway and analogs and derivatives (including fragments) thereof; antibodies thereto and fragments or other derivatives of such antibodies containing the binding region thereof; nucleic acids encoding the proteins and derivatives or analogs; as well as proteins and derivatives and analogs thereof which bind to or otherwise interact with Notch proteins or other proteins

15   in the Notch pathway such that Notch pathway activity is promoted. Such agonists include but are not limited to Notch proteins and derivatives thereof comprising the intracellular domain, Notch nucleic acids encoding the foregoing, and proteins comprising the Notch-interacting domain of Notch ligands (e.g., the extracellular domain of Delta or Serrate). Other agonists include but are not limited to

20   RBPJκ/Suppressor of Hairless or Deltex. Fringe can be used to enhance Notch activity, for example in conjunction with Delta protein. These proteins, fragments and derivatives thereof can be recombinantly expressed and isolated or can be chemically synthesized.

        In another specific embodiment, the Notch agonist is a cell which recombinantly

25   expresses a protein or fragment or derivative thereof, which agonizes Notch. The cell expresses the Notch agonist in such a manner that it is made available to the CB Stem Cells in which Notch signal transduction is to be activated, e.g., it is secreted, expressed on the cell surface, etc.

        In yet another specific embodiment, the agonist of Notch is a peptidomimetic or

30   peptide analog or organic molecule that binds to a member of the Notch signaling pathway. Such an agonist can be identified by binding assays selected from those known in the art, for example the cell aggregation assays described in Rebay et al., 1991, Cell 67:687-699 and in International Patent Publication No. WO 92/19734.

In a preferred embodiment the agonist is a protein consisting of at least a
fragment of a protein encoded by a Notch-interacting gene which mediates binding to a
Notch protein or a fragment of Notch, which fragment of Notch contains the region of
Notch responsible for binding to the agonist protein, *e.g.*, epidermal growth factor-like
5   repeats 11 and 12 of Notch. Notch interacting genes, as used herein, shall mean the
genes Notch, Delta, Serrate, RBPJκ, Suppressor of Hairless and Deltex, as well as other
members of the Delta/Serrate family or Deltex family which may be identified by virtue
of sequence homology or genetic interaction and more generally, members of the "Notch
cascade" or the "Notch group" of genes, which are identified by molecular interactions
10   (*e.g.*, binding *in vitro*, or genetic interactions (as depicted phenotypically, *e.g.*, in
Drosophila). Exemplary fragments of Notch-binding proteins containing the region
responsible for binding to Notch are described in U.S. Pat. Nos. 5,648,464; 5,849,869;
and 5,856,441.

The Notch agonists utilized by the methods of the invention can be obtained
15   commercially, produced by recombinant expression, or chemically synthesized.

In a specific embodiment, exposure of the cells to a Notch agonist is not done by
incubation with other cells recombinantly expressing a Notch ligand on the cell surface
(although in other embodiments, this method can be used), but rather is by exposure to a
cell-free Notch ligand, *e.g.*, incubation with a cell-free ligand of Notch, which ligand is
20   immobilized on the surface of a solid phase, *e.g.*, immobilized on the surface of a tissue
culture dish.

In specific embodiments, Notch activity is promoted by the binding of Notch
ligands (*e.g.*, Delta, Serrate) to the extracellular portion of the Notch receptor. Notch
signaling appears to be triggered by the physical interaction between the extracellular
25   domains of Notch and its ligands that are either membrane-bound on adjacent cells or
immobilized on a solid surface. Full length ligands are agonists of Notch, as their
expression on one cell triggers the activation of the pathway in the neighboring cell
which expresses the Notch receptor. Soluble truncated Delta or Serrate molecules,
comprising the extracellular domains of the proteins or Notch-binding portions thereof,
30   that have been immobilized on a solid surface, such as a tissue culture plate, are
particularly preferred Notch pathway agonists. Such soluble proteins can be
immobilized on a solid surface by an antibody or interacting protein, for example an
antibody directed to an epitope tag with which Delta or Serrate is expressed as a fusion

protein (*e.g.*, a myc epitope tag, which is recognized by the antibody 9E10) or a protein
which interacts with an epitope tag with which Delta or Serrate is expressed as a fusion
protein (*e.g.*, an immunoglobulin epitope tag, which is bound by Protein A).

5   In another specific embodiment, and as described in U.S. Pat. No. 5,780,300 to
Artavanis-Tsakonas *et al.*, Notch agonists include reagents that promote or activate
cellular processes that mediate the maturation or processing steps required for the
activation of Notch or a member of the Notch signaling pathway, such as the furin-like
convertase required for Notch processing, Kuzbanian, the metalloprotease-disintegrin
(ADAM) thought to be required for the activation of the Notch pathway upstream or
10   parallel to Notch (Schlondorff and Blobel, 1999, J. Cell Sci. 112:3603-3617), or, more
generally, cellular trafficking and processing proteins such as the rab family of GTPases
required for movement between cellular compartments (for a review on Rab GTPases,
see Olkkonen and Stenmark, 1997, Int. Rev. Cytol. 176:1-85). The agonist can be any
molecule that increases the activity of one of the above processes, such as a nucleic acid
15   encoding a furin, Kuzbanian or rab protein, or a fragment or derivative or dominant
active mutant thereof, or a peptidomimetic or peptide analog or organic molecule that
binds to and activates the function of the above proteins.

           U.S. Pat. No. 5,780,300 further discloses classes of Notch agonist molecules (and
methods of their identification) which can be used to activate the Notch pathway in the
20   practice of the present invention, for example molecules that trigger the dissociation of
the Notch ankyrin repeats with RBP-Jκ, thereby promoting the translocation of RBP-Jκ
from the cytoplasm to the nucleus.

6.3.2   Growth Factors/Cytokines

25   In a preferred embodiment of the present invention, the CB Stem Cells are
expanded by culturing the cells in the presence of an agonist of Notch function,
discussed *supra*, and one of more growth factors or cytokines for a given period of time.
Alternatively, the CB Stem Cells are expanded by culturing the cells in the presence of
one of more growth factors or cytokines for a given period of time. Wherein expansion
30   of the CB Stem Cells without differentiation is to be achieved, the CB Stem Cells of the
invention are cultured in the presence of growth factors that support growth but not

differentiation. The growth factor can be any type of molecule, such as a protein or a chemical compound, that promotes cellular proliferation and/or survival.

Exposing the CB Stem Cells to one or more growth factors can be done prior to, concurrently with, or following exposure of the cells to a Notch agonist.

5   In specific exemplary embodiments, the growth factors present in the expansion medium include one or more of the following growth factors: stem cell factor (SCF), also known as the c-kit ligand or mast cell growth factor, Flt-3 ligand (Flt-3L), interleukin-6 (IL-6), interleukin-3 (IL-3), interleukin-11 (IL-11) and thrombopoietin (TPO), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte
10   colony stimulating factor (G-CSF), angiopoietin-like proteins (Angptls) (Angptl2, Angptl3, Angptl5, Angptl7, and Mfap4), insulin growth factor-2 (IFG-2), fibroblast growth factor-1 (FGF-1). The amount of SCF, Flt-3L, IL-6, or TPO can be in the range of 10-1000 ng/ml, more preferably about 50-500 ng/ml, most preferably about 100-300 ng/ml. In certain specific embodiments, the amount of SCF, Flt-3L, IL-6, or TPO is
15   100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425 or 450 ng/ml. The amount of Il-3, IL-11, G-CSF, or GM-CSF can be in the range of 2-100 ng/ml, more preferably about 5-50 ng/ml, more preferably about 7.5-25 ng/ml, most preferably about 10-15 ng/ml. In certain specific embodiments, the amount of Il-3, IL-11, G-CSF, or GM-CSF is 5, 6, 7, 8, 9, 10, 12.5, or 15 ng/ml.

20   In a preferred embodiment for expanding CB Stem Cells, the cells are cultured in a tissue culture dish onto which an extracellular matrix protein is bound. In a preferred mode of the embodiment, the extracellular matrix protein is fibronectin (FN), or a fragment thereof. Such a fragment can be but is not limited to CH-296 (Dao *et al.*, 1998, Blood 92(12):4612-21) or RetroNectin® (a recombinant human fibronectin fragment)
25   (Clontech Laboratories, Inc., Madison, WI).

In a specific embodiment for expanding CB Stem Cells of the present invention, the cells are cultured on a plastic tissue culture dish containing immobilized Delta ligand, *e.g.*, the extracellular domain of Delta, and fibronectin in the presence of 100 ng/ml of each of SCF and TPO, and 10 ng/ml GM-CSF. In another specific
30   embodiment for expanding CB Stem Cells, the cells are cultured on a plastic tissue culture dish containing immobilized Delta ligand and fibronectin in the presence of 100 ng/ml of each of SCF, Flt-3L, TPO and IL-6 and 10 ng/ml of IL-3. In another specific

embodiment for expanding Stem Cells of the present invention, the cells are cultured on a plastic tissue culture dish containing immobilized Delta ligand and fibronectin in the presence of 100 ng/ml of each of SCF and Flt-3L and 10 mg/ml of each of G-CSF and GM-CSF. In another specific embodiment for expanding CB Stem Cells, the cells are

5   cultured on a plastic tissue culture dish containing immobilized Delta ligand and fibronectin in the presence of 100 ng/ml of each of SCF, Flt-3L and TPO and 10 mg/ml of GM-CSF. In yet another specific embodiment for expanding CB Stem Cells, the cells are cultured on a plastic tissue culture dish containing immobilized Delta ligand and fibronectin in the presence of 300 ng/ml of each of SCF and Flt-3L, 100 ng/ml of each

10   of TPO and IL-6, and 10 mg/ml of IL-3. In another embodiment for expanding CB Stem Cells, the cells are cultured on a plastic tissue culture dish containing immobilized Delta ligand and fibronectin in the presence of 100 ng/ml of each of SCF, Flt-3L, and TPO and 10 mg/ml of each of G-CSF and GM-CSF. In alternative embodiments to the foregoing culture conditions, fibronectin is excluded from the tissue culture dishes or is

15   replaced by another extracellular matrix protein. See also U.S. Patent No. 7,399,633 B2 to Bernstein *et al.* for additional exemplary culture conditions for CB Stem Cell expansion.

The growth factors utilized by the methods of the invention can be obtained commercially, produced by recombinant expression, or chemically synthesized. For

20   example, Flt-3L (human), IGF-1 (human), IL-6 (human and mouse), IL-11 (human), SCF (human), TPO (human and murine) can be purchased from Sigma (St. Louis, Mo.). IL-6 (human and murine), IL-7 (human and murine), and SCF (human) can be purchased from Life Technologies, Inc. (Rockville, Md.).

In other embodiments, the growth factors are produced by recombinant

25   expression or by chemical peptide synthesis (*e.g.* by a peptide synthesizer). Growth factor nucleic acid and peptide sequences are generally available from GenBank.

Preferably, but not necessarily, the growth factor(s) used to expand the CB Stem Cells in the presence of a Notch agonist by the methods of the invention is derived from the same species as the CB Stem Cells.

30   The amount or concentration of growth factors suitable for expanding the CB Stem Cells of the present invention will depend on the activity of the growth factor preparation, and the species correspondence between the growth factors and the CB

Stem Cells, etc. Generally, when the growth factor(s) and the CB Stem Cells are of the same species, the total amount of growth factor in the culture medium ranges from 1 ng/ml to 5 µg/ml, more preferably from 5 ng/ml to 1 µg/ml, and most preferably from about 10 ng/ml to 200 ng/ml. In one embodiment, the CB Stem Cells are expanded by exposing the CB Stem Cells to a Notch agonist and 100 ng/ml of SCF. In another embodiment, the CB Stem Cells are expanded by exposing the CB Stem Cells to a Notch agonist and 100 ng/ml of each of Flt-3L, IL-6 and SCF and 10 ng/ml of IL-11.

6.4   CRYOPRESERVATION AND THAWING
6.4.1   Cryopreservation

Once the Expanded CB Stem Cell population is obtained after expanding CB Stem Cells from cord blood, the Expanded CB Stem Cell population can be cryopreserved. In one embodiment, an Expanded CB Stem Cell population can be divided and frozen in one or more bags (or units), before pooling (and pooling upon subsequent thawing). In another embodiment, two or more Expanded CB Stem Cell populations can be pooled and frozen, or optionally pooled, divided into separate aliquots, and each aliquot is frozen. In a preferred embodiment, a maximum of approximately 4 billion nucleated cells is frozen in a single bag. In a preferred embodiment, the Expanded CB Stem Cells are fresh, i.e., they have not been previously frozen prior to expansion or cryopreservation. The terms "frozen/freezing" and "cryopreserved/cryopreserving" are used interchangeably in the present application. Cryopreservation can be by any method in known in the art that freezes cells in viable form. The freezing of cells is ordinarily destructive. On cooling, water within the cell freezes. Injury then occurs by osmotic effects on the cell membrane, cell dehydration, solute concentration, and ice crystal formation. As ice forms outside the cell, available water is removed from solution and withdrawn from the cell, causing osmotic dehydration and raised solute concentration which eventually destroy the cell. For a discussion, see Mazur, P., 1977, Cryobiology 14:251-272.

These injurious effects can be circumvented by (a) use of a cryoprotective agent, (b) control of the freezing rate, and (c) storage at a temperature sufficiently low to minimize degradative reactions.

Cryoprotective agents which can be used include but are not limited to dimethyl sulfoxide (DMSO) (Lovelock and Bishop, 1959, Nature 183:1394-1395; Ashwood-Smith, 1961, Nature 190:1204-1205), glycerol, polyvinylpyrrolidine (Rinfret, 1960, Ann. N.Y. Acad. Sci. 85:576), polyethylene glycol (Sloviter and Ravdin, 1962, Nature

5   196:548), albumin, dextran, sucrose, ethylene glycol, i-erythritol, D-ribitol, D-mannitol (Rowe *et al.*, 1962, Fed. Proc. 21:157), D-sorbitol, i-inositol, D-lactose, choline chloride (Bender *et al.*, 1960, J. Appl. Physiol. 15:520), amino acids (Phan The Tran and Bender, 1960, Exp. Cell Res. 20:651), methanol, acetamide, glycerol monoacetate (Lovelock, 1954, Biochem. J. 56:265), and inorganic salts (Phan The Tran and Bender, 1960, Proc.

10   Soc. Exp. Biol. Med. 104:388; Phan The Tran and Bender, 1961, in Radiobiology, Proceedings of the Third Australian Conference on Radiobiology, Ilbery ed., Butterworth, London, p. 59). In a preferred embodiment, DMSO is used, a liquid which is nontoxic to cells in low concentration. Being a small molecule, DMSO freely permeates the cell and protects intracellular organelles by combining with water to

15   modify its freezability and prevent damage from ice formation. Addition of plasma (*e.g.*, to a concentration of 20-25%) can augment the protective effect of DMSO. After addition of DMSO, cells should be kept at 0° C until freezing, since DMSO concentrations of about 1% are toxic at temperatures above 4° C.

A controlled slow cooling rate can be critical. Different cryoprotective agents

20   (Rapatz *et al.*, 1968, Cryobiology 5(1):18-25) and different cell types have different optimal cooling rates (see *e.g.*, Rowe and Rinfret, 1962, Blood 20:636; Rowe, 1966, Cryobiology 3(1):12-18; Lewis, *et al.*, 1967, Transfusion 7(1):17-32; and Mazur, 1970, Science 168:939-949 for effects of cooling velocity on survival of marrow-stem cells and on their transplantation potential). The heat of fusion phase where water turns to ice

25   should be minimal. The cooling procedure can be carried out by use of, *e.g.*, a programmable freezing device or a methanol bath procedure.

Programmable freezing apparatuses allow determination of optimal cooling rates and facilitate standard reproducible cooling. Programmable controlled-rate freezers such as Cryomed or Planar permit tuning of the freezing regimen to the desired cooling

30   rate curve. For example, for marrow cells in 10% DMSO and 20% plasma, the optimal rate is 1° to 3° C/minute from 0° C to -80° C. In a preferred embodiment, this cooling rate can be used for the neonatal cells of the invention. The container holding the cells must be stable at cryogenic temperatures and allow for rapid heat transfer for effective

control of both freezing and thawing. Sealed plastic vials (*e.g.*, Nunc, Wheaton cryules) or glass ampules can be used for multiple small amounts (1-2 ml), while larger volumes (100-200 ml) can be frozen in polyolefin bags (*e.g.*, Delmed) held between metal plates for better heat transfer during cooling. Bags of bone marrow cells have been

5   successfully frozen by placing them in -80° C freezers which, fortuitously, gives a cooling rate of approximately 3° C/minute).

In an alternative embodiment, the methanol bath method of cooling can be used. The methanol bath method is well-suited to routine cryopreservation of multiple small items on a large scale. The method does not require manual control of the freezing rate

10   nor a recorder to monitor the rate. In a preferred embodiment, DMSO-treated cells are pre-cooled on ice and transferred to a tray containing chilled methanol which is placed, in turn, in a mechanical refrigerator (*e.g.*, Harris or Revco) at -80° C. Thermocouple measurements of the methanol bath and the samples indicate the desired cooling rate of 1° to 3° C/minute. After at least two hours, the specimens have reached a temperature of

15   -80° C and can be placed directly into liquid nitrogen (-196° C) for permanent storage.

After thorough freezing, the Expanded CB Stem Cells can be rapidly transferred to a long-term cryogenic storage vessel. In a preferred embodiment, samples can be cryogenically stored in liquid nitrogen (-196° C) or its vapor (-165° C). Such storage is greatly facilitated by the availability of highly efficient liquid nitrogen refrigerators,

20   which resemble large Thermos containers with an extremely low vacuum and internal super insulation, such that heat leakage and nitrogen losses are kept to an absolute minimum.

Suitable racking systems are commercially available and can be used for cataloguing, storage, and retrieval of individual specimens.

25   Considerations and procedures for the manipulation, cryopreservation, and long-term storage of the hematopoietic stem cells, particularly from bone marrow or peripheral blood, are largely applicable to the Expanded CB Stem Cells of the invention. Such a discussion can be found, for example, in the following references, incorporated by reference herein: Gorin, 1986, Clinics In Haematology 15(1):19-48; Bone-Marrow

30   Conservation, Culture and Transplantation, Proceedings of a Panel, Moscow, July 22-26, 1968, International Atomic Energy Agency, Vienna, pp. 107-186.

Other methods of cryopreservation of viable cells, or modifications thereof, are available and envisioned for use (*e.g.*, cold metal-mirror techniques; Livesey and Linner, 1987, Nature 327:255; Linner *et al.*, 1986, J. Histochem. Cytochem. 34(9):1123-1135; see also U.S. Pat. No. 4,199,022 by Senkan *et al.*, U.S. Pat. No. 3,753,357 by Schwartz,
5   U.S. Pat. No. 4,559,298 by Fahy).

6.4.2 Thawing

Frozen cells are preferably thawed quickly (*e.g.*, in a water bath maintained at 37°-41° C) and chilled immediately upon thawing. In a specific embodiment, the vial
10   containing the frozen cells can be immersed up to its neck in a warm water bath; gentle rotation will ensure mixing of the cell suspension as it thaws and increase heat transfer from the warm water to the internal ice mass. As soon as the ice has completely melted, the vial can be immediately placed in ice.

In an embodiment of the invention, the Expanded CB Stem Cell sample as
15   thawed, or a portion thereof, can be infused for providing hematopoietic function in a human patient in need thereof. Several procedures, relating to processing of the thawed cells are available, and can be employed if deemed desirable.

It may be desirable to treat the cells in order to prevent cellular clumping upon thawing. To prevent clumping, various procedures can be used, including but not
20   limited to, the addition before and/or after freezing of DNase (Spitzer *et al.*, 1980, Cancer 45:3075-3085), low molecular weight dextran and citrate, hydroxyethyl starch (Stiff *et al.*, 1983, Cryobiology 20:17-24), *etc.*

The cryoprotective agent, if toxic in humans, should be removed prior to therapeutic use of the thawed Expanded CB Stem Cells. In an embodiment employing
25   DMSO as the cryopreservative, it is preferable to omit this step in order to avoid cell loss, since DMSO has no serious toxicity. However, where removal of the cryoprotective agent is desired, the removal is preferably accomplished upon thawing.

One way in which to remove the cryoprotective agent is by dilution to an insignificant concentration. This can be accomplished by addition of medium, followed
30   by, if necessary, one or more cycles of centrifugation to pellet cells, removal of the supernatant, and resuspension of the cells. For example, intracellular DMSO in the thawed cells can be reduced to a level (less than 1%) that will not adversely affect the

recovered cells. This is preferably done slowly to minimize potentially damaging osmotic gradients that occur during DMSO removal.

After removal of the cryoprotective agent, cell count (e.g., by use of a hemocytometer) and viability testing (e.g., by trypan blue exclusion; Kuchler, 1977,

5   Biochemical Methods in Cell Culture and Virology, Dowden, Hutchinson & Ross, Stroudsburg, Pa., pp. 18-19; 1964, Methods in Medical Research, Eisen et al., eds., Vol. 10, Year Book Medical Publishers, Inc., Chicago, pp. 39-47) can be done to confirm cell survival. The percentage of viable antigen (e.g., CD34) positive cells in a sample can be determined by calculating the number of antigen positive cells that exclude 7-AAD (or

10   other suitable dye excluded by viable cells) in an aliquot of the sample, divided by the total number of nucleated cells (TNC) (both viable and non-viable) in the aliquot of the sample. The number of viable antigen positive cells in the sample can be then determined by multiplying the percentage of viable antigen positive cells by TNC of the sample.

15   Prior to cryopreservation and/or after thawing, the total number of nucleated cells, or in a specific embodiment, the total number of $CD34^+$ or $CD133^+$ cells can be determined. For example, total nucleated cell count can be performed by using a hemocytometer and exclusion of trypan blue dye. Specimens that are of high cellularity can be diluted to a concentration range appropriate for manual counting. Final cell

20   counts for products are corrected for any dilution factors. Total nucleated cell count = viable nucleated cells per mL x volume of product in mL. The number of $CD34^+$ or $CD133^+$ positive cells in the sample can be determined, e.g., by the use of flow cytometry using anti-CD34 or anti-CD133 monoclonal antibodies conjugated to a fluorochrome.

25   Optionally, the Expanded CB Stem Cell sample can undergo HLA typing either prior to cryopreservation and/or after cryopreservation and thawing. HLA typing can be performed using serological methods with antibodies specific for identified HLA antigens, or using DNA-based methods for detecting polymophisms in the HLA antigen-encoding genes for typing HLA alleles. In a specific embodiment, HLA typing can be

30   performed at intermediate resolution using a sequence specific oligonucleotide probe method for HLA-A and HLA-B or at high resolution using a sequence based typing method (allele typing) for HLA-DRB1.

In certain embodiments, the identity and purity of the starting umbilical cord blood and/or placental blood, the CB Stem Cells, and the Expanded CB Stem Cells prior to cryopreservation, or the Expanded CB Stem Cells after thawing can be subjected to multi-parameter flow cytometric immunophenotyping, which provides the percentage of

5   viable antigen positive cells present in a sample. Each sample can be tested for one or more of the following cell phenotypes using a panel of monoclonal antibodies directly conjugated to fluorochromes:

1. $CD34^+$ HPC

2.   T cells ($CD3^+$, including both $CD4^+$ and $CD8^+$ subsets)

10   3.   B cells ($CD19^+$ or $CD20^+$)

4.   NK cells ($CD56^+$)

5.   Monocytes ($CD14^+$)

6.   Myelomonocytes ($CD15^+$)

7.   Megakaryocytes ($CD41^+$)

15   8.   Dendritic Cells (lineage negative/HLA-DRbright and CD123bright, or lineage negative/HLA-DRbright and CD11cbright).

6.5   GENETICALLY ENGINEERED STEM CELLS

In a preferred embodiment, the Expanded CB Stem Cells administered to the

20   patient are non-recombinant. However, in a different embodiment, the CB Stem Cells prior to expansion or the Expanded CB Stem Cells can be genetically engineered to produce gene products beneficial upon transplantation of the genetically engineered cells to a subject. Such gene products include but are not limited to anti-inflammatory factors, *e.g.*, anti-TNF, anti-IL-1, anti-IL-2, etc. The CB Stem Cells can be genetically

25   engineered for use in gene therapy to adjust the level of gene activity in a subject to assist or improve the results of transplantation or to treat a disease caused by, for example, a deficiency in the recombinant gene. The CB Stem Cells are made recombinant by the introduction of a recombinant nucleic acid into the CB Stem Cells or into the Expanded CB Stem Cells.

30   In its broadest sense, gene therapy refers to therapy performed by the administration of a nucleic acid to a subject. The nucleic acid, either directly or indirectly via its encoded protein, mediates a therapeutic effect in the subject. The present invention provides methods of gene therapy wherein a nucleic acid encoding a

protein of therapeutic value (preferably to humans) is introduced into the CB Stem Cells, before or after expansion, such that the nucleic acid is expressible by the Stem Cells and/or their progeny, followed by administration of the recombinant Expanded CB Stem Cells to a subject.

5   The recombinant CB Stem Cells of the present invention can be used in any of the methods for gene therapy available in the art. Thus, the nucleic acid introduced into the cells may encode any desired protein, e.g., a protein missing or dysfunctional in a disease or disorder. The descriptions below are meant to be illustrative of such methods. It will be readily understood by those of skill in the art that the methods illustrated

10   represent only a sample of all available methods of gene therapy.

For general reviews of the methods of gene therapy, see Gardlik et al., 2005, Med. Sci. Monit. 11:RA110-121; Lundstrom, 1999, J. Recept. Signal Transduct. Res. 19:673-686; Robbins and Ghivizzani, 1998, Pharmacol. Ther.80:35-47; Pelegrin et al., 1998, Hum. Gene Ther. 9:2165-2175; Harvey and Caskey, 1998, Curr. Opin. Chem.

15   Biol. 2:512-518; Guntaka and Swamynathan, 1998, Indian J. Exp. Biol. 36:539-535; Desnick and Schuchman, 1998, Acta Paediatr. Jpn. 40:191-203; Vos, 1998, Curr. Opin. Genet. Dev. 8:351-359; Tarahovsky and Ivanitsky, 1998, Biochemistry (Mosc) 63:607-618; Morishita et al., 1998, Circ. Res. 2:1023-1028; Vile et al., 1998, Mol. Med. Today 4:84-92; Branch and Klotman,1998, Exp. Nephrol. 6:78-83; Ascenzioni et al., 1997,

20   Cancer Lett. 118:135-142; Chan and Glazer, 1997, J. Mol. Med. 75:267-282. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

25   In an embodiment in which recombinant CB Stem Cells are used in gene therapy, a gene whose expression is desired in a subject is introduced into the CB Stem Cells such that it is expressible by the cells and/or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect.

Recombinant Expanded CB Stem Cells can be used in any appropriate method of

30   gene therapy, as would be recognized by those in the art upon considering this disclosure. The resulting action of recombinant cell populations administered to a

subject can, for example, lead to the activation or inhibition of a pre-selected gene in the subject, thus leading to improvement of the diseased condition afflicting the subject.

In this embodiment, the desired gene is introduced into the CB Stem Cell or its progeny prior to administration *in vivo* of the resulting recombinant cell. Such

5   introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, lipofection, calcium phosphate mediated transfection, infection with a viral or bacteriophage vector containing the gene sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the

10 introduction of foreign genes into cells (see *e.g.*, Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen *et al.*, 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the gene

15 to the cell, so that the gene is expressible by the cell and preferably heritable and expressible by its cell progeny. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

20   More detail about retroviral vectors can be found in Boesen *et al.*, 1994, Biotherapy 6:291-302, Clowes *et al.*, 1994, J. Clin. Invest. 93:644-651; Kiem *et al.*, 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

Adenoviruses are also of use in gene therapy. See Kozarsky and Wilson, 1993,

25 Current Opinion in Genetics and Development 3:499-503, Rosenfeld *et al.*, 1991, Science 252:431-434; Rosenfeld *et al.*, 1992, Cell 68:143-155; and Mastrangeli *et al.*, 1993, J. Clin. Invest. 91:225-234.

It has been proposed that adeno-associated virus (AAV) be used in gene therapy (Walsh *et al.*, 1993, Proc. Soc. Exp. Biol. Med. 204:289-300). It has also been proposed

30 that alphaviruses be used in gene therapy (Lundstrom, 1999, J. Recept. Signal Transduct. Res. 19:673-686).

Other methods of gene delivery in gene therapy include the use of mammalian artificial chromosomes (Vos, 1998, Curr. Op. Genet. Dev. 8:351-359); liposomes (Tarahovsky and Ivanitsky, 1998, Biochemistry (Mosc) 63:607-618); ribozymes (Branch and Klotman, 1998, Exp. Nephrol. 6:78-83); and triplex DNA (Chan and Glazer, 1997,

5   J. Mol. Med. 75:267-282).

A desired gene can be introduced intracellularly and incorporated within CB Stem Cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra *et al.*, 1989, Nature 342:435-438).

10   In a specific embodiment, the desired gene recombinantly expressed in the CB Stem Cells or their progeny after expansion to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the recombinant gene is controllable by controlling the presence or absence of the appropriate inducer of transcription.

15

6.6   SELECTION OF EXPANDED CORD BLOOD CELLS

In accordance with the present invention, a pool of Expanded CB Stem Cell samples is selected for administration to a human patient in need thereof in order to provide hematopoietic function to the patient, wherein the samples in the pool

20   collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient. By the phrase "the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient," what is meant is that when tallying the HLA mismatches to those typed in the patient over all the cells in the samples in the pool, no more than 2 mismatches are present.

25   Thus, for example, if 6 HLA antigens/alleles are typed in the patient, and 1 sample in the pool mismatches at 2 antigens/alleles, no other samples in the pool can mismatch at any of the 4 remaining matched antigens/alleles (the other samples in the pool can mismatch only at zero, or one or both of the same 2 mismatched antigens/alleles). In specific embodiments, the patient is typed at 1, 2, 3, 4, 5, or 6 HLA antigens/alleles, preferably at

30   at least 4 HLA antigens/alleles, and most preferably at 6 HLA antigens/alleles.

In an embodiment wherein pooling of samples occurs prior to freezing (and generally to patient identification), selection of samples for pooling occurs, before

freezing, wherein only those samples that when pooled collectively do not mismatch at more than 2 of the typed HLA antigens or alleles in the samples that are selected for pooling.

5   In an embodiment wherein pooling of samples occurs after thawing (and generally after patient identification), selection of a pooled sample for administration to the patient involves selecting samples to be pooled together to form the pool. Such selecting of samples to form the pool comprises selecting samples such that the samples in the pool collectively will not mismatch the patient at more than 2 of the HLA antigens/alleles typed in the patient. Thus, for example, if a first sample is accepted for

10   pooling that differs at 2 out of the antigens/alleles typed in the expanded human cord blood stem cell sample, no one other sample can be accepted for pooling that differs at HLA antigens/alleles other than those 2. As another example, if a first sample is accepted with a mismatch at a first HLA antigen/allele, and a second sample is accepted with a mismatch at a second HLA antigen/allele that differs from the first HLA

15   antigen/allele, other samples accepted for pooling can mismatch the patient only at the first and/or second antigen/allele.

In one embodiment of the invention, a method for providing hematopoietic function to a human patient in need thereof is provided, which method comprises selecting a pool of expanded human cord blood stem cell samples for administration to

20   the patient from a plurality of pools of expanded human cord blood stem cell samples, wherein the pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles

25   typed in the patient; and (b) administering the selected pool, or an aliquot thereof, to the patient. In a preferred embodiment, the selecting is from a plurality of different pools of samples (e.g., at least 100, 200, 250, 500, 750, 1000, 1500, 2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000 different pools of expanded cord blood stem cell samples), preferably stored frozen in a bank.

30   Optional parameters for consideration in the selection of samples to be pooled, or selection of a pool of Expanded CB Stem Cell samples, for use in a method of treatment according to the present invention include, but are not limited to one or more of total nucleated cell count, total CD34$^+$ (or other suitable antigen) cell count, age of sample,

age of patient, race or ethnic background of donor, weight of the patient, type of disease to be treated and its level of severity in a particular patient, presence of $CD3^+$ cells in the Expanded CB Stem Cell samples or pool thereof, panel reactive antibody result of the patient, etc. For example, in a specific embodiment, a pool of Expanded CB Stem Cell

5   samples can be rejected (or individual Expanded CB Stem Cell samples can be rejected for forming a pool for use), and thus not selected for use in a method of treatment if there are more than 500,000 $CD3^+$ cells per kilogram (patient weight) in the pool.

In a specific embodiment, the selecting can be computer-implemented, whereby the selection software can take into account any one or more of the foregoing

10 information characterizing the sample or pool of samples, e.g., by filtering out (rejecting) samples or pools of samples that do not meet certain criteria, e.g., that do not contain threshold amounts of $CD34^+$ cells (e.g., at least 75 million $CD34^+$ cells, preferably, at least 100 million, 150 million, 200 million, 250 million, 300 million, 350 million, most preferably at least 250 million), and/or that contain more than a threshold

15 amount of $CD3^+$ cells (e.g., more than 500,000 $CD3^+$ per kilogram patient weight). In a specific embodiment, pools of samples left after filtering are selected, for example, by choosing the sample stored for the longest period, or at random, or based on any characteristic useful to the skilled practitioner.

The selection of the sample can be carried out by a suitably programmed

20 computer by selecting an appropriate identifier for the frozen, Expanded CB Stem Cell sample or pools of samples, from among a plurality of identifiers stored in a computer database, each identifying a different frozen, Expanded CB Stem Cell sample, or pool of samples. Each identifier is preferably associated with the information for its corresponding sample or pool of samples as described above (one or more of total

25 nucleated cell count, total $CD34^+$ cell count, etc.), so that the software can take into account the information as described above in the selection process.

6.7   THERAPEUTIC METHODS

The pool of Expanded CB Stem Cell samples, whether recombinantly expressing

30 a desired gene or not, can be administered into a human patient in need thereof for hematopoietic function for the treatment of disease or injury or for gene therapy by any method known in the art which is appropriate for the Expanded CB Stem Cells and the

transplant site. Preferably, a pool of Expanded CB Stem Cell samples is transplanted (infused) intravenously. In one embodiment, the Expanded CB Stem Cell samples differentiate into cells of the myeloid lineage in the patient. In another embodiment, the Expanded CB Stem Cell samples differentiate into cells of the lymphoid lineage in the

5   patient. Administration of a pool of two or more Expanded CB Stem Cell samples is only where the Expanded CB Stem Cell samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient. In one embodiment, one HLA antigen or allele is different collectively between the pool of expanded human cord blood stem cell samples and the recipient patient among those

10   HLA antigens or alleles typed. In another embodiment, 2 HLA antigens or alleles are different collectively between the pool of expanded human cord blood stem cell samples and the recipient patient among those HLA antigens or alleles typed.

         In specific embodiments, a pool of Expanded CB Stem Cell samples is not administered to the patient within 12 hours of administration of a myeloid progenitor

15   cell population as defined in International Patent Publication Nos. WO 2006/047569 A2 and/or WO 2007/095594 A2. In other specific embodiments, a pool of Expanded CB Stem Cell samples is not administered to the patient within 18 or 24 or 36 or 48 or 72 or 96 hours or within 7, 10, 14, 21, 30 days of administration of such a myeloid progenitor cell population to the patient.

20   In a specific embodiment, the methods of the invention described herein, involving administration of a pool of Expanded CB Stem Cell samples, further comprise administering one or more umbilical cord blood/placental blood samples (hereinafter called "Grafts" or "cord blood transplants"). Such Grafts are umbilical cord blood and/or placental blood samples from humans that are whole blood samples, except that

25   red blood cells have been removed from the whole blood samples, but which samples have not been further fractionated and have not been expanded. In a specific embodiment, the Grafts have been cryopreserved and are thawed prior to administration. In a specific embodiment, at least 4 of the HLA antigens or alleles of the Grafts are typed. In a preferred embodiment, 6 HLA antigens or alleles (e.g., each of the 2 HLA-

30   A, HLA-B and HLA-DR alleles) are typed. In a preferred embodiment, the one or more Grafts administered to the patient match the patient at at least 4 out of 6 HLA antigens or alleles. In a specific embodiment, the Graft is administered without matching the HLA-type of the Graft with the HLA-type of the patient. The Grafts can be administered

concurrently with, sequentially with respect to, before, or after the pool of Expanded CB Stem Cell samples is administered to the patient. In a specific embodiment, the pool of Expanded CB Stem Cell samples that is administered to the patient is administered within 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days of administering the one or more Grafts. In a

5   specific embodiment, the pool of Expanded CB Stem Cell samples is administered before administering the one or more Grafts. In another specific embodiment, the pool of Expanded CB Stem Cell samples is administered after administering the one or more Grafts. In a specific embodiment, the pool of Expanded CB Stem Cell samples is administered 1 to 24 hours, 2 to 12 hours, 3 to 8 hours, or 3 to 5 hours before or after

10   administering the one or more Grafts. In other specific embodiments, the pool of Expanded CB Stem Cell samples is administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, or 24 hours before or after administering the one or more Grafts. In a preferred embodiment, the pool of Expanded CB Stem Cell samples is administered about 4 hours after administering the one or more Grafts. In a specific embodiment, a single Graft is

15   administered that is derived from the cord and/or placental blood of a single human individual. In a specific embodiment, two Grafts are administered, each derived from the cord and/or placental blood of a different human individual. In another specific embodiment, a single Graft is administered that is a combination of cord and/or placental blood derived from two or more different human individuals. In the foregoing

20   embodiments, the pool of Expanded CB Stem Cell samples is intended to provide temporary hematopoietic benefit to the patient, while the Graft is intended to provide long-term engraftment.

        Other suitable methods of administration of the Expanded CB Stem Cell samples, or pools thereof, are encompassed by the present invention. The Expanded CB

25   Stem Cell samples, or pools thereof, can be administered by any convenient route, for example by infusion or bolus injection, and may be administered together with other biologically active agents. Administration can be systemic or local.

        The titer of Expanded CB Stem Cells administered which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder

30   or condition, and can be determined by standard clinical techniques. In addition, *in vitro* and *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided

according to the judgment of the practitioner and each subject's circumstances. In specific embodiments, suitable dosages of Expanded CB Stem Cells, or pools thereof, for administration are generally about at least 5×10^6, 10^7, 5×10^7, 75×10^6, 10^7, 5×10^7, 10^8, 5×10^8, 1×10^9, 5×10^9, 1×10^{10}, 5×10^{10}, 1×10^{11}, 5×10^{11} or 10^{12} CD34$^+$

5 cells per kilogram patient weight, and most preferably about 10^7 to about 10^{12} CD34$^+$ cells per kilogram patient weight, and can be administered to a patient once, twice, three or more times with intervals as often as needed.

The patient is a human patient, preferably an immunodeficient human patient.

In one embodiment, the individual samples in the pool are all derived from

10 umbilical cord blood and/or placental blood of individuals of the same race, e.g., African-American, Caucasian, Asian, Hispanic, Native-American, Australian Aboriginal, Inuit, Pacific Islander, or are all derived from umbilical cord blood and/or placental blood of individuals of the same ethnicity, e.g., Irish, Italian, Indian, Japanese, Chinese, Russian, etc.

15

6.8   PHARMACEUTICAL COMPOSITIONS

The invention provides methods of treatment by administration to a patient of a pharmaceutical (therapeutic) composition comprising a therapeutically effective amount of recombinant or non-recombinant pool of Expanded CB Stem Cell samples produced

20 by the methods of the present invention as described herein above, wherein the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient. Preferably, a myeloid progenitor cell population is not administered to the patient within 12 hours of the administering of the pool of expanded human cord blood stem cell samples, wherein a majority of the cells in the myeloid

25 progenitor cell population do not produce lymphoid cells in cell culture. In other embodiments, a myeloid progenitor cell population is not administered to the patient within 18, 20, 24, 36, 48, 72 hours or within 1 week of the administering of the pool of expanded human cord blood stem cell samples, wherein a majority of the cells in the myeloid progenitor cell population do not produce lymphoid cells in cell culture. In a

30 specific embodiment, a majority of the cells in the myeloid cell population express the cell surface marker CD33 and/or do not express the cell surface marker CD45RA.

The present invention provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Expanded CB Stem Cell sample, or pool therof, and a pharmaceutically acceptable carrier or excipient. Such a carrier can be but is not limited to saline, buffered saline, dextrose, water, glycerol,

5 ethanol, and combinations thereof. The carrier and composition preferably are sterile. The formulation should suit the mode of administration. The pharmaceutical composition is acceptable for therapeutic use in humans. The composition, if desired, can also contain pH buffering agents.

In a preferred embodiment, the composition is formulated in accordance with

10 routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection.

15 The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more pools of the Stem Cell or Expanded CB Stem Cell populations produced by the methods of the invention and/or reagents to prepare said cells, or with reagents for the genetic manipulation of the cells.

In a preferred embodiment, a kit of the invention comprises in one or more

20 containers one or more purified growth factors that promote proliferation but not differentiation of a precursor cell and a purified Notch agonist, which growth factors and Notch agonist are together effective to expand Stem Cells exposed to them in culture. Optionally, cell culture medium is also provided.

Optionally associated with such container(s) can be a notice in the form

25 prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

6.9   THERAPEUTIC USES OF THE EXPANDED CB STEM CELLS

30 The pools of two or more different Expanded CB Stem Cell samples of the present invention can be used to provide hematopoietic function to a patient in need thereof, preferably a human patient, wherein the samples in the pool collectively do not

mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient. The pools of Expanded CB Stem Cell samples that are administered to a patient in need thereof can be derived from the umbilical cord blood and/or placental blood of at least 2 different humans at birth. In one embodiment, administration of a pool of Expanded CB

5   Stem Cell samples of the invention is for the treatment of immunodeficiency. In a preferred embodiment, administration of a pool of Expanded CB Stem Cell samples of the invention is for the treatment of pancytopenia or for the treatment of neutropenia. The immunodeficiency in the patient, for example, pancytopenia or neutropenia, can be the result of an intensive chemotherapy regimen, myeloablative regimen for

10   hematopoietic cell transplantation (HCT), or exposure to acute ionizing radiation. Exemplary chemotherapeutics that can cause prolonged pancytopenia or prolonged neutropenia include, but are not limited to alkylating agents such as cisplatin, carboplatin, and oxaliplatin, mechlorethamine, cyclophosphamide, chlorambucil, and ifosfamide. Other chemotherapeutic agents that can cause prolonged pancytopenia or

15   prolonged neutropenia include azathioprine, mercaptopurine, vinca alkaloids, e.g., vincristine, vinblastine, vinorelbine, vindesine, and taxanes. In particular, a chemotherapy regimen that can cause prolonged pancytopenia or prolonged neutropenia is the administration of clofarabine and Ara-C.

        In one embodiment, the patient is in an acquired or induced aplastic state.

20   The immunodeficiency in the patient also can be caused by exposure to acute ionizing radiation following a nuclear attack, e.g., detonation of a "dirty" bomb in a densely populated area, or by exposure to ionizing radiation due to radiation leakage at a nuclear power plant, or exposure to a source of ionizing radiation, raw uranium ore.

        Transplantation of the pools of Expanded CB Stem Cell samples of the invention

25   can be used in the treatment or prevention of hematopoietic disorders and diseases. In one embodiment, the pools of Expanded CB Stem Cell samples are used to treat or prevent a hematopoietic disorder or disease characterized by a failure or dysfunction of normal blood cell production and cell maturation. In another embodiment, the pools of Expanded CB Stem Cell samples are used to treat or prevent a hematopoietic disorder or

30   disease resulting from a hematopoietic malignancy. In yet another embodiment, the pools of Expanded CB Stem Cell samples are used to treat or prevent a hematopoietic disorder or disease resulting from immunosuppression, particularly immunosuppression in subjects with malignant, solid tumors. In yet another embodiment, the pools of

Expanded CB Stem Cell samples are used to treat or prevent an autoimmune disease affecting the hematopoietic system. In yet another embodiment, the pools of Expanded CB Stem Cell samples are used to treat or prevent a genetic or congenital hematopoietic disorder or disease.

5   Examples of particular hematopoietic diseases and disorders which can be treated by the Expanded CB Stem Cell samples, or pools thereof, of the invention include but are not limited to those listed in Table I, *infra*.

TABLE I

DISEASES OR DISORDERS WHICH CAN BE TREATED BY ADMINISTERING EXPANDED CB STEM CELLS OF THE INVENTION

I.   Diseases Resulting from a Failure or Dysfunction
       of Normal Blood Cell Production and Maturation

hyperproliferative stem cell disorders
aplastic anemia
pancytopenia
agranulocytosis
thrombocytopenia
red cell aplasia
Blackfan-Diamond syndrome due to drugs, radiation, or infection
Idiopathic

II.   Hematopoietic malignancies

acute lymphoblastic (lymphocytic) leukemia
chronic lymphocytic leukemia
acute myelogenous leukemia
chronic myelogenous leukemia
acute malignant myelosclerosis
multiple myeloma
polycythemia vera
agnogenic myelometaplasia
Waldenstrom's macroglobulinemia
Hodgkin's lymphoma
non-Hodgkin's lymphoma

III.   Immunosuppression in patients with malignant, solid tumors

malignant melanoma
carcinoma of the stomach
ovarian carcinoma
breast carcinoma
small cell lung carcinoma

DISEASES OR DISORDERS WHICH CAN BE TREATED BY ADMINISTERING EXPANDED CB STEM CELLS OF THE INVENTION

retinoblastoma
testicular carcinoma
glioblastoma
rhabdomyosarcoma
neuroblastoma
Ewing's sarcoma
lymphoma

IV   Autoimmune diseases

rheumatoid arthritis
diabetes type I
chronic hepatitis
multiple sclerosis
systemic lupus erythematosus

V.   Genetic (congenital) disorders

anemias
familial aplastic
Fanconi's syndrome
Bloom's syndrome
pure red cell aplasia (PRCA)
dyskeratosis congenital
Blackfan-Diamond syndrome
congenital dyserythropoietic syndromes I-IV
Chwachmann-Diamond syndrome
dihydrofolate reductase deficiencies
formamino transferase deficiency
Lesch-Nyhan syndrome
congenital spherocytosis
congenital elliptocytosis
congenital stomatocytosis
congenital Rh null disease
paroxysmal nocturnal hemoglobinuria
G6PD (glucose-6-phosphate dehydrogenase) variants 1, 2, 3
pyruvate kinase deficiency
congenital erythropoietin sensitivity deficiency
sickle cell disease and trait
thalassemia alpha, beta, gamma
met-hemoglobinemia
congenital disorders of immunity
severe combined immunodeficiency disease (SCID)
bare lymphocyte syndrome
ionophore-responsive combined immunodeficiency
combined immunodeficiency with a capping abnormality
nucleoside phosphorylase deficiency

DISEASES OR DISORDERS WHICH CAN BE TREATED BY ADMINISTERING EXPANDED CB STEM CELLS OF THE INVENTION
granulocyte actin deficiency
infantile agranulocytosis
Gaucher's disease
adenosine deaminase deficiency
Kostmann's syndrome
reticular dysgenesis
congenital leukocyte dysfunction syndromes

VI. Others

osteopetrosis
myelosclerosis
acquired hemolytic anemias
acquired immunodeficiencies
infectious disorders causing primary or secondary
immunodeficiencies
bacterial infections (*e.g.*, Brucellosis, Listerosis, tuberculosis, leprosy)
parasitic infections (*e.g.*, malaria, Leishmaniasis)
fungal infections
disorders involving disproportions in lymphoid cell sets and impaired immune functions
due to aging
phagocyte disorders
Kostmann's agranulocytosis
chronic granulomatous disease
Chediak-Higachi syndrome
neutrophil actin deficiency
neutrophil membrane GP-180 deficiency
metabolic storage diseases
mucopolysaccharidoses
mucolipidoses
miscellaneous disorders involving immune mechanisms
Wiskott-Aldrich Syndrome
A1-antitrypsin deficiency

In one embodiment, the Expanded CB Stem Cells, or pools thereof, are administered to a patient with a hematopoietic deficiency. Hematopoietic deficiencies whose treatment with the Expanded CB Stem Cells of the invention is encompassed by the methods of the invention include but are not limited to decreased levels of either myeloid, erythroid, lymphoid, or megakaryocyte cells of the hematopoietic system or combinations thereof, including those listed in Table I.

Among conditions susceptible to treatment with the Expanded CB Stem Cells, or pools thereof, of the present invention is leukopenia, a reduction in the number of circulating leukocytes (white cells) in the peripheral blood. Leukopenia may be induced

by exposure to certain viruses or to radiation. It is often a side effect of various forms of cancer therapy, *e.g.*, exposure to chemotherapeutic drugs, radiation and of infection or hemorrhage.

5   Expanded CB Stem Cells, or pools thereof, also can be used in the treatment or prevention of neutropenia and, for example, in the treatment of such conditions as aplastic anemia, cyclic neutropenia, idiopathic neutropenia, Chediak-Higashi syndrome, systemic lupus erythematosus (SLE), leukemia, myelodysplastic syndrome, myelofibrosis, thrombocytopenia. Severe thrombocytopenia may result from genetic defects such as Fanconi's Anemia, Wiscott-Aldrich, or May-Hegglin syndromes and

10   from chemotherapy and/or radiation therapy or cancer. Acquired thrombocytopenia may result from auto- or allo-antibodies as in Immune Thrombocytopenia Purpura, Systemic Lupus Erythromatosis, hemolytic anemia, or fetal maternal incompatibility. In addition, splenomegaly, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, infection or prosthetic heart valves may result in thrombocytopenia.

15   Thrombocytopenia may also result from marrow invasion by carcinoma, lymphoma, leukemia or fibrosis.

Many drugs may cause bone marrow suppression or hematopoietic deficiencies. Examples of such drugs are AZT, DDI, alkylating agents and anti-metabolites used in chemotherapy, antibiotics such as chloramphenicol, penicillin, gancyclovir, daunomycin

20   and sulfa drugs, phenothiazones, tranquilizers such as meprobamate, analgesics such as aminopyrine and dipyrone, anticonvulsants such as phenytoin or carbamazepine, antithyroids such as propylthiouracil and methimazole and diuretics. Transplantation of the Expanded CB Stem Cells, or pools thereof, can be used in preventing or treating the bone marrow suppression or hematopoietic deficiencies which often occur in subjects

25   treated with these drugs.

Hematopoietic deficiencies may also occur as a result of viral, microbial or parasitic infections and as a result of treatment for renal disease or renal failure, *e.g.*, dialysis. Transplantation of Expanded CB Stem Cell samples, or pools thereof, may be useful in treating such hematopoietic deficiency.

30   Various immunodeficiencies, *e.g.*, in T and/or B lymphocytes, or immune disorders, *e.g.*, rheumatoid arthritis, may also be beneficially affected by treatment with the Expanded CB Stem Cells, or pools thereof. Immunodeficiencies may be the result of

viral infections (including but not limited to HIVI, HIVII, HTLVI, HTLVII, HTLVIII),
severe exposure to radiation, cancer therapy or the result of other medical treatment.

6.10   BANKS OF FROZEN, EXPANDED CORD BLOOD STEM CELLS

5   Since according to the present invention, only limited matching of HLA-type is
necessary for therapeutic use of the Expanded CB Stem Cells, it is now practical to store
frozen Expanded CB Stem Cells since the present invention teaches that useful amounts
can practically be stored. In the prior art, since it was expected that HLA matching to
the recipient would generally be necessary to find a useful sample of Expanded CB Stem

10   Cells for therapeutic use, an unattainably large number of different Expanded CB Stem
Cell samples had to be stored to make it feasible generally to find a match for a patient,
the large numbers making it impractical to store expanded samples, due to the even
larger amount of storage space needed to store expanded units. In contrast, and in
accordance with the present invention, limited HLA matching is required, and thus, the

15   generation of a "bank" of CB Stem Cell samples which have been pooled, expanded and
then cryopreserved, or expanded, pooled and then cryopreserved, useful for the general
human population to use in stem cell transplantation, is feasible, since any pool of
Expanded CB Stem Cell samples in the bank could feasibly be used with many
recipients in a therapeutic method of the invention. It is noted that the pool of CB Stem

20   Cell samples or pool of Expanded CB Stem Cell samples collectively do not mismatch
at more than 2 HLA antigens or alleles typed in the samples.

          Once the Expanded CB Stem Cells are obtained and cryopreserved, the
cryopreserved samples, or pool of samples, can be stored in a bank (a repository for the
collection of samples). The bank can consist of one or more physical locations. Thus,

25 .   the present invention is also directed to a collection of frozen Expanded CB Stem Cell
samples or pools of samples in a bank. The collection can comprise at least 50, 100,
200, 250, 300, 400, 500, 600, 700, 750, 800, 1,000, 2,000, 3,000, 5,000, 7,500, 10,000,
25,000, 50,000 or 100,000 samples of Expanded CB Stem Cells and/or pools of such
samples as described above (which do not collectively mismatch at more than 2 of the

30   HLA antigens or alleles typed in the samples), each sample derived from the umbilical
cord blood and/or placental cord blood of a human at birth. In a specific embodiment,
the bank comprises frozen mixtures of two or more different Expanded CB Stem Cell

samples, each different sample derived from the umbilical cord blood and/or placental
cord blood of a different human at birth, *e.g.*, pooled as described above. The Expanded
CB Stem Cell samples are stored at a temperature no warmer than -20° C, preferably at -
80° C. In a preferred embodiment, samples can be cryogenically stored in liquid
5   nitrogen (-196° C) or its vapor (-165° C).

        In certain embodiments, individual samples of Expanded CB Stem Cells can be
mixed prior to cryopreservation.

        In a preferred embodiment, all or most of the samples of Expanded CB Stem
Cells, or all or most of the pooled samples thereof, present in the bank have greater than
10   75 million viable CD34$^+$ cells, as determined prior to cryopreservation.

6.11   APPARATUS, COMPUTER AND COMPUTER PROGRAM PRODUCT IMPLEMENTATIONS

        The selection of an appropriate pool of frozen Expanded CB Stem Cell samples
15   and/or of such samples to be pooled, for administration to a patient can be implemented
by use of a computer program product that comprises a computer program mechanism
embedded in a computer readable storage medium. Some embodiments of the present
invention provide a computer system or a computer program product that encodes or has
instructions for performing selecting and outputting an identifier and optionally robotic
20   retrieval of a frozen stored Expanded CB Stem Cell sample, or pool of samples. The
identifier distinguishes one frozen Expanded CB Stem Cell sample or frozen pool of
Expanded CB Stem Cell samples from other frozen Expanded CB Stem Cell samples
and/or pools thereof that are stored in a bank of frozen Expanded CB Stem Cell samples
and/or pools thereof, as described above, and thus the identifier is unique to each sample
25   or pool. Preferably, the collection of identifiers is stored in one or more computer
databases, wherein each identifier is preferably associated with information on the
physical location of the Expanded CB Stem Cell sample or pool of Expanded CB Stem
Cell samples, as the case may be, associated with the identifier, and/or with information
on one or more other characteristics of the pool or sample, including but not limited to,
30   total hematopoietic stem cell or hematopoietic stem and progenitor cell count (*e.g.*, total
CD34$^+$ cell count) of the pool or sample, total nucleated cell count of the pool or sample,
percentage hematopoietic stem cell or hematopoietic stem and progenitor cells (*e.g.*,
percentage of CD34$^+$ cells), volume of the pool or sample, sex of the donor, date of

freezing of the pool or sample, HLA type of the pool or sample, as described in Section
6.6. Thus, one or more databases store data on each frozen Expanded CB Stem Cell
sample or pool of samples. The database stores one or more of the following
characteristics of the stored, frozen Expanded CB Stem Cell sample or pool of samples,
5   including but not limited to, total $CD34^+$ cell count of the pool or sample, total nucleated
cell count of the pool or sample, volume of the pool or sample, sex of the donor, race or
ethnicity of the pool or sample, date of freezing of the pool or sample, HLA type of the
pool or sample.

Executable instructions for carrying out the selecting and outputting of
10   identifiers, and/or robotic retrieval of the sample can be stored on a CD-ROM, DVD,
magnetic disk storage product, or any other computer readable data or program storage
product. Such methods can also be embedded in permanent storage, such as ROM, one
or more programmable chips, or one or more application specific integrated circuits
(ASICs). Such permanent storage can be localized in a server, 802.11 access point,
15   802.11 wireless bridge/station, repeater, router, mobile phone, or other electronic
devices. Such methods encoded in the computer program product can also be
distributed electronically, via the Internet or otherwise, by transmission of a computer
data signal (in which the software modules are embedded) either digitally or on a carrier
wave.

20   Some embodiments of the present invention provide a computer program product
that contains any or all of the program modules shown in Fig. 1. These program
modules can be stored on a CD-ROM, DVD, magnetic disk storage product, or any other
computer readable data or program storage product. The program modules can also be
embedded in permanent storage, such as ROM, one or more programmable chips, or one
25   or more application specific integrated circuits (ASICs). Such permanent storage can be
localized in a server, 802.11 access point, 802.11 wireless bridge/station, repeater,
router, mobile phone, or other electronic devices. The software modules in the computer
program product can also be distributed electronically, via the Internet or otherwise, by
transmission of a computer data signal (in which the software modules are embedded)
30   either digitally or on a carrier wave.

Figure 1 illustrates a system 11 that is operated in accordance with one
embodiment of the present invention. System 11 comprises at least one computer 10.
Computer 10 comprises standard components including a central processing unit 22,

memory 36, non-volatile storage 14 accessed via controller 12 for storage of programs

and data, user input/output device 32, a network interface 20 for coupling computer 10

to other computers via a communication network (*e.g.*, wide area network 34), power

source 24, and one or more busses 30 that interconnect these components. User

5   input/output device 32 comprises one or more user input/output components such as a

mouse, display 26, and keyboard 28.

Memory 36 comprises a number of modules and data structures that are used in

accordance with the present invention. It will be appreciated that, at any one time during

operation of the system, a portion of the modules and/or data structures stored in

10   memory 36 can be stored in random access memory while another portion of the

modules and/or data structures can be stored in non-volatile storage 14. In a typical

embodiment, memory 36 comprises an operating system 40. Operating system 40

comprises procedures for handling various basic system services and for performing

hardware dependent tasks. Memory 36 further comprises a file system 42 for file

15   management. In some embodiments, file system 42 is a component of operating system

40.

Memory 36 further discloses a number of modules including a selecting module

70 for selecting an identifier from a plurality of (preferably of at least 50, 100, 200, 250,

500, 750, 1000, 1500, 2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000)

20   identifiers stored in a computer database, each identifier identifying a frozen, stored

Expanded CB Stem Cell sample derived from the umbilical cord blood and/or placental

blood of a different individual at birth, or pool of such samples, an outputting or

displaying module 72 for outputting or displaying the identifier and optionally

associated information to a user, an internal or external component of a computer, a

25   remote computer, or to storage on a computer readable medium, and an optional

retrieval module 74 for robotically retrieving the identified frozen Expanded CB Stem

Cell sample, or pool of Expanded CB Stem Cell samples. The selection module can

carry out computer-implemented selecting as described in Section 6.6 above. It will be

appreciated that one or more of these modules can be run on computer 10 or any other

30   computer that is addressable by computer 10. Thus, the present invention encompasses

systems 11 that have more than one computer, with each such computer optionally

storing some or all of the Expanded CB Stem Cell sample or pool of Expanded CB Stem

Cell samples data 44 and performing any or all of the methods disclosed herein. In some embodiments, system 11 is a cluster of computers.

In one embodiment of the invention, a computer-implemented method for selecting a frozen expanded human cord blood stem cell sample for use in providing

5   hematopoietic function to an immunodeficient human patient is provided, which method comprises the following steps performed by a suitably programmed computer: (a) selecting an identifier from a plurality of at least 50, 100, 200, 250, 300, 400, 500, 600, 700, 750, 800, 1,000, 2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000 identifiers stored in a computer database, each identifier identifying an expanded human

10   cord blood stem cell sample derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the sample does not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein the selecting is for administration of the expanded human cord blood stem cell sample, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and (b)

15   outputting or displaying the selected identifier. In another embodiment of the invention, the computer-implemented method comprises the following steps performed by a suitably programmed computer: (a) selecting an identifier from a plurality of at least 50, 100, 200, 250, 300, 400, 500, 600, 700, 750, 800, 1,000, 2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000 identifiers stored in a computer database, each

20   identifier identifying a frozen stored pool of expanded human cord blood stem cell samples, wherein each pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA

25   antigens or alleles typed in the patient, wherein the selecting is to identify a pool of expanded human cord blood stem cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and (b) outputting or displaying the selected identifier. In particular embodiments, the identifier is outputted or displayed to a user, an internal or external component of a computer, a

30   remote computer, or to storage on a computer readable medium. The outputting or displaying can also output or display information on the physical location of the expanded human cord blood stem cell sample, or pool of samples identified by the identifier. In a specific embodiment, the method further comprises implementing

robotic retrieval of the identified frozen, expanded human cord blood stem cell sample or pool of samples.

In a specific embodiment where the selecting is from already pooled samples, the selecting further comprises rejecting pools of samples that do not contain at least 75

5   million $CD34^+$ cells. In another embodiment, the selecting further comprises rejecting pools of samples that contain more than 500,000 $CD3^+$ cells per kilogram patient weight. In yet another embodiment, the selecting further comprises accepting pools of samples containing samples having 0, 1, or 2 HLA antigen or allele collective mismatches between the patient and the pool of samples of the HLA antigens or alleles typed in the

10   patient. In another embodiment, the selecting further comprises accepting pools of samples containing samples having 1 or 2 HLA antigen or allele collective mismatches between the patient and the pool of samples of the HLA antigens or alleles typed in the patient. In another embodiment, the selecting can be as described in Section 6.6 above.

In a specific embodiment where the selecting is from stored, individual (non-

15   pooled) samples to be pooled prior to administration, the method comprises sequentially considering samples to be selected to be pooled until the pool reaches the earlier of (a) greater than 2 collective HLA antigen or allele mismatches in the pool relative to the HLA antigens and alleles typed in the patient to whom the pool will be administered; and (b) the preselected maximum number of individual samples that will be used to form

20   the pool. Thus, before the maximum number of individual samples have been accepted to form the pool, the method comprises the step of considering whether a sample, if added to the pool, would give the pool greater than 2 collective mismatches relative to the HLA antigens and alleles typed in the patient. If adding that sample to the pool would give the pool greater than 2 collective mismatches, that sample is rejected and a

25   next sample is considered.

In another embodiment of the invention, a computer program product is provided for use in conjunction with a computer system, which computer program product comprises a computer readable storage medium and a computer program mechanism embedded therein, the computer program comprising (a) executable instructions for

30   selecting an identifier from a plurality of at least 50, 100, 200, 250, 300, 400, 500, 600, 700, 750, 800, 1,000, 2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000 identifiers stored in a computer database, each identifier identifying a frozen, stored expanded human cord blood stem cell sample derived from the umbilical cord blood

and/or placental blood of a different human at birth, wherein the sample does not

mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient,

wherein the selecting is for administration of the expanded human cord blood stem cell

sample, or an aliquot thereof, identified by said identifier to a human patient in need

5   thereof; and (b) executable instructions for outputting or displaying the selected

identifier. In another embodiment of the invention, the computer program comprises (a)

executable instructions for selecting an identifier from a plurality of at least 50, 100,

200, 250, 300, 400, 500, 600, 700, 750, 800, 1,000, 2,000, 3,000, 5,000, 7,500, 10,000,

25,000, 50,000 or 100,000 identifiers stored in a computer database, each identifier

10   identifying a frozen, stored pool of expanded human cord blood stem cell samples,

wherein each pool comprises two or more different expanded human cod blood stem cell

samples, each different sample in the pool being derived from the umbilical cord blood

and/or placental blood of a different human at birth, wherein the samples in the pool

collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles

15   typed in the patient, wherein the selecting is to identify a pool of expanded human cord

blood stem cell samples for administration of the pool, or an aliquot thereof, identified

by said identifier to a human patient in need thereof; and (b) executable instructions for

outputting or displaying the selected identifier. In particular embodiments, the identifier

is outputted or displayed to a user, an internal or external component of a computer, a

20   remote computer, or to storage on a computer readable medium. In a specific

embodiment, the computer program product further comprises executable instructions

for implementing robotic retrieval of the identified frozen expanded human cord blood

stem cell sample, or pool of samples.

In a specific embodiment where the selecting is from already pooled samples, the

25   selecting further comprises rejecting pools of samples that do not contain at least 75

million CD34$^+$ cells. In another embodiment, the selecting further comprises rejecting

pools of samples that contain more than 500,000 CD3$^+$ cells per kilogram patient weight.

In yet another embodiment, the selecting further comprises accepting pools of samples

containing samples having 0, 1, or 2 HLA antigen or allele collective mismatches

30   between the patient and the pool of samples of the HLA antigens or alleles typed in the

patient. In another embodiment, the selecting further comprises accepting pools of

samples containing samples having 1 or 2 HLA antigen or allele mismatches collective

between the patient and the pool of samples of the HLA antigens or alleles typed in the patient. In another embodiment, the selecting can be as described in Section 6.6 above.

In a specific embodiment where the selecting is from stored, individual (non-pooled) samples to be pooled prior to administration, the executable instructions for

5   selecting an identifier include instructions for sequentially considering samples to be selected to be pooled until the pool reaches the earlier of (a) greater than 2 collective HLA antigen or allele mismatches in the pool relative to the HLA antigens and alleles typed in the patient to whom the pool will be administered; and (b) the preselected maximum number of individual samples that will be used to form the pool. Thus, before

10   the maximum number of individual samples have been accepted to form the pool, the instructions include the step of considering whether a sample, if added to the pool, would give the pool greater than 2 collective mismatches relative to the HLA antigens and alleles typed in the patient. If adding that sample to the pool would give the pool greater than 2 collective mismatches, that sample is rejected and a next sample is

15   considered.

In yet another embodiment, the present invention provides an apparatus comprising a processor; a memory, coupled to the processor, the memory storing a module, the module comprising (a) executable instructions for selecting an identifier from a plurality of at least 50, 100, 200, 250, 300, 400, 500, 600, 700, 750, 800, 1,000,

20   2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000 identifiers stored in a computer database, each identifier identifying a frozen, stored expanded human cord blood stem cell sample derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the sample does not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein the selecting is for

25   administration of the expanded human cord blood stem cell sample, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and (b) executable instructions for outputting or displaying the selected identifier. In another embodiment, the apparatus comprises a processor; a memory, coupled to the processor, the memory storing a module, the module comprising (a) executable instructions for

30   selecting an identifier from a plurality of at least 50, 100, 200, 250, 300, 400, 500, 600, 700, 750, 800, 1,000, 2,000, 3,000, 5,000, 7,500, 10,000, 25,000, 50,000 or 100,000 identifiers stored in a computer database, each identifier identifying a frozen, stored pool of expanded human cord blood stem cell samples, wherein each pool comprises two or

more different expanded human cod blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein

5   the selecting is to identify a pool of expanded human cord blood stem cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and (b) executable instructions for outputting or displaying the selected identifier. In particular embodiments, the identifier is outputted or displayed to a user, an internal or external component of a computer, a remote computer, or to

10   storage on a computer readable medium. In a specific embodiment, the apparatus further comprises executable instructions for implementing robotic retrieval of the identified frozen, expanded human cord blood stem cell samples or pool of samples.

In a specific embodiment where the selecting is from already pooled samples, the selecting further comprises rejecting pools of samples that do not contain at least 75

15   million $CD34^+$ cells. In another embodiment, the selecting further comprises rejecting pools of samples that contain more than 500,000 $CD3^+$ cells per kilogram patient weight. In yet another embodiment, the selecting further comprises accepting pools of samples containing samples having 0, 1, or 2 HLA antigen or allele collective mismatches between the patient and the pool of samples of the HLA antigens or alleles typed in the

20   patient. In another embodiment, the selecting further comprises accepting pools of samples containing samples having 1 or 2 HLA antigen or allele collective mismatches between the patient and the pool of samples of the HLA antigens or alleles typed in the patient. In another embodiment, the selecting can be as described in Section 6.6 above.

In a specific embodiment where the selecting is from stored, individual (non-

25   pooled) samples to be pooled prior to administration, the executable instructions for selecting an identifier include instructions for sequentially considering samples to be selected to be pooled until the pool reaches the earlier of (a) greater than 2 collective HLA antigen or allele mismatches in the pool relative to the HLA antigens and alleles typed in the patient to whom the pool will be administered; and (b) the preselected

30   maximum number of individual samples that will be used to form the pool. Thus, before the maximum number of individual samples have been accepted to form the pool, the instructions include the step of considering whether a sample, if added to the pool, would give the pool greater than 2 collective mismatches relative to the HLA antigens

and alleles typed in the patient. If adding that sample to the pool would give the pool greater than 2 collective mismatches, that sample is rejected and a next sample is considered.

5   Alternative embodiments for implementing the methods and producing the Stem Cell and Expanded CB Stem Cell populations or pools thereof of the present invention will be apparent to one of skill in the art and are intended to be comprehended within the accompanying claims. The experimental examples in Sections 7-10, *infra*, are offered by way of illustration and not by way of limitation.

10   **7.   ALTERNATIVE EMBODIMENTS OF THE INVENTION**

In alternative embodiments of the invention applicable to all aspects of the invention described herein, the pool of 2 or more different expanded human cord blood stem cell samples, instead of being characterized in that "the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles

15   typed in the patient" is characterized such that at least 1 sample in the pool matches the patient at 3, 4, 5 or 6 of the 6 HLA antigens or alleles in the patient and in the sample; in such alternative embodiments, the other samples in the pool, if any, are administered without matching, or without regard to matching, the HLA antigens or alleles in the patient.

20

8.   EXAMPLE: ENRICHMENT AND EXPANSION OF CD34$^+$ CELLS

This data presented herein supports the usefulness of CD34$^+$ cord blood stem cells which have been expanded *ex vivo* with an agonist of Notch function as an off-the-shelf, non-HLA matched product to provide rapid but transient myeloid engraftment and

25   to potentially facilitate autologous hematopoietic recovery in immunodeficient patients. In the prior art, it was not feasible to perform *ex vivo* expansion in advance as the need for HLA-matching required an unattainable number of pre-expanded units in order for an individual patient to find a suitably matched unit. In contrast, where no, or limited, HLA-matching, is required for the expanded stem cell product, generation of a "bank" of

30   pre-expanded and then cryopreserved cells is possible, and the products would be available for immediate use.

8.1   CD34$^+$/CD38$^-$ versus CD34$^+$

Growth characteristics and generation of SCID repopulating cells (SRC) starting from CD34$^+$ or CD34$^+$CD38$^-$ human cord blood progenitor cell populations were compared. Enriched CD34$^+$CD38$^-$ cord blood progenitors cells were used as the starting

5   cell population for Notch-mediated expansion as described in Delaney *et al.*, 2005, Blood 106:1784-1789. Cells were cultured for 17-21 days in the presence of fibronectin fragments and immobilized engineered Notch ligand (Delta1$^{ext-IgG}$) or control human IgG in serum free conditions supplemented with cytokines (SCF 300ng/ml, Flt3L 300 ng/ml, TPO 100ng/ml, IL-6 100ng/ml, and IL-3 10ng/ml, denoted as "5GF"). Delta1^{ext-}

10   ^{IgG} consists of the extracellular domain of Delta1 fused to the Fc domain of human IgG1. No significant difference was observed in absolute numbers of CD34$^+$ cells generated, with a CD34$^+$ cell fold expansion of 138 ±64 and 163±64, (mean±sem, p=0.1612, data not shown) for the CD34$^+$ versus the CD34$^+$CD38$^-$ starting cell population, respectively. Assessment of *in vivo* NOD/SCID repopulating ability at 3, 6 and 10 weeks post

15   infusion did, however, reveal enhanced human engraftment in the marrow of recipient mice when a CD34$^+$ starting cell population was used as compared to a CD34$^+$CD38$^-$ starting cell population (mean CD45% in CD34$^+$ versus CD34$^+$CD38$^-$ starting cell populations cultured in the presence of Delta1$^{ext-IgG}$: 3 weeks; 6.7% versus 1.6%, p=0.02 and 10 weeks: 1.0% vs 0.2%, p=0.1). It was further determined that the 5GF

20   combination was superior to use of combinations utilizing fewer cytokines with respect to both *in vitro* generation of CD34$^+$ cells and SRC frequency as determined by limiting dilution analysis (data not shown).

8.2   Culture of cord blood progenitor cells with Delta1
25   **results in increased SCID repopulating cell (SRC) frequency**

Having identified the optimal conditions for Notch-mediated generation of UCB repopulating cells, 5 independent experiments were carried out to test this closed system for generation of stem/progenitor cells. Human CD34$^+$ cord blood cells were cultured for 17 days with Delta1 immobilized to the surface of the tissue culture vessel together

30   with CH-296 fibronectin fragments in the presence of cytokines (IL-3 at 10 ng/ml, IL-6 and TPO at 100 ng/ml, SCF and Flt3 ligand at 300 ng/ml) and low density lipoproteins (LDL at 20 ng/ml) in serum free medium. The number of repopulating cells generated was determined using quantitative limit dilution assays in which groups of 8 to 15 mice

received 1.5×10^5, 3×10^4, or 6×10^3 non-cultured cells or the cultured progeny of 3×10^4,

6×10^3 or 1.2×10^3 cells. Of note, mice that received non-cultured cells also received

2×10^5 irradiated CD34⁻ cells as accessory supporting cells to facilitate engraftment.

Such accessory cells have not been required for cultured cells as their function is

5   provided by differentiated myeloid cells in the culture. The frequency of repopulating

cells in the starting cell population, determined using Poisson analysis with L-Calc™

software, demonstrated a 15.8 fold increase in SRC frequency in Delta1-cultured cells

compared to non-cultured cells at 3 weeks (p=0.0001) post infusion of cells and a 6.3

fold increase in SRC frequency at 9 weeks (p=0.0001) (Figure 2a), thus indicating a

10   significant increase in repopulating ability after culture with Delta1.

In addition, the fold expansion of the human $CD34^+$ cells and the *in vivo* level of

human engraftment including lineage assessment (lymphoid versus myeloid) of the

human cells present was determined. In these 5 experiments, there was a mean fold

expansion of $CD34^+$ cells of 230 ± 53 (mean ± sem) for the Delta1 cultured cells versus

15   65 ± 31 (mean ± sem) for the control cultured cells (p=0.03) (data not shown) and

demonstrated significantly higher engraftment of human $CD45^+$ cells, as well as $CD33^+$

myeloid and $CD19^+$ B cells from the Delta1-cultured cells (Figure 2b). Although cells

cultured with Delta1 led to increased overall hematopoietic reconstitution at 9 versus 3

weeks, this was due primarily to an increase in engrafted lymphoid cells, presumably

20   due to expansion of mature cells, whereas myeloid engraftment decreased suggesting at

least a portion of engrafting cells were short term in nature.

8.3   Early engrafting potential of Delta1-cultured UCB progenitors

In three independent experiments, in which the engraftment of $\text{Delta1}^{\text{ext-IgG}}$-

25   cultured human umbilical cord blood stem and progenitor cells produced as described in

Section 8.2, *supra*, was compared to engraftment of non-$\text{Delta1}^{\text{ext-IgG}}$-cultured stem and

progenitor cells, there was no measurable contribution to engraftment 10 days post

transplant in mice receiving non-cultured cord blood cells, whereas the mice that

received Delta1-cultured cells all engrafted at a level of >0.5% human engraftment

30   consisting of >95% myeloid cells as measured by co-expression of the human antigens,

CD33/CD45 (Figure 3). Taken together, the above data suggests that culture of cord

blood progenitors with Delta1 dramatically enhances the *in vitro* generation and

frequency of NOD/SCID repopulating cells resulting in improvement in the kinetics and level of human engraftment in a NOD/SCID mouse model.

8.4   *In vivo* repopulating ability is retained following cryopreservation of the expanded cell product

Using an immunodeficient mouse model, the ability of *ex vivo* expanded cryopreserved progenitor cells to engraft was evaluated. The cells were expanded according to the method set forth in Section 8.2, *infra*. Initial experiments compared *in vivo* repopulating ability of human expanded cells that were directly infused into immunodeficient mice upon harvest versus those that were harvested post expansion and cryopreserved for future use. There were no significant differences observed in the *in vivo* repopulating ability of cells that were cultured, cryopreserved (in standard media used for hematopoietic cell cryopreservation containing DMSO), and then thawed prior to transplant when compared to expanded progenitor cells that were harvested at the end of culture and freshly infused (Figure 4a). Additional experiments have confirmed that repopulating ability of the expanded cell product is retained following cryopreservation. As shown in Figure 4b, all mice that received human expanded cryopreserved cells engrafted (defined >0.5% human CD45 in the marrow) with an average overall human engraftment of 8% at 2 weeks post infusion and 7% at 4 weeks post infusion. Lastly, various thawing methodologies were compared (thaw and wash, thaw and dilute with dextran/HSA and thaw and directly infuse) and have also not seen a significant difference in the three methods evaluated (Figure 4c).

8.5   Murine hematopoietic progenitor cells cultured with Delta1$^{\text{ext-IgG}}$ provide short-term engraftment in H-2 mismatched recipients and facilitates autologous recovery following radiation exposure

The studies described below with a murine model show that expanded numbers of progenitor cells derived from murine hematopoietic progenitors (LSK cells) are capable of providing short term engraftment when transplanted in an H-2 mismatched recipient. LSK cells from C57BL/6.J Ly5.1 (CD45.1) mice were cultured as previously described for four weeks on Delta1$^{\text{ext-IgG}}$ (Dallas *et al.*, 2007, Blood 109:3579-3587). For the congenic transplant, lethally irradiated C57BL/6 (H-2b, CD45.1) mice received 10^6 Delta1$^{\text{ext-IgG}}$-cultured Ly5.2 (H-2b, CD45.2) primary LSK cells along with 10^5

C57BL/6 (H-2b, CD45.1) syngeneic whole bone marrow. For the allogeneic transplant, lethally irradiated BALB.c (H-2d, CD45.1) recipients received 10^6 Ly5.2 (H-2b, CD45.2) Delta1$^{ext-IgG}$-cultured LSK cells along with 10^5 BALB.c (H-2d, CD45.1) syngeneic whole bone marrow. Peripheral blood from mice were analyzed by FACS

5   analysis at various times after transplantation to evaluate for donor chimerism (Figure 5). The data show that the Delta cultured cells are able to provide short-term donor engraftment in transplant with major H-2 mismatch.

Furthermore, the data indicate that Delta1$^{ext-IgG}$-cultured cells have enhanced hematopoietic engraftment early after irradiation compared to LSK bone marrow cells

10   (which are depleted of T cells potentially able to cause graft-versus-host disease) in a competitive repopulation assay. This data demonstrates higher levels of early bone marrow repopulation following infusion of cells cultured with Delta1$^{ext-IgG}$, compared to non-cultured precursors. The marrow of mice receiving allogeneic cells following culture with Delta1$^{ext-IgG}$ contained a significantly greater number of the allogeneic

15   donor cells than mice that received non-cultured allogeneic donor LSK cells. Furthermore, assessment of engraftment derived from syngeneic cells, provided to ensure survival of the recipient mice, demonstrated facilitation of engraftment of the syngeneic cells when co-transplanted with Delta1$^{ext-IgG}$-cultured allogeneic cells. The number of cells derived from the host was higher in recipients of the Delta1$^{ext-IgG}$-

20   cultured cells compared to non-cultured allogeneic cells (Figure 6). Thus, this data indicates that engraftment by cultured cells can occur in mismatched settings, and moreover the Delta1$^{ext-IgG}$-cultured cells can facilitate engraftment of syngeneic cells, further suggesting their potential for facilitating recovery of autologous residual stem cells remaining after radiation.

25   In separate experiments, murine Ly5a Lin-Sca-1 c-kit+ cells (H-2b, CD45.1) ("LSK") cells obtained from the bone marrow of C57 black mice by flow cytometric sorting (10^3) were expanded by culturing the cells with growth medium and immobilized Delta1$^{ext-IgG}$ (expanded LSK cells). Control (unexpanded) LSK cells were cultured with IgG. The growth medium (Iscoves modified Dulbecco medium) was supplemented with

30   20% FBS and 4 growth factors (4GF): murine stem cell factor, human Flt-3 ligand, and human IL-6, each at 100 ng/mL, and human IL-11 at 10 ng/mL (PeproTech, Rocky Hill, NJ). Cell density was maintained at approximately 2.5×10^5 cells/cm^2 by transferring the cultures to larger vessels every 3 to 5 days during the first 2 weeks (see Dallas *et al.*,

2007, Blood 109:3579-3587). After 14 days of culture, the cells were harvested and transplanted into irradiated Balb-c (H-2d, CD45.2) mice. Figure 7 is a schematic drawing of this experimental protocol. Figures 8a-8b depict the level of engraftment of the expanded and non-expanded LSK cells in either bone marrow (Figure 8a) or

5   peripheral blood (Figure 8b) of lethally irradiated Balb-c mice as a result of carrying out the protocol set forth in Figure 7, measured as donor percent (percentage of donor cells in bone marrow or peripheral blood as determined by immunophenotyping and FACS analysis). The results confirmed that effective engraftment was achieved when expanded stem and progenitor cells were infused in a mismatched setting after a single

10   dose of radiation. In a similar experiment, 5×10^6 cryopreserved LSK cells, expanded as described above, were infused into mice exposed to 7.5 Gy or 8 Gy of radiation. Figures 9a-9b show that mice infused with the expanded LSK cells (indicated as "Delta") had a greater survival rate as compared to a control group infused with saline. Similarly, the overall survival of mice lethally irradiated at 8.5 Gy was increased after infusion of

15   either 3×10^6, 5×10^6, or 10×10^6 Delta1$^{ext-IgG}$-cultured (expanded) LSK cells as compared to 1×10^6 or 3×10^6 IgG-cultured (non-expanded) LSK cells (Figure 10). In another experiment, following the protocol set forth in Figure 7, donor engraftment of mismatched expanded LSK cells (DXI) was enhanced with increasing dose of radiation as measured by the percentage of donor cells (donor percent) in bone marrow and

20   peripheral blood, as determined by immunophenotyping and FACS analysis (Figures 11a-11b).

8.6   Preliminary Results of a Phase I Clinical CBT Trial

Direct clinical translation of the above has resulted in an ongoing Phase I cord

25   blood transplantation trial (FHCRC Protocol 2044) using *ex vivo* expanded cord blood progenitor cells following myeloablative conditioning. Results from the current Phase I trial have not only demonstrated safety of this protocol, but more importantly have demonstrated rapid myeloid engraftment derived from *ex vivo* expanded hematopoietic progenitors and consequently, a significant reduction in median time to an absolute

30   neutrophil count of 500/μl to just 14 days. This is a statistically significant (p=0.002) improvement in time to engraftment when compared to a cohort of patients (N=20) with the same treatment regimen at our institution but with two non-manipulated cord blood units who engrafted at a median of 26 days (Figure 12). The two cohorts did not differ

significantly in age, weight, diagnosis or infused cell doses as provided by the non-manipulated units. It has been suggested that an ANC threshold of >100/μl is strongly associated with a survival benefit post allogeneic stem cell transplant (Offner et al., 1996, Blood 88:4058). Among enrolled patients median time to achieve an ANC

5   >100/μl was 9 days versus 19 days in the conventional setting (as above) (p=0.006, data not shown).

In the 11 patients analyzed to date, there has been no failure to ex vivo expand the absolute numbers of CD34$^+$ cells available for infusion. The average fold expansion of CD34$^+$ cells was 163 (±43 SEM, range 41-471) and 590 (±124 SEM, range 146 -

10   1496) for the total cell numbers, correlating with a significantly higher infused CD34 cell dose (CD34$^+$ cells/kg recipient body weight) derived from the expanded cord blood graft averaging 6 x10^6 CD34/kg (range 0.93 to 13 x10^6) versus 0.24 x10^6 CD34/kg (range 0.06 to 0.54 x10^6) from the non-manipulated cord blood graft. It is important to note that the unit subjected to ex vivo expansion is CD34-selected and therefore T cell

15   depleted prior to culture initiation. Additional details of the final harvested product, including viability and additional immunophenotyping, can be found in Table II below. As demonstrated in Table II, no CD3$^+$/CD4$^+$ or CD3$^+$/CD8$^+$ cells were identified. No mature T cells are generated during culture. Also, as discussed below, even in this setting where the expanded cells were at least 4/6 HLA-matched to the recipient, there

20   was no contribution to CD3 engraftment from the expanded unit. CD4$^+$/CD3$^-$/CD8$^-$ cells were observed in culture and consistent with monocytes.

Table II: Selected Immunophenotyping of Expanded Cell Product at Harvest

	Percent (range)	Cells/kg (range)
CD34	14.5 (6.2 - 26)	6.1×10^6 (0.9 - 13.6)
CD7	8.1 (5.9 – 12)	3.9×10^6 (0.3 - 9.1)
CD14	11.3 (1.8 – 23)	5.6×10^6 (0.1 - 14.6)
CD15	20.5 (6 – 36)	9.0×10^6 (1.1 - 23)
CD34$^+$/56$^+$	2.9 (1.4 – 5.8)	1.7×10^6 (0.08 – 5.3)
CD3$^-$/CD16$^+$/56$^+$	5.4 (2.2 – 13.6)	2.7×10^6 (0.1 – 12.4)
CD20	0.1 (0 – 0.2)	3.6×10^4 (0 – 14)
CD3	0.2 (0 – 0.6)	4.8×10^4 (0 – 13)
CD4^{lo}/CD3^{neg}/CD8^{neg}	40.6 (16 – 67)	1.7×10^7 (0.2 – 6.1)
CD8	0.1 (0 – 0.5)	3.4×10^4 (0 – 17)

Contribution to donor engraftment as derived from the expanded or non-manipulated grafts was determined weekly in the first month beginning at day 7 post-

5   transplant on peripheral blood sorted cell fractions. In eight of the nine engrafted patients there were sufficient numbers of peripheral blood sorted myeloid cells for evaluation and in each of these patients revealed a predominance of donor cell engraftment derived from the expanded cell graft in both the CD33 and CD14 cell fractions. Contribution to early myeloid recovery at day 7 was derived almost entirely

10   from the expanded cell graft, but generally did not persist beyond day 14 to 21 post-transplant. Despite this, time to engraftment was decreased significantly, indicating of a potential facilitating effect exerted by the *ex vivo* expanded cells on the non-manipulated unit. In all but one patient, as expected, the non-manipulated donor graft has emerged as the source of sustained donor engraftment.

15   Longer-term in vivo persistence of the expanded cell graft was observed in two patients. In one patient, analysis at day 240 post transplant revealed a portion (10 - 15%) of the donor CD14, CD56 and CD19 cells were derived from the expanded graft but was no longer present by one year. In the second patient at day 180 post transplant, contribution to engraftment from the expanded cell population at day 180 post transplant

20   in CD33, CD14, CD56 and CD19 cells ranged from 25 to 66% of total donor engraftment. However, the expanded graft did not contribute to CD3$^+$ cell engraftment.

9.   EXAMPLE: CLINICAL ENRICHMENT AND EXPANSION

The following section describes the production and storage of an expanded human cord blood stem cell samples, as depicted as a flow chart in Figure 13.

5   The umbilical cord blood/placental blood unit(s) were collected from a single human at birth. The collected blood was then mixed with an anti-coagulant to prevent clotting. The blood was stored under quarantine at 4° C in a monitored refrigerator. The received unit(s) were assessed, and which unit(s) will be processed for expansion was determined. The following information was collected on the units: date received, age in hours of the unit, gestational age of the donor in weeks, sex of the donor, and volume of
10   the unit. Further, total nucleated cell count and total $CD34^+$ cell count of each unit was determined and percent $CD34^+$ cells was calculated. If the unit had less than 3.5 million $CD34^+$ cells, the unit was discarded. When a unit was selected for expansion, it was removed from quarantine and assigned a unique Lot Number identifier, which it retains throughout the manufacturing process.

15   Prior to planned initiation of expansion cultures, tissue culture vessels were first coated overnight at 4° C or a minimum of 2 hours at 37° C with $Delta1^{ext-IgG}$ at 2.5 μg/ml and RetroNectin® (a recombinant human fibronectin fragment) (Clontech Laboratories, Inc., Madison, WI) at 5 μg/ml in phosphate buffered saline (PBS). The flasks were then washed with PBS and then blocked with PBS-2% Human Serum Albumin (HSA). The
20   fresh cord blood unit was processed to select for $CD34^+$ cells using the CliniMACS® Plus Cell Separation System. Prior to CD34 selection, an aliquot of the fresh cord blood unit was checked for total cell count and CD34 content. Both $CD34^+$ and $CD34^-$ cell fractions were recovered after processing. After enrichment, the percentage of $CD34^+$ cells in the sample increased by 88- to 223-fold relative to the percentage of $CD34^+$ cells
25   in the sample prior to enrichment. DNA was extracted from a sample of the $CD34^-$ cell fraction for initial HLA typing. The enriched $CD34^+$ cell fraction was resuspended in final culture media, which consists of STEMSPAN™ Serum Free Expansion Medium (StemCell Technologies, Vancouver, British Columbia) supplemented with rhIL-3 (10 ng/ml), rhIL-6 (50 ng/ml), rhTPO (50 ng/ml), rhFlt-3L (50 ng/ml), rhSCF (50 ng/ml).

30   The $CD34^+$ enriched cells were added to the specifically labeled and prepared tissue culture vessels at a concentration of ≤ 1.8 x 10^4 total nucleated cells/cm2 of vessel surface area, and then placed into individually monitored and alarmed incubators dedicated solely to that lot of product. After 2-4 days of culture, 50% of the original

volume of fresh culture media (as above) was added to the vessels. The cell culture vessels were removed from the incubator periodically (every 1-3 days), and examined by inverted microscope for cell growth and signs of contamination. On day 5-8, the vessel was gently agitated to mix the cells, and a 1 ml sample was removed for in process

5   testing. The sample of cells was counted and phenotyped for expression of CD34, CD7, CD14, CD15 and CD56. Throughout the culture period, cells were transferred to additional flasks as needed when cell density increases to $\geq 8 \times 10^5$ cells/ml. On the day prior to harvesting the cells for cryopreservation, fresh media was added.

On day 14-16, the expanded cell population was harvested for cryopreservation.

10   The vessels were agitated and the entire contents transferred to sterile 500 ml centrifuge tubes. The harvested cells were centrifuged and then washed one time by centrifugation in PBS and resuspended in a cryoprotectant solution containing HSA, Normosol-R (Hospira, ake Forrest, IL) and Dimethylsulfoxide (DMSO). Samples for completion of release testing were taken. The Expanded CB Stem cell population product was frozen

15   in a controlled-rate freezer and transferred to storage in a vapor-phase liquid nitrogen (LN2) freezer.

At the end of the culture period, the resulting cell population was heterogeneous, consisting of CD34$^+$ progenitor cells and more mature myeloid and lymphoid precursors, as evidenced by flow cytometric analysis for the presence of CD34, CD7,

20   CD14, CD15 and CD56 antigens. Typical flow cytometry characterization of the expanded cells at the end of the expansion period is presented in Table III below.

Table III : Expanded Cell Phenotype (N= 9, *N=5)

	Mean Percent (range)
CD34	12.8 (4.9-25)
CD7	9.7 (4.3-21)
CD14	7.7 (3.5-22)
CD15	42 (23-66)
CD34$^+$/56$^+$	1.8 (0.7-3.1)
CD3$^-$/CD16$^+$/56$^+$	3.5 (0-9.5)
CD20$^+$	0.4 (0-1.2)
CD3$^+$4$^+$*	0.7 (0.04-1.4)
CD3$^+$8$^+$*	0
TNC Fold Expansion	1586 (617-3337)
CD34 Fold Expansion	204 (100-387)

There was a significant increase of CD34$^+$ and total cell numbers during the culture period, ranging from 100 to 387 fold expansion of CD34$^+$ cells and 617 to 3337 fold expansion of total cell numbers (N= 9 individual cord blood units, processed per the final clinical expansion procedures as described above). There was essentially a complete lack of T cells as measured by immunophenotyping. Functionally, these cells are capable of multi-lineage human hematopoietic engraftment in a NOD/SCID mouse model as described above.

Data from ten full-scale *ex vivo* expansions are presented in Table IV below. The average fold expansion for total cell numbers was 1723±230 (mean± sem) and for CD34$^+$ cells was 179±30 (mean ± sem).

Table IV: Pre- and Post-expansion absolute cell numbers and fold expansion

Run	TNC (total number cells)			CD34			Number banked (cryopreserved) Cells				
	Starting Number	Ending Number	Fold Expansion	Starting Number	Ending Number	Fold Expansion	TNC	CD34#	# Bags	TNC/Bag	CD34/Bag
1	1.9 x 10^6	2.01 x 10^9	1068	1.66 x 10^6	2.13 x 10^8	129	n/a	n/a	n/a	n/a	n/a
2	1.76 x 10^6	1.20 x 10^9	690	1.41 x 10^6	3.04 x 10^8	216	n/a	n/a	n/a	n/a	n/a
3	2.60 x 10^6	5.47 x 10^9	2104	2.29 x 10^6	2.69 x 10^8	117	4.20 x 10^9	2.06 x 10^8	2	2.10 x 10^9	1.03 x 10^8
4	2.40 x 10^6	4.67 x 10^9	1944	2.04 x 10^6	6.07 x 10^8	298	2.90 x 10^9	3.77 x 10^8	1	2.90 x 10^9	3.77 x 10^8
5	2.17 x 10^6	3.22 x 10^9	1484	1.76 x 10^6	2.71 x 10^8	154	2.12 x 10^9	1.78 x 10^8	1	2.12 x 10^9	1.78 x 10^8
6	1.90 x 10^6	2.59 x 10^9	1364	1.70 x 10^6	1.70 x 10^8	100	2.00 x 10^9	1.32 x 10^8	1	2.00 x 10^9	1.32 x 10^8
7	4.80 x 10^6	1.60 x 10^{10}	337	4.32 x 10^6	1.69 x 10^9	387	1.29 x 10^{10}	1.35 x 10^9	4	3.23 x 10^9	3.38 x 10^8
8	4.86 x 10^6	9.94 x 10^9	2045	4.23 x 10^6	7.28 x 10^8	172	1.02 x 10^{10}	7.47 x 10^8	3	3.40 x 10^9	2.49 x 10^8
9	1.70 x 10^6	2.55 x 10^9	1499	1.39 x 10^6	1.46 x 10^8	105	2.25 x 10^9	1.29 x 10^8	1	2.25 x 10^9	1.29 x 10^8
10	2.06 x 10^6	3.48 x 10^9	1692	1.77 x 10^6	1.92 x 10^8	108	2.75 x 10^9	1.51 x 10^8	1	2.75 x 10^9	1.51 x 10^8
average	2.62 x 10^6	5.11 x 10^9	1723	2.26 x 10^6	4.59 x 10^8	179	4.92 x 10^9	4.09 x 10^8		2.59 x 10^9	2.07 x 10^8

n/a: not available

Table V sets forth the starting, ending and fold expansion numbers for total nucleated cells and CD34⁺ cells post-expansion for 19 full scale *ex vivo* expansions.

TABLE V

Unit ID	CD34			CD34 Purity		TNC		
Product #	Starting #	Ending #	Fold Expansion	Starting %	Ending %	Starting #	Ending #	Fold Expansion
S001	2.29×10^6	2.69×10^8	117	88	4.9	2.60×10^6	5.47×10^9	2104
S002	2.04×10^6	6.07×10^8	298	85	13	2.40×10^6	4.67×10^9	1944
S003	1.76×10^6	2.71×10^8	154	81	8.4	2.17×10^6	3.22×10^9	1484
S004	1.70×10^6	1.70×10^8	100	91	6.6	1.90×10^6	2.59×10^9	1364
S005	4.32×10^6	1.69×10^9	387	90	10.4	4.80×10^6	1.60×10^{10}	3337
S006	4.23×10^6	7.28×10^8	172	87	7.3	4.86×10^6	9.94×10^9	2045
S007	1.39×10^6	1.46×10^8	105	82	5.7	1.70×10^6	2.55×10^9	1499
S008	1.77×10^6	1.92×10^8	108	86	5.5	2.06×10^6	3.48×10^9	1692
S009	2.70×10^6	4.74×10^8	176	88	8.8	3.07×10^6	5.42×10^9	1765
S010	2.02×10^6	7.92×10^8	392	75	11.6	2.69×10^6	6.84×10^9	2543
S011	1.64×10^6	4.25×10^8	259	82	15.2	2.00×10^6	2.79×10^9	1395
S012	1.64×10^6	4.25×10^8	259	82	15.2	2.82×10^6	2.12×10^9	752
S013	1.97×10^6	2.25×10^8	114	70	10.6	2.96×10^6	6.25×10^9	2111
S014	2.28×10^6	6.49×10^8	285	77	10.4	2.60×10^6	2.15×10^9	827
S015	1.74×10^6	1.42×10^8	82	67	6.63	2.50×10^6	2.97×10^9	1187
S016	1.88×10^6	2.80×10^8	149	75	9.4	4.46×10^6	7.65×10^9	1715
S017	3.75×10^6	1.04×10^9	276	84	13.6	6.90×10^6	4.07×10^9	590
S018	$6.28E \times 10^6$	2.91×10^8	46	91	7.14	2.34×10^6	2.18×10^9	932
S019	$1.78E \times 10^6$	2.29×10^8	129	76%	10.52	2.16×10^6	2.00×10^9	926

These 19 expanded human cord blood stem cells were cryopreserved in one or more bags. Table VI sets forth total nucleated cell (TNC) and CD34⁺ cell counts for each of the expanded human cord blood stem cell sample and cell viability prior to cryopreservation, and TNC and CD34⁺ cell counts in each frozen bag.

TABLE VI

Unit ID	Banked Cells					Final Viability Trypan Blue
Product #	TNC	CD34 #	# Bags	TNC/Bag	CD34#/Bag	
S001	4.20×10^9	2.06×10^8	2	2.10×10^9	1.03×10^8	67%
S002	2.90×10^9	3.77×10^8	1	2.90×10^9	3.77×10^8	62%
S003	2.12×10^9	1.78×10^8	1	2.12×10^9	1.78×10^8	69%
S004	2.00×10^9	1.32×10^8	1	2.00×10^9	1.32×10^8	55%
S005	1.29×10^{10}	1.35×10^9	4	3.23×10^9	3.38×10^8	67%
S006	1.02×10^{10}	7.47×10^8	3	3.40×10^9	2.49×10^8	57%
S007	2.25×10^9	1.29×10^8	1	2.25×10^9	1.29×10^8	70%
S008	2.75×10^9	1.51×10^8	1	2.75×10^9	1.51×10^8	79%
S009	6.30×10^9	5.51×10^8	2	3.15×10^9	2.76×10^8	59%
S010	4.93×10^9	5.70×10^8	2	2.47×10^9	2.85×10^8	66%
S011	1.82×10^9	2.77×10^8	1	1.82×10^9	2.77×10^8	57%
S012	1.70×10^9	1.81×10^8	1	1.70×10^9	1.81×10^8	59%
S013	5.14×10^9	5.34×10^8	2	2.57×10^9	2.67×10^8	68%
S014	1.50×10^9	9.91×10^7	1	1.50×10^9	9.91×10^7	68%
S015	1.94×10^9	1.83×10^8	1	1.94×10^9	1.83×10^8	62%
S016	4.08×10^9	5.53×10^8	2	2.04×10^9	2.76×10^8	54%
S017	3.90×10^9	2.78×10^8	1	3.90×10^9	2.78×10^8	65%
S018	1.23×10^9	1.29×10^8	1	1.23×10^9	1.29×10^8	68%
S019	2.19×10^9	2.23×10^8	1	2.19×10^9	2.23×10^8	

Further, an additional 12 samples of enriched CD34$^+$ cells were expanded with Delta1$^{\text{ext-IgG}}$, and showed an average 141-fold expansion (SEM 17) of CD34$^+$ cells, prior to cryopresevation.

10. EXAMPLE: TREATMENT OF PATIENTS WITH AML

10.1: DESIGN

The below discussion describes the design of a clinical trial aimed at providing rapid restoration of hematopoietic function to patients suffering from acute myelogenous leukemia (AML) who have been treated with intensive induction chemotherapy by administering expanded human cord blood stem cells. This study will enroll in three cohorts at 10-15 patients per cohort, each with separate inclusion criteria based on disease status. Cohort A will enroll first for a total of 10 patients. If safety criteria are met, enrollment will occur in cohort B for a total of 15 patients; if safety criteria are again met, enrollment will occur in cohort C for a total of 15 patients.

Cohort A: Diagnosis of acute myeloid leukemia by WHO criteria, either relapsed or refractory. Acute promyelocytic leukemia [Acute promyelocytic leukemia with t(15;17)(q22;q12) and variants] will be eligible only after failure of a regimen containing arsenic trioxide. Patients in this cohort must have had an initial remission duration of < 1 year and can not have received any prior salvage chemotherapy.

Cohort B: Untreated AML patients with cytogenetic or molecular abnormalities associated with poor prognosis.

Cohort C: Untreated AML patients with intermediate prognosis.

In addition to disease criteria established above, all patients must meet inclusion criteria listed below:

1.   The first three patients enrolled in each cohort must be less than 60 years old. Thereafter, patients aged $\geq$ 18 and $\leq$ 70 are eligible.

2.   The first three patients enrolled in each cohort must have an ECOG performance status of 0 -1. Thereafter, ECOG performance status of 0-2 is required.

3.   The patients must have adequate renal and hepatic functions as indicated by the following laboratory values:

a.   Serum creatinine ≤ 1.0 mg/dL; if serum creatinine >1.0 mg/dl, then the estimated glomerular filtration rate (GFR) must be >60 mL/min/1.73 m2 as calculated by the Modification of Diet in Renal Disease equation where predicted GFR (ml/min/1.73 m2)= 186 X (Serum Creatinine)-1.154 X (age in years)-0.023 X (0.742 if patient is female) X (1.212 if patient is black).

b.   Serum total or direct bilirubin ≤ 1.5 x upper limit of normal (ULN), aspartate transaminase (AST)/alanine transaminase (ALT) $\leq 2.5 \times$ ULN, alkaline phosphatase ≤ 2.5 x ULN

4.   Capable of understanding the investigational nature, potential risks and benefits of the study, and able to provide valid informed consent.

5.   Female patients of childbearing potential must have a negative serum pregnancy test within 2 weeks prior to enrollment.

6.   Male and female patients must be willing to use an effective contraceptive method during the study and for a minimum of 6 months after study treatment.

7.   Panel reactive antibody (PRA) negative or with specific antibodies characterized and known to not be donor-directed against cord blood antigens.

The following individuals are excluded from this trial:

1.   Allogeneic transplant recipients.

2.   Current concomitant chemotherapy, radiation therapy, or immunotherapy other than as specified in the protocol.

3.   Have any other severe concurrent disease, or have a history of serious organ dysfunction or disease involving the heart, kidney, liver (including symptomatic hepatitis, veno-occlusive disease), or other organ system dysfunction.

4.   Patients with a systemic fungal, bacterial, viral, or other infection not controlled (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment).

5.   Pregnant or lactating patients.

6.   Any significant concurrent disease, illness, or psychiatric disorder that would compromise patient safety or compliance, interfere with consent, study participation, follow up, or interpretation of study results.

The expanded cord blood stem cells to be used for this trial will be selected from a bank of previously expanded cord blood progenitors that have been cryopreserved for future clinical use. Each individual progenitor cell product is derived from a single cord blood unit (donor) that is CD34 selected (and therefore T cell depleted), *ex vivo* expanded in the presence of Notch ligand and then cryopreserved as described above. The fresh cord blood units are obtained through a collaboration with the Cord Blood (CB) Program at the Puget Sound Blood Center/Northwest Tissue Center (PSBC/NTC). Selection of the expanded cord blood stem cells will be based on the following:

A.   Panel Reactive Antibody (PRA) to be performed on all enrolled patients, and product selected based on the specificity of donor directed antibodies when present.

PRA negative patients may receive any product that fits cell dose restrictions. HLA matching will not be considered outside of PRA+ patients.

        1.   For patients eligible for a second dose of expanded cell product: PRAs will be repeated prior to selection of cord blood progenitor cell products.

B.   Cell Doses:

        1.   Infused TNC/kg cell dose will not exceed 1×10^8 TNC/kg recipient body weight.

        2.   No upper limit will be placed on the $CD34^+$ cells/kg infused.

        3.   All expanded products are evaluated by immunophenotyping for the presence of $CD3^+$ cells prior to freezing. While there has been no convincing evidence of a $CD3^+$ cell population, if a product has evidence of a T cell ($CD3^+$) population, this product will not be used unless the dose of $CD3^+$ cells is $<5 \times 10^5$ $CD3^+$ cells/kg (recipient weight).

C.   Repeat Infusions of Expanded Progenitors: Patients with severe infusional toxicities are not eligible for repeat infusions.

Figure 14 depicts the plan for treating enrolled patients suffering from AML.

Patients will receive one cycle of induction chemotherapy followed by infusion of expanded cord blood progenitors, with the possibility of a second cycle with infusion of expanded cell product beginning on day 21 to 28 post chemotherapy, provided the following conditions are met:

        1.   The patient does not have residual leukemia, defined as <5% marrow blasts by morphology.

        2.   The patient has not experienced any extramedullary grade 3 -4 toxicities.

        3.   The neutrophil count has recovered to 500/μl (on or off G-CSF).

        4.   The patient has no uncontrolled infections.

        5.   The patient has not had a history of severe infusional toxicities with the first expanded product infusion.

Plan for Consolidation Cycles: Only patients who achieve a remission (defined as <5% blasts by morphology) after reinduction (without expanded cells) or cycle 2 (with expanded cells) as per Figure 14 will be eligible to receive additional consolidation therapy. Eligible patients will receive a maximum of two cycles of consolidation

5   therapy. Whether or not a patient receives consolidation therapy will depend on whether the patient will be undergoing additional therapy such as a stem cell transplant. Consolidation will be offered without the use of expanded cord blood progenitor cells.

D.   Induction Therapy (See also Figure 14):

1.   All patients will receive an initial induction cycle followed by

10   infusion of expanded cord blood progenitors ("Cycle 1" in diagram, Figure 14. Patients will receive a second infusion of expanded cord blood progenitors with induction cycle 2 only if eligible, as outlined in "Eligibility for repeat expanded cell infusion with induction cycle 2" in Figure 14. All other patients will undergo reinduction therapy without infusion of expanded cord blood progenitors, as per Figure 14.

15   2.   The dosing of clofarabine, Ara-C and G-CSF is the same for induction cycles 1 and 2, regardless of whether the patient is eligible for a second infusion of expanded progenitor cells.

3.   G-CSF: 5 µg/kg subcutaneously (SQ), rounded up to nearest vial size, beginning 24 hours prior to chemotherapy and continued daily through day 5.

20   Infusion of the expanded cell product will occur on day 6 and G-CSF will be held that day. G-CSF will be resumed on day 7 and continued daily until ANC > 2000 for two consecutive days.

4.   Clofarabine: A dose of 25mg/m2 will be administered as a 1 hour intravenous infusion once daily for 5 days.

25   5.   Ara-C: A dose of 2 gm/m2 will be administered as a 2-hour intravenous infusion once daily for 5 days, starting 4 hours after the start of the clofarabine infusion.

6.   Infusion of expanded, cryopreserved cord blood stem cells of the invention:

30   a.   Dosage and selection of expanded product: Infused total nuclear cell count(TNC)/kg cell dose will not exceed 1 x 10^8 TNC/kg recipient body

weight. CD34 cell dose: No upper limit will be placed on the CD34 cells/kg infused.
All expanded products are evaluated by immunophenotyping for the presence of $CD3^+$
cells prior to freezing. While there has been no convincing evidence of a $CD3^+$ cell
population, if a product has evidence of a T cell ($CD3^+$) population, this product will not
5   be used unless the dose of $CD3^+$ cells is $<5 \times 10^5$ CD3 cells/kg (recipient weight).

The infusion rate of the expanded cord blood stem cells of the invention is infuse
at a rate of 3-5 ml/min for the first 4 minutes. If tolerated, the rate is increased to "wide
open". No medications or fluids should be given piggyback through the catheter that is
being used for the expanded cell infusion.

10

Table VII: Induction Therapy

Day	Cycle 1 and 2 (with expanded cells)	"Reinduction" (without expanded cells)
0	G-CSF 5 µg/kg SQ	G-CSF 5 µg/kg SQ
1-5	Clofarabine 25 mg/m² IV over 1 hour Ara-C 2 gm/m² IV over 2 hours G-CSF 5 µg/kg SQ	Clofarabine 25 mg/m² IV over 1 hour Ara-C 2 gm/m² IV over 2 hours G-CSF 5 µg/kg SQ
6	Hold GCSF Infusion of expanded cord blood progenitors	Continue GCSF until ANC >2000 for two consecutive days
7	Resume GCSF and continue until ANC >2000 for two consecutive days	

E.   Consolidation Therapy

1.   Patients in remission (defined as <5% marrow blasts by
15   morphology) will be eligible to receive up to two cycles of consolidation, depending on
whether the patient will be going on to receive additional therapy such as a stem cell
transplant. Patients with refractory disease after cycle 2 with expanded cell infusion or
reinduction without expanded cell infusion will be removed from the study.

2.   G-CSF: 5 µg/kg subcutaneously (SQ), rounded up to nearest vial
20   size, beginning 24 hours prior to chemotherapy and continued daily until ANC > 2000
for two consecutive days.

3.   Clofarabine: A dose of 20 mg/m2 will be administered as a 1 hour intravenous infusion once daily for 5 days.

4.   Ara-C: 1 gm/m2 as a two hour intravenous infusion once daily for five days, starting four hours after the start of the clofarabine infusion. Patients in remission (defined as <5% marrow blasts by morphology) will be eligible to receive up to two cycles of consolidation, depending on whether the patient will be going on to receive additional therapy such as a stem cell transplant. Patients with refractory disease after cycle 2 with expanded cell infusion or reinduction without expanded cell infusion will be removed from the study.

Table VIII: Consolidation Therapy:

Day 0	G-CSF 5 μg/kg SQ
Day 1- 5	Clofarabine 20 mg/m^2 IV over 1 hour Ara-C 1 gm/m^2 IV over 2 hours G-CSF 5 μg/kg SQ
Day 6	Continue G-CSF until ANC >2000 for two consecutive days

Evaluation Guidelines:

A.   Pre-treatment evaluation

1.   Complete physical examination.

2.   Medical history: Detailed documentation of disease and treatment history with outcomes.

3.   ECOG performance status

4.   12 lead EKG

5.   Hematology: CBC with differential and platelet count and peripheral blood smear.

6.   Serum chemistries: Electrolytes (sodium, potassium, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, glucose, and liver function

tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH).

       7.   Panel Reactive Antibody (PRA).

       8.   Adverse event assessment from time of first dose of G-CSF.

       9.   Initial standard of care diagnostic bone marrow reports, including hematopathology, cytogenetics/FISH, and flow cytometry.

       10.   To subsequently determine post transplant chimerism, heparinized peripheral blood from the patient will be obtained and chimerism analysis by DNA analysis will be performed.

    B.   Evaluation to be completed the morning of expanded progenitor infusion:

       1.   Physical exam and review of systems done by provider

       2.   Weight by nursing

       3.   CBC [CBD] (includes HCT, HGB, WBC,RBC, indices, platelets, DIFF/SMEAR EVAL)

       4.   [SRFM] and [SHFL] (HSCT Renal function panel with magnesium and HSCT Hepatic function panel with LD; SRFM includes NA, K, CL, CO_2, GLU, BUN, CRE, CA, P, ALB, MG and SHFL includes ALT, AST, ALK, BILIT/D, TP, ALB, LD)

       5.   Complete urinalysis

    C.   Evaluation during infusion of *ex vivo* expanded cord blood progenitors

       1.   RN must be in attendance during infusion.

       2.   MD or PA must be available on the inpatient unit.

       3.   If any changes in cardiac status, notify physician and obtain ECG.

       4.   Obtain and record vital signs including temperature, BP, HR, Respirations, and O2 saturation at the following time points:

TABLE IX

Pre-infusion	1 hour after the start of infusion
15 minutes after the start of infusion	2 hours after the start of infusion
30 minutes after the start of infusion	4 hours after the start of infusion
45 minutes after the start of infusion	24 hours after the start of infusion

     5.   Dipstick for HGB/protein every voided urine for 24 hours after infusion of expanded cells. Record HGB and Protein.

     D.   Evaluation 24 hours post infusion of *ex vivo* expanded cord blood progenitors

     1.   CBC [CBD] (includes HCT, HGB, WBC, RBC, indices, platelets, DIFF/SMEAR EVAL)

     2.   [SRFM] and [SHFL] (HSCT Renal function panel with magnesium and HSCT Hepatic function panel with LD; SRFM includes NA, K, CL, CO_2, GLU, BUN, CRE, CA, P, ALB, MG and SHFL includes ALT, AST, ALK, BILIT/D, TP, ALB, LD

     3.   Complete Urinalysis

     E.   Post-treatment evaluation

     1.   Engraftment studies: Contribution to hematopoietic recovery from the expanded cell product will be assessed from sorted peripheral blood (cell sorted for $CD3^+$, $CD33^+$, $CD14^+$, and $CD56^+$ cell fractions) on day 7, 14, 21, 28 and 56 following the infusion of expanded cells (or days 13, 20, 27, 34 and 62 of the chemotherapy cycle). If the patient is 100% host at the day 14 time point, all subsequent analyses will not be performed. However, should there be persistent evidence of engraftment derived from the expanded cell infusion at day 56, donor-host chimerism studies will be performed every 2 to 4 weeks as necessary to follow donor-host kinetics of engraftment. The percentages of donor-host chimerism will be evaluated by polymerase chain reaction (PCR)-based amplification of variable-number tandem repeat (VNTR) sequences unique to donors and hosts and quantified by phosphoimaging analyses.

2.   Alloimmunization: Repeat PRA to evaluate for the development of anti-HLA antibodies will be performed upon count recovery or prior to the next cycle of chemotherapy.

3.   Hematology: CBC with differential and platelet count and

5   peripheral blood smear daily while in hospital and/or until hematopoietic recovery, then at each outpatient clinic visit during the induction, re-induction, and consolidation cycles.

4.   Serum chemistries: Electrolytes (sodium, potassium, chloride, and bicarbonate), BUN, creatinine, glucose, and liver function tests (AST, ALT, ALP,

10   total bilirubin, LDH) twice weekly while in hospital, then weekly during the induction, re-induction, and consolidation cycles.

5.   Bone marrow evaluations: Post induction cycles or reinduction: Marrow evaluations will be performed for hematopathology, cytogenetics/FISH, flow cytometry and whole marrow chimerism evaluations on day 8 and 15 (if necessary)

15   following the infusion of expanded cells (or days 14 and 21 (if necessary) of the chemotherapy cycle). Additional marrows will be done as clinically indicated. If there is no count recovery by day 42 post chemotherapy, a bone marrow evaluation will be performed for hematopathology, cytogenetics/FISH, flow cytometry and whole marrow chimerism to rule out aplasia induced by a graft-versus-host phenomenon from the

20   expanded cell population versus aplasia due to persistent disease or chemotherapy induced aplasia. Post consolidation cycles (if received): Marrow evaluations will be performed for hematopathology, cytogenetics/FISH, and flow cytometry evaluations on day 21 and upon hematopoietic recovery (if necessary).

6.   Host and Donor Immunologic Interaction Studies

25   a.   Prior to the start of chemotherapy (and after consent obtained): 40 ml of peripheral blood will be collected in green top tubes to generate EBV transformed LCL lines from the patient for research studies evaluating donor/host immunologic reactions.

                b.   Post infusion of expanded cells on up to five occasions, 30

30   to 40 ml of peripheral blood may be collected in green top tubes to assay for immune mediated responses occurring between the host and donors. Investigator discretion on the timing of samples is provided to allow investigators to obtain samples once

individual hematopoietic recovery has occurred and to avoid obtaining samples if patients have been placed on steroids for treatment of a GVHD (steroids interfere with the studies).

       c.   Samples should be drawn on Monday through Friday only.

7.   Adverse Events: Adverse events will be evaluated and recorded.

8.   GVHD: All patients will be monitored for development of potential transfusion related GVHD. If signs or symptoms of acute GVHD occur, patients will be assessed as per Appendix C. Treatment of GVHD will be per institutional guidelines, but only if biopsy proven GVHD is present.

9.   Follow-up through 6 months:

       a.   Complete blood count, renal function, and liver function tests obtained for clinical reasons for a period of 6 months, as needed to define toxicity or duration of response.

       b.   Disease free and overall survival data will be assessed by contacting the referring MD or the patient every three to six months for the first two years, then annually for 3 years.

F.   Supportive Care Guidelines

1.   Blood Products: All blood products are to be irradiated and leukocyte-reduced. Also, CMV-negative patients will receive blood products as outlined by institutional standard practice guidelines. Transfusions will be administered for symptomatic anemia, or below standard threshold levels appropriate to the clinical setting.

2.   Infection Prophylaxis: Prophylactic oral acyclovir and levofloxacin will be used during the period of neutropenia. To the extent possible, use of nephrotoxic (*e.g.*, vancomycin, amphotericin B, etc.) and hepatotoxic (*e.g.*, voriconazole, cyclosporine, etc) agents is to be avoided during clofarabine administration for all treatment cycles.

3.   Treatment of Fever and Neutropenia: Standard diagnostic testing will be performed as per institutional guidelines, and empiric antibiotic coverage will be utilized. Specific antibiotics will be used for positive cultures.

4.   Colony Stimulating Factors: G-CSF will be utilized as per protocol during induction and consolidation chemotherapy as outlined above. Erythropoietic stimulating agents will not be utilized during induction or consolidation.

5.   Concomitant Therapy: No concomitant cytotoxic therapy or investigational therapy is allowed during the study with the exception of intrathecal therapy for leukemic meningitis. Intrathecal therapy must not be given during or within 24 hours of any 5 day Clofarabine/Cytarabine treatment period.

G.   Duration of Therapy: Patients will receive one to four cycles of study treatment. Expanded cord blood progenitors will be used with induction cycle #1 and cycle #2 unless:

1.   There is a history of severe infusional toxicity associated with the expanded cell product, in which case the patient will not be eligible to receive additional doses of the expanded cell product.

2.   There is evidence of disease progression.

3.   General or specific changes in the patient's condition render the patient unacceptable for further treatment per the investigator's judgment.

4.   The patient chooses to withdraw from the study.

5.   The patient becomes pregnant or fails to use adequate birth control if able to conceive.

6.   The patient is not able to comply with the protocol requirement.

10.2: IMPLEMENTATION

Frequent infections are a common complication of induction chemotherapy and salvage regimens used in the treatment of AML, and, in fact, are a leading cause of treatment failure. Use of clofarabine and high dose ara-c, in combination with granulocyte colony stimulating factor (G-CSF) has been studied in a phase I/II trial in the treatment of AML (Becker *et al.*, 2008, Blood 112 ASH Annual Meeting Abstracts) (such a chemotherapy cycle is referred to herein as "GCLAC"). Clofarabine has potent anti-leukemic activity, and clofarabine and high dose ara-c, in combination with G-CSF appears to be at least as effective as the more commonly used combination of idarubicin

and ara-C. However, clofarabine is also profoundly immunosuppressive and, in
conjunction with ara-C, is highly myelosuppressive, with periods of prolonged
neutropenia post-GCLAC of greater than three weeks. The combined immune- and
myelosuppressive effects of clofarabine and the delayed hematopoietic recovery results
5   in frequent infections and prevents dose intensive therapy. In a cohort of patients treated
at our center, >50% of patients experienced infectious complications post GCLAC, and
approximately 13% of patients experienced grade 4 infections (Becker *et al.*, 2008,
Blood 112 ASH Annual Meeting Abstracts). Importantly, infusion of expanded human
cord blood stem and progenitor cells can help overcome both of these challenges.
10   Additionally, the immunosuppression caused by the clofarabine-based regimen increases
the likelihood that the expanded human cord blood stem cell sample may temporarily
engraft and provide clinical benefit.

       To date, nine adult patients with relapsed (n=7) or primary refractory (n=2) AML
have been enrolled according to the criteria set out in Section 10.1, *supra*. The age
15   range was 40 to 55 years. Patients received their first cycle of chemotherapy:
clofarabine 25 mg/m^2/day for 5 days, ara-C 2gm/m^2/day for 5 days, G-CSF 5
mcg/kg/day for 6 days ("GCLAC"), followed approximately 24 hours after completion
of GCLAC by infusion of an expanded human cord blood stem cell sample without
regard to matching the HLA-type of the expanded human cord blood stem cell sample to
20   the HLA-type of the patient. The expanded human cord blood stem cell sample was
produced according to the method set forth in Section 9, *supra*. If response to GCLAC
was demonstrated by achievement of morphologic remission (based on bone marrow
aspirate), patients were eligible to receive a second cycle of GCLAC and a second
expanded human cord blood stem cell sample.

25   A total of twelve expanded human cord blood stem cell samples were infused
into the nine patients. Of the nine patients treated, four patients were refractory to
GCLAC therapy, and therefore were non-evaluable for neutrophil recovery. Three of
the five patients who achieved remission received a second cycle of GCLAC and a
second expanded human cord blood stem cell sample. Two out of the five patients who
30   achieved remission after the first cycle of GCLAC and expanded cord blood stem cell
sample, were given a hematopoietic stem cell transplant of a type determined by the
treating physician. For these remaining 5 patients (2 male, 3 female), the average time
to achieve an absolute neutrophil count (ANC) > 500 per μl was 19 days (see Figure 15),

comparing favorably to 21 days in a historical cohort of patients receiving GCLAC only
without expanded cells. Importantly, in the nine patients there have been no safety
issues with the infusion of the expanded human cord blood stem cell samples, or serious
adverse events attributed to the expanded human cord blood stem cell samples to date.

5   Only 2 of the 9 enrolled patients have experienced clinically significant
infections (e.g., bacteremia, fungal infections, pneumonia) compared to 17 out of 28
patients in the comparison cohort. Finally, three out of three patients who received a
second cycle of GCLAC with a second expanded human cord blood stem cell sample
were found to have transient donor contribution (as measured by peripheral blood cell
10   sorted DNA chimerism studies) to myeloid recovery one week after infusion of the cells,
ranging from 85 to 100% donor in the CD33/CD14 cell lineages. In these three patients,
the cells were also able to home to the marrow as evidenced by transient myeloid
engraftment of donor origin in the marrows of recipients (day 7 after infusion of the
cells) ranging from 3 to 15% (Figure 16).

15

11.   EXAMPLE: TREATMENT OF PATIENTS WITH HODGKIN'S LYMPHOMA, NON-HODGKIN'S LYMPHOMA AND MULTIPLE MYELOMA

The below discussion describes the design of a clinical trial aimed at providing
20   rapid restoration of hematopoietic function to patients suffering from Hodgkin's
lymphoma, non-Hodgkin's lymphoma and multiple myeloma who have been treated
with intensive chemotherapy and/or radiation therapy in preparation for autologous
transplant by administering expanded human cord blood stem cells. This study will
enroll patients will the following characteristics:

25   Patients with Hodgkin's or non-Hodgkin's lymphoma and multiple myeloma are
eligible. Histologically confirmed Hodgkin's or non-Hodgkin's lymphoma who have
failed at least 1 prior therapy. Histologically confirmed, symptomatic multiple myeloma
who have received at least 1 line of conventional chemotherapy. Failure to collect an
optimum number of PBSC after at least 1 attempt at mobilization. For purposes of this
30   trial this shall be defined as $<3 \times 10^6$ CD34$^+$ cells/kg, however, the first 3 patients
enrolled will have 1 to 2 x 106 CD34$^+$ cells/kg. Patients may have more than 1 attempt
at mobilization as long as the total dose is $<3 \times 106$ CD34$^+$ cells/kg. Patients must have
at least 1 x 106 CD34$^+$ cells/kg PBSC product available to be eligible for this trial.

The patients will be between the ages of 18 and 70 and will have 0-2 ECOG performance status results. Further, the patients will have adequate renal and hepatic functions as indicated by the following laboratory values: Serum creatinine ≤2.0 mg/dL; if serum creatinine >2.0 mg/dl, then the estimated glomerular filtration rate (GFR) must

5　be >60 mL/min/1.73 m2 as calculated by the Modification of Diet in Renal Disease equation where predicted GFR (ml/min/1.73 m2)= 186 X (Serum Creatinine)-1.154 X (age in years)-0.023 X (0.742 if patient is female) X (1.212 if patient is black). Serum total or direct bilirubin ≤1.5 × upper limit of normal (ULN), aspartate transaminase (AST)/alanine transaminase (ALT) ≤2.5 × ULN, alkaline phosphatase ≤ 2.5 × ULN.

10　The patients will be capable of understanding the investigational nature, potential risks and benefits of the study, and able to provide valid informed consent. Female patients of childbearing potential must have a negative serum pregnancy test within 2 weeks prior to enrollment. Male and female patients must be willing to use an effective contraceptive method during the study and for a minimum of 6 months after study

15　treatment. Panel reactive antibody (PRA) negative or with specific antibodies characterized for product selection will be performed (to avoid donor-directed antibodies against the potential cord blood product). All eligible patients will have a preliminary donor search conducted prior to the initiation of therapy to identify potential donors (related or unrelated and including suitable cord blood units) in the event of graft failure.

20　　　The following types of patients are excluded: Allogeneic transplant recipients, current concomitant chemotherapy, radiation therapy, or immunotherapy other than as specified in the protocol, use of other investigational agents within 30 days or any anticancer therapy within 2 weeks before study entry. Other exclusion factors are any other severe concurrent disease, or have a history of serious organ dysfunction or disease

25　involving the heart, kidney, liver (including symptomatic hepatitis, veno-occlusive disease), or other organ system dysfunction, history of HIV infection, patients with a systemic fungal, bacterial, viral, or other infection not controlled (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment), pregnant or lactating patients, patients having

30　any significant concurrent disease, illness, or psychiatric disorder that would compromise patient safety or compliance, interfere with consent, study participation, follow up, or interpretation of study results, having central nervous system involvement

with malignancy, and patients having no potential donor available (based on preliminary search) for allogeneic transplant in the event of graft failure.

The expanded cord blood progenitors to be used for this trial will be selected from a bank of previously expanded cord blood progenitors that have been

5   cryopreserved for future clinical use. Each individual progenitor cell product is derived from a single cord blood unit (donor) that is CD34 selected (and therefore T cell depleted), *ex vivo* expanded in the presence of Notch ligand (as described above in Section 8) and then cryopreserved. The fresh cord blood units are obtained through a collaboration with the Cord Blood (CB) Program at the Puget Sound Blood

10   Center/Northwest Tissue Center (PSBC/NTC).

Selection of the expanded progenitors will be based on the following:

A   Panel Reactive Antibody (PRA) to be performed on all enrolled patients, and product selected based on the specificity of donor directed antibodies when present. PRA negative patients may receive any product that fits cell dose restrictions. HLA

15   matching will not be considered outside of PRA+ patients.

B.   Cell Doses:

1.   TNC/kg pre-cryopreservation cell dose will not exceed 1.2×10^9 TNC/kg recipient body weight, to account for an anticipated approximate 20% cell loss upon thaw with the goal of maintaining cell doses at $\leq 1 \times 10^9$ TNC/kg.

20   2.   CD34 cell dose: No upper limit will be placed on the CD34 cells/kg infused.

3.   All expanded products are evaluated by immunophenotyping for the presence of $CD3^+$ cells prior to freezing. While there has been no convincing evidence of a $CD3^+$ cell population, if a product has evidence of a T cell ($CD3^+$)

25   population, this product will not be used unless the dose of $CD3^+$ cells is $<5 \times 10^5$ $CD3^+$ cells/kg (recipient weight).

The patient will be referred for treatment of lymphoma or multiple myeloma. The patient will be completely evaluated. The protocol will be discussed thoroughly with the patient and family, including requirement for data collection and release of

30   medical records, and all known significant risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible

and the risks and hazards of the procedure explained to the patient. Informed consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. A summary of the conference should be dictated for the medical record detailing what was covered.

5   The patients will be treated according to the following plan:

A.   Peripheral Blood Stem Cell Collection: Peripheral blood stem cells (PBSC) will be collected by serial leukaphereses by any know mobilization method). At least 1.0×10^6 CD34$^+$ cells/kg must be available for transplant.

B.   High dose conditioning regimens:

10   Multiple myeloma patients

Standard conditioning using melphalan 200 mg/m2 will be utilized for all patients.

TABLE X

Treatment	Day						
	-5	-4	-3	-2	-1	0	+1
Allopurinol (300 mg)		X	X	X	X		
Bactrim (1 DS tab BID)	X	X	X	X			
Melphalan 200 mg/m²				X			
Infusion: autologous PBSC + expanded cord blood						X	
G-CSF 5 mcg/kg/d until ANC > 2000 for two consecutive days							X

15   Lymphoma patients

TBI-based regimen for patients who have not received prior dose limiting TBI (>20 Gy to any critical normal organ (*e.g.* lung, liver, spinal cord).

TABLE XI

Treatment	Day													
	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1	+2
Palifermin 60 mcg/kg/day	X	X	X										X	X	X
TBI 1.5 Gy BID (total dose 12 Gy)				X	X	X	X							
Etoposide 60 mg/kg IV								X						
Rest									X					
Cyclophosphamide 100 mg/kg IV										X				
Rest											X			
Infusion: autologous PBSC + expanded cord blood												X		
G-CSF 5 mcg/kg/d until ANC > 2000 for two consecutive days													X	X

Cyclophosphamide Dosage: Cyclophosphamide will be administered at a dose
of 100 mg/kg/day IV on day 2 of conditioning. Preparation, administration and
5   monitoring will be according to standard methods. Dosing in patients >100% of IBW
will be per standard practice. MESNA will be given for bladder prophylaxis according
to standard practice. Continuous bladder irrigation is an alternative for bladder
prophylaxis at the attending physician's discretion. Hydration and monitoring for
toxicities will be according to standard practice.

10   Total Body Irradiation.: Total body irradiation (TBI), 1.5 Gy BID x 4 days (for a
total dose of 12 Gy) is delivered via a linear accelerator at a dose rate of 8 Gy/min.

IV Hydration and Antiemetic Therapy: IV hydration should be given at 2
liters/m2/24hrs. Scheduled doses of antiemetics per standard practice.

BEAM conditioning regimen: Patients ineligible for a TBI based regimen will
15   receive high dose therapy with a BEAM conditioning regimen.

TABLE XII

Treatment	Day								
	-7	-6	-5	-4	-3	-2	-1	0	+1
BCNU 300 mg/m2 IV x 1d	X								
Etoposide 100 mg/m2 IV BID x 4d		X	X	X	X				
Ara-C 100 mg/m2 IV BID x 4d		X	X	X	X				
Melphalan 140 mg/m2 x 1d						X			
Rest							X		
Infusion: Autologous PBSC + expanded cord blood								X	
G-CSF 5 mcg/kg/d until ANC > 2000 for two consecutive days									X

BCNU (Carmustine):

Dosage: Carmustine 300 mg/m2 IV x 1 will be infused over 3 hours on day -7 of

5   conditioning. Carmustine should not be infused with solutions or tubing containing or

previously containing bicarbonate solution.

Chemistry: Carmustine, a nitrosourea derivative, is generally considered to be an

alkylating agent. The drug is available as a white lyophilized powder at 4°C. It is

slightly soluble in water, freely soluble in alcohol, and highly soluble in lipids.

10   Administration: Carmustine is available as a sterile powder as 100 mg vials.

The drug is reconstituted by dissolving the contents of the 100 mg vial in 3ml of sterile

dehydrated (absolute) alcohol, followed by the addition of 27 ml of sterile water for

injection. The resultant solution contains 3.3 ml of carmustine per ml of 10% alcohol.

This solution may be further diluted with 0.9% sodium chloride or 5% dextrose injection

15   to a final concentration of 0.2 mg/ml in glass containers. Only glass containers are

recommended to be used for administration of this drug. Carmustine is rapidly degraded

in aqueous solutions at a pH greater than 6.

After IV administration, carmustine is rapidly cleared from the plasma with no

intact drug detectable after 15 minutes. Carmustine is rapidly metabolized, although the

20   mechanism is not fully elucidated. Excretion of the metabolites occurs mainly through

the urine and some metabolites are known to be active.

Maintenance hydration: Normal saline plus 20 mEq KCL is to be started at 2 liters/m2/day pre-Carmustine and continued until 24 hours after the last dose of Melphalan.

Pharmacokinetics: Because of their high lipid solubility, carmustine and/or its
5 metabolites readily cross the blood-brain barrier. Substantial CSF concentrations occur almost immediately after administration of carmustine, and CSF concentrations of radiolabeled-BCNU have been variously reported to range from 15-70% of concurrent plasma concentrations. Carmustine metabolites are distributed into milk, but in concentrations less than those in maternal plasma.

10   Etoposide (VP-16, Vepesid):

Dosage: Etoposide 100 mg/m2 IV BID will be administered in 500-1000 cc normal saline over 2 hours on days -6, -5, -4, and -3 of conditioning for a total dose of 800 mg/m2.

Chemistry and mechanism of action: Etoposide is a semi-synthetic
15 podophyllotoxin. The epipodophyllotoxins exert phase-specific spindle poison activity with metaphase arrest and, in contrast to the vinca alkaloids, have an additional activity of inhibiting cells from entering mitosis. Suppression of tritiated thymidine, uridine, and leucine incorporation in human cells in tissue culture suggests effects against DNA, RNA, and protein synthesis.

20   Storage and stability: Unopened vials of VP-16 are stable for 24 months at room temperature. Vials diluted as recommended to a concentration of 0.2 or 0.4 mg/mL are stable for 96 and 24 hours, respectively, at room temperature (25° C) under normal room fluorescent light in both glass and plastic containers. Undiluted VP-16 in plastic syringes has been reported to be stable for up to 5 days.

25   Availability, reconstitution and administration: Etoposide is commercially available in 100 mg/5 ml, 150 mg/7.5 ml, 500 mg/25 ml or 1000 mg/50 ml sterile multiple dose vials. VP-16 should be diluted prior to use with either 5% Dextrose Injection, USP, or 0.9% Sodium Chloride Injection, USP, to give a final concentration of 0.2 or 0.4 mg/ml. Precipitation may occur at solutions above 0.4 mg/ml concentration. It
30 is recommended that VP-16 solution be administered IV over 2 hours. However, a longer duration of administration may be used when infusing large volumes of fluid. VP-16 should not be infused rapidly. To avoid large volumes, VP-16 can be given

undiluted, with special equipment and precautions. If VP-16 is administered undiluted, a 4-way stopcock and tubing made with "chemo resistant" (not containing acrylic or ABS components) plastic must be used. Undiluted VP-16 cannot be infused without concurrent IV solution infusing through the Hickman catheter. Infusion of undiluted
5   VP-16 alone will cause Hickman catheter occlusion.

        Supportive Care: Appropriate anti-emetics and sedatives should be given before the infusion begins. Before and 2 hours into the infusion, the patient is to receive 25 mg of diphenhydramine, and 100 mg of hydrocortisone to prevent allergic reactions. Normal saline plus 20 mEq KCL is to be continued at 2 liters/ m2/day. If necessary, diuretics
10   may be given. Since in rare cases metabolic acidosis has been observed after high dose VP-16, additional $NaHCO_3$ may be added to hydration, though not infused while VP-16 is infusing.

        Cytarabine(Ara-C):

        Dosage: Cytarabine 100 mg/m2 IV BID will be infused over 3 hours on days -6,
15   -5, -4 and -3 of conditioning.

        Chemistry: Cytarabine is a synthetic pyrimidine nucleoside and pyrimidine antagonist anti-metabolite.

        Availability and administration: Cytarabine is available in a reconstituted form in solutions containing 20, 50 and 100 mg of cytarabine per ml. These solutions have
20   been reconstituted from a sterile powder with bacteriostatic water containing 0.945% benzyl alcohol for injection. The manufacturers state that the reconstituted solutions with water for injection may be diluted with 0.9% sodium chloride or 5% dextrose. The diluted solutions containing 0.5 mg of cytarabine per mL are stable for at least 8 days at room temperature.

25   Pharmacokinetics: Cytarabine is not effective when administered orally. Continuous IV infusions produce relatively constant plasma concentrations of the drug in 8-24 hours. Cytarabine is rapidly and widely distributed into tissues and fluids, including liver, plasma, and peripheral granulocytes and crosses the blood-brain barrier to a limited extent. The drug apparently crosses the placenta. It is not known if
30   cytarabine is distributed in milk. After rapid IV injection, plasma drug concentrations appear to decline in a biphasic manner with a half-life of about 10 minutes in the initial phase and about 1-3 hours in the terminal phase. Cytarabine is rapidly and extensively

metabolized mainly in the liver but also in the kidneys, gastrointestinal mucosa,
granulocytes, and to a lesser extent in other tissues by the enzyme cytidine deaminase,
producing the inactive metabolite 1-B-d-arabinofuranosyluracil (ara-U). Cytarabine and
ara-U are excreted in urine. After rapid IV, IM, SQ, or IT injection or continuous IV

5   infusion of cytarabine, about 70-80% of the dose is excreted in the urine within 24
hours.

        Melphalan:

        Dosage: Melphalan will be administered at a dose of 140 mg/m2 IV x 1 infused
over 30 minutes on day -2 of conditioning.

10   Chemistry: Melphalan (L-phenylalanine mustard) is a typical alkylating agent
that can be given intravenously or orally.

        Administration: Melphalan is available in 50 mg vials and when reconstituted
with 10 ml sterile water results in a concentration of 5 mg/ml. The reconstituted
melphalan is diluted in 250 cc normal saline to a concentration not greater than 0.5

15   mg/ml. Melphalan is administered over 15 minutes, not to exceed 60 minutes.

        Pharmacokinetics: Plasma melphalan levels are highly variable after oral dosing,
both with respect to the time of the first appearance of melphalan in plasma (range: 0 to
336 minutes) and to the peak plasma concentration (range: 0.166 to 3.741 mg/mL)
achieved. These results may be due to incomplete intestinal absorption, a variable "first

20   pass" hepatic metabolism, or to rapid hydrolysis.

        C.   Cell Infusion:

        1.   Autologous PBSC will be thawed and infused on the morning of
day 0.

        2.   Expanded cell infusion: Expanded cells will be thawed and

25   infused as per standard guidelines and infused approximately 4 hours after infusion of
the autologous stem cell graft.

        D.   Supportive care:

        G-CSF: 5 mcg/kg subcutaneously (SQ), rounded up to nearest vial size,
beginning the day after autologous stem cell infusion and expanded cell product

30   infusion. G-CSF will be continued daily until ANC > 2000 for two consecutive days.

Evaluation Guidelines

A.   Pre-transplant evaluation

      1.   History, physical exam, Karnofsky score.

      2.   CBC, serum sodium, potassium, CO_2, BUN, creatinine, uric acid, LDH, calcium, bilirubin, alkaline phosphatase, AST, ALT, hepatitis screen, ABO/RH typing, blood crossmatch, CMV, VZV, HSV, HIV, and toxoplasmosis serology.

      3.   CT/PET (lymphoma).

      4.   MRI of skeleton, and osseous survey needed for staging (myeloma).

      5.   Bone marrow aspirations and biopsies; samples for pathology, flow cytometry and cytogenetics including FISH.

      6.   Serum protein electrophoresis and immunofixation (myeloma).

      7.   Quantitative serum immunoglobulin levels, beta 2 microglobulin.

      8.   24 hour urine collection to determine creatinine clearance and total protein excretion, urine protein electrophoresis, quantitative Bence Jones excretion and immunofixation (myeloma).

      9.   PFTS, MUGA.

      10.   Clinical immune reconstitution studies.

B.   Evaluation during conditioning:

      1.   Daily CBC until ANC >500/ul and platelet count >20,000/ul following the nadir.

      2.   Electrolyte panel (sodium,potassium, chloride, CO_2, calcium, magnesium, phosphorus, albumin, BUN creatinine) 3x per week at a minimum.

      3.   Liver function tests (ALT, AST, ALK phos, bilirubin, and LDH) 2X per week at a minimum.

C.   Evaluation to be completed the morning of autologous PBSC and expanded progenitor infusion:

      1.   Physical exam and review of systems done by provider

2.   Weight by nursing

3.   CBC [CBD] (includes HCT, HGB, WBC,RBC, indices, platelets, DIFF/SMEAR EVAL)

4.   [SRFM] and [SHFL] (HSCT Renal function panel with magnesium and HSCT Hepatic function panel with LD; SRFM includes NA, K, CL, CO_2, GLU, BUN, CRE, CA, P, ALB, MG and SHFL includes ALT, AST, ALK, BILIT/D, TP, ALB, LD)

5.   Complete urinalysis

D.   Evaluation during infusion of *ex vivo* expanded cord blood progenitors

1.   RN must be in attendance during infusion.

2.   MD or PA must be available on the inpatient unit.

3.   If any changes in cardiac status, notify physician and obtain ECG.

4.   Obtain and record vital signs including temperature, BP, HR, Respirations, and O2 saturation at the following time points:

TABLE XIII

Pre-infusion	1 hour after the start of infusion
15 minutes after the start of infusion	2 hours after the start of infusion
30 minutes after the start of infusion	4 hours after the start of infusion
45 minutes after the start of infusion	24 hours after the start of infusion

5.   Dipstick for HGB/protein every voided urine for 24 hours after infusion of expanded cells. Record HGB and Protein.

E.   Evaluation 24 hours following the infusion of expanded cord blood progenitors:

1.   CBC [CBD] (includes HCT, HGB, WBC, RBC, indices, platelets, DIFF/SMEAR EVAL)

2.   [SRFM] and [SHFL] (HSCT Renal function panel with magnesium and HSCT Hepatic function panel with LD; SRFM includes NA, K, CL, CO2, GLU, BUN, CRE, CA, P, ALB, MG and SHFL includes ALT, AST, ALK, BILIT/D, TP, ALB, LD.

5   3.   Complete Urinalysis

F.   Evaluation from day 0 to day 60:

1.   Daily CBC until ANC >500/ul and platelet count >20,000/μl following the nadir. Thereafter CBC 3x per week until day +28 and 2x per week until day +60.

10   2.   Electrolyte panel (sodium,potassium, chloride, CO2, calcium, magnesium, phosphorus, albumin, BUN creatinine) 3x per week until day 60.

3.   Liver function tests (ALT, AST, ALK phos, bilirubin, and LDH) 2X per week until day 28 then weekly until day 60.

4.   Engraftment studies: Contribution to hematopoietic recovery
15 from the expanded cell product will be assessed from sorted peripheral blood (cell sorted for $CD3^+$, $CD33^+$, $CD14^+$, and $CD56^+$ cell fractions) on day 7, 14, 21, 28 and 60 following the infusion. If at any time point the patient is 100% host, all subsequent analyses will not be performed. If there is evidence of engraftment from expanded cell product which persists at day 60, chimerism studies will be continued at 2-4 week
20 intervals until the patient is 100% host. The percentages of donor-host chimerism will be evaluated by polymerase chain reaction (PCR)-based amplification of variable-number tandem repeat (VNTR) sequences unique to donors and hosts and quantified by phosphoimaging analyses.

5.   Alloimmunization: Repeat PRA to evaluate for the development
25 of anti-HLA antibodies will be performed upon count recovery.

6.   GVHD: All patients will be monitored for development of potential transfusion related GVHD. If signs or symptoms of acute GVHD occur, patients will be assessed. Treatment of GVHD will be per institutional guidelines, but only if biopsy proven GVHD is present.

30   7.   Bone Marrow Evaluations: Marrow evaluations will be performed for hematopathology, cytogenetics/FISH, flow cytometry and whole marrow

chimerism evaluations on day 14 and 21 (if necessary). In the event of graft failure, a marrow evaluation will be performed to rule out aplasia due to graft versus host effect. Additional marrows will be done as clinically indicated.

8.   Adverse event monitoring until day 60 evaluation.

9.   Clinical immune reconstitution studies.

G.   Day 60 re-staging evaluation

1.   History, physical exam, Karnofsky score.

2.   CBC, serum sodium, potassium, CO2, BUN, creatinine, uric acid, LDH, calcium, bilirubin, alkaline phosphatase, AST, ALT, hepatitis screen.

3.   CT/PET imaging (lymphoma).

4.   Osseous survey use skeletal survey for re-staging, and MRI of skeleton (myeloma).

5.   Bone marrow aspiration and biopsy; samples for pathology, flow cytometry and cytogenetics including FISH. If there is persistence of the expanded cord blood cells (as demonstrated on peripheral blood chimerism analysis), this marrow will be sent for whole marrow chimerism as well.

6.   Quantitative serum immunoglobulin levels.

7.   Serum protein electrophoresis and immunofixation (myeloma).

8.   24 hour urine collection to determine creatinine clearance and total protein excretion, urine protein electrophoresis, quantitative Bence Jones excretion and immunofixation (myeloma).

9.   Serum B2 microglobulin.

10.   Repeat PRA to evaluate for the development of anti-HLA antibodies.

11.   Adverse event monitoring

12.   Clinical immune reconstitution studies

H.   Immune Reconstitution:

Clinical Studies (to be performed as possible):

1.   Quantitative immunoglobulin levels (IgG, IgA, IgM) will be assessed at Day 28, 60, 100, 6 months, 1 year and 2 years.

2.   Total T lymphocytes and subset enumeration (Lymphocytes panel) will be assessed pre-transplant and at Day 28, 60, 100, 6 months, 1 year and 2 years.

I.   Follow-up

1.   Complete blood count, renal function, and liver function tests obtained for clinical reasons for a period of 6 months, as needed to define toxicity or duration of response.

2.   Disease free and overall survival data will be assessed by contacting the referring MD or the patient every three to six months for the first two years, then annually for 3 years.

J.   Supportive Care Guidelines

1.   Blood Products: All blood products are to be irradiated and leukocyte-reduced. Also, CMV-negative patients will receive CMV-safe blood products. Transfusions will be administered for symptomatic anemia, or below standard threshold levels appropriate to the clinical setting.

2.   Infection Prophylaxis: Prophylactic oral levofloxacin will be used during the period of neutropenia. Acyclovir and bactrim prophylaxis will be used according to standard practice guidelines.

3.   Treatment of Fever and Neutropenia; Standard diagnostic testing will be performed as per institutional guidelines, and empiric antibiotic coverage will be utilized. Specific antibiotics will be used for positive cultures.

4.   Colony Stimulating Factors: G-CSF will be utilized as outlined above. Erythropoietic stimulating agents will not be utilized.

5.   Concomitant Therapy: No concomitant cytotoxic therapy or investigational therapy is allowed during the study with the exception of prophylactic intrathecal therapy per standard practice guidelines.

12.   EXAMPLE: TREATMENT OF AML PATIENTS
WITH EXPANDED HUMAN CORD BLOOD STEM
CELLS AND CORD BLOOD TRANSPLANT

This protocol involves the administration of one or more umbilical cord

5   blood/placental blood units ("Grafts" or "cord blood transplants") in combination with

an expanded cord blood stem cell sample of the invention for the treatment of acute

myelogenous leukemia (AML) in human patients. The cord blood transplants were cord

and/or placental whole blood, except that red blood cells were removed.

To date, six patients with leukemia at high risk of relapse have been enrolled and

10   received treatment per the treatment protocol set forth in Figure 17, which is a

myeloablative, total body irradiation (TBI)-based cord blood transplant (CBT) protocol

for patients with hematologic malignancy ("CSA/MMF" refers to cyclosporin and

micophenylatemofetil, a conventional immune-suppressive treatment to prevent graft vs.

host disease (GVHD)). The conditioning and post-transplant immune suppression

15   regimens in this study are identical to the *ex vivo* expansion trial described in Section 10,

supra, and are considered standard of care for myeloablative cord blood transplant. The

patients received two previously cryopreserved cord blood transplants (depleted of red

blood cells) with a minimum of 1.5×10^7 total nucleated cells ("TNC")/kg (depending

on algorithm of cell dose and HLA typing) and one expanded cord blood stem cell

20   sample produced as described in Section 9, *supra*.

To date, all patients treated received a double cord blood transplant (cord blood

transplants from the cord and/or placental blood of two different individuals) followed

by infusion of a previously cryopreserved and thawed, expanded human cord blood stem

cell sample without regard to HLA matching, on day 0. No toxicities were observed at

25   the time of infusion and no serious adverse events have been attributed to the expanded

human cord blood stem cell sample to date. The first patient was infused on September

22. 2010, and the sixth patient is now 2 months post transplant. The infused TNC and

$CD34^+$ cell doses are presented in the table below.

Table XIV: Demographics, Infused Cell Doses and Engraftment, Patients 1-6

Pt #	Age	Wt (kg)	Diagnosis	Infused TNC* (x10⁷/kg)	Infused TNC** (x10⁷/kg)	Infused CD34* (x10⁶/kg)	Infused CD34** (x10⁶/kg)	1ˢᵗ Day ANC >100	1ˢᵗ Day ANC >500	Date Platelet >20k
1	35	67.1	AML	8.9	2.7	0.31	4.1	8	26	35
2	5	27.4	ALL	7.3	10.0	0.37	9.8	13	15	26
3	24	76.4	ALL	4.0	6.9	0.12	9.2	9	19	37
4	21	74	ALL	4.8	4.7	0.16	4.9	16	21	35
5	13	57	ALL	4.2	7.2	0.15	8.8	7	26	45
6	33	72	AML	5.2	6.8	0.16	11.1	9	19	33
Mean	21.2	61.2	—	5.7	6.5	0.21	8	10	21	35

*Total of both unmanipulated units. ** Based on pre-freeze total of off-the-shelf expanded unit.

The kinetics of hematopoietic recovery and the relative contribution of the expanded cells and cord blood graft cells to engraftment were determined beginning on day 7 post transplant. All patients treated to date have engrafted. It has been previously demonstrated that an ANC ≥100 is a critical threshold to reduce the risk of death prior to

5   day 100 post hematopoietic cell transplant (Offner *et al.*, 1996, Blood 88:4058). Furthermore, in our own single center analysis of severe neutropenia following cord blood transplant, both ANC <100 and time to engraftment as a time-dependent covariate, correlates significantly with both day 200 transplant related mortality ("TRM") and overall survival. In this analysis of 88 patients undergoing cord blood

10   transplant, at any given time point, an ANC <100 is associated with a 4.77-fold increase in the risk of overall mortality compared to an ANC >100 (1.74 – 13.11, p=0.002) and an 8.95-fold increase in risk of day 200 TRM (2.59-30.89, p=0.00095). This is similar to findings when modeling the time to engraftment (ANC >500), such that engraftment at a specific time point is associated with a 0.23-fold risk of death as compared to lack of

15   engraftment at this time (0.08 – 0.62, p=0.004), and a 0.11-fold risk of day 200 TRM (0.03 – 0.38, p=0.0005). Therefore, the time to achieve an ANC ≥ 100 and the time to achieve ANC ≥ 500 were evaluated in patients who underwent a myeloablative double cord blood transplant with administration of a previously cryopreserved expanded human cord blood stem cell sample without regard to HLA matching (off-the-shelf +

20   unmanipulated), compared (i) to a concurrent cohort of patients who received a conventional myeloablative double cord blood transplant (conventional dCBT), and (ii) to a cohort of patients who received a myeloablative double cord blood transplant with administration of a partially HLA matched expanded human cord blood stem cell sample that was not cryopreserved (expanded + unmanipulated), as described in Delaney *et al.*,

25   2010 Nature Med. 16:232-236.

While the patient numbers were small, an advantage for earlier myeloid recovery is suggested in the patients treated to date with the cryopreserved expanded human cord blood stem cell sample without regard to HLA matching (off-the shelf + unmanipulated), and in the patients treated with the partially HLA matched expanded

30   human cord blood stem cell sample (expanded + unmanipulated) compared to the conventional double cord blood transplant (conventional dCBT) (see Figure 18 and Figure 19). In one of the six patients administered the expanded human cord blood stem cell sample without regard to HLA matching, *in vivo* persistence of the expanded cord

blood stem cells continues to persist when last checked at day 56. Retrospectively, this patient was found to be fortuitously matched at 3/6 HLA antigens to the off-the-shelf product.

5   Early myeloid recovery at day 7 was derived almost entirely from the previously cryopreserved expanded human cord blood stem cell sample that was administered to all 6 patients without regard to HLA matching, but generally such recovery did not persist beyond day 14 post-transplant (see Figure 20). This result is in accord with results seen when infusing a freshly harvested (not cryopreserved) and partially HLA matched expanded human cord blood stem cell sample, as described in Delaney *et al.*, 2010

10 Nature Med. 16;232-236.

It is envisioned that future patients will receive one, two or more cord blood transplants in addition to the expanded human cord blood stem cell sample.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those

15 described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications, including patents, patent application publications, and scientific literature, are cited herein, the disclosures of which are incorporated by

20 reference in their entireties for all purposes.

WHAT IS CLAIMED:

1.   A method for providing hematopoietic function to a human patient in need thereof, comprising administering a pool of expanded human cord blood stem cell samples, or an aliquot thereof, to the patient

5   wherein the pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, and

10   wherein each different expanded human cord blood stem cell sample in the pool has been subjected to an expansion technique that has been shown to result in an at least 50-fold increase hematopoietic stem cells or hematopoietic stem and progenitor cells in an aliquot of a human cord blood stem cell sample subjected to the expansion technique, relative to an aliquot of the human cord blood cell stem cell sample

15   prior to being subjected to the expansion technique.

2.   The method of claim 1, wherein the hematopoietic stem cells or hematopoietic stem and progenitor cells are CD34$^+$.

3.   The method of claim 1 or 2, wherein the two or more samples in the pool mismatch the patient at 1 or 2 of the HLA antigens or alleles typed in the patient and

20   typed in the samples.

4.   The method of any one of claims 1-3, wherein the two or more samples in the pool mismatch the patient at 2 of the HLA antigens or alleles typed in the patient.

5.   A method for providing hematopoietic function to a human patient in need thereof, comprising:

25   (a)   selecting a pool of expanded human cord blood stem cell samples for administration to the patient from a plurality of pools of expanded human cord blood stem cell samples, wherein the pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the

samples in the pool collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient; and

(b)   administering the selected pool, or an aliquot thereof, to the patient.

6.   The method of claim 5, wherein the selecting further comprises rejecting pools of samples containing samples having more than 2 HLA antigen or allele mismatches with the patient of the HLA antigens or alleles typed in the patient.

7.   The method of claim 5 or 6, wherein the selecting further comprises accepting pools of samples containing samples having 1 or 2 HLA antigen or allele mismatches with the patient of the HLA antigens or alleles typed in the patient.

8.   The method of any one of claims 5-7, wherein the selecting further comprises accepting pools of samples containing samples having 1 or 2 HLA antigen or allele mismatches with the patient of the HLA antigens or alleles typed in the patient.

9.   The method of any one of claims 5-8, wherein the selecting is from among at least 50 frozen expanded human cord blood stem cell pools of samples.

10.   The method of any one of claims 1-9, wherein all the samples in each pool are derived from the umbilical cord blood and/or placental blood of humans of the same race.

11.   The method of any one of claims 1-9, where all the samples in each pool are derived from the umbilical cord blood and/or placental blood of humans of the same ethnicity.

12.   The method of any one of claims 1-11, wherein each pool of expanded human cord blood stem cell samples contains at least 75 million viable CD34$^+$ cells.

13.   The method of any one of claims 1-12, wherein the method further comprises producing each different expanded human cord blood stem cell sample in each pool by a method comprising expanding *ex vivo* isolated human cord blood stem cells obtained from the umbilical cord blood and/or placental blood of a human at birth.

14.   The method of any one of claims 5-13, wherein each different expanded human cord blood stem cell sample in each pool has been subjected to an expansion technique that has been shown to result in an at least 50-fold increase hematopoietic stem cells or hematopoietic stem and progenitor cells in an aliquot of a human cord blood stem cell sample subjected to the expansion technique, relative to an aliquot of the human cord blood cell stem cell sample prior to being subjected to the expansion technique.

15.   The method of claim 14, wherein the hematopoietic stem cells or hematopoietic stem and progenitor cells are positive $CD34^+$.

16.   The method of claim 13, wherein the expanding step comprises contacting the human cord blood stem cells with an agonist of Notch function.

17.   The method of any one of claims 1-13, wherein each different expanded human cord blood stem cell sample in each pool has been subjected to an expansion technique that has been shown to increase the number of SCID repopulating cells in a human cord blood stem cell sample subject to the expansion technique, relative to the human cord blood stem cell sample prior to being subject to the expansion technique.

18.   The method of claim 14, wherein the expansion technique has been shown to result in an at least 50-fold increase in $CD34^+$ cells in a human cord blood stem cell sample subjected to the expansion technique, relative to the human cord blood cell stem cell sample prior to being subjected to the expansion technique.

19.   The method of any one of claims 1-18, wherein each pool of expanded human cord blood stem cell samples is frozen prior to said administering step, and wherein the method further comprises a step of thawing each pool prior to said administering.

20.   A method for providing hematopoietic function to a human patient in need thereof, comprising:

(a) pooling at least two umbilical cord blood and/or placental blood samples, wherein each sample is obtained at birth of a different human to produce pooled cord blood;

(b) enriching for hematopoietic stem cells or hematopoietic stem and progenitor cells from pooled cord blood to produce a population enriched in hematopoietic stem cells or hematopoietic stem and progenitor cells;

(c) expanding *ex vivo* the population enriched in hematopoietic stem cells or hematopoietic stem and progenitor cells to produce an expanded stem cell sample; and

(d) administering the expanded stem cell sample, or an aliquot thereof, to a human patient in need of hematopoietic function, wherein the expanded stem cell sample collectively does not mismatch at more than 2 of the HLA antigens or alleles typed in the patient.

21. The method of claim 20, wherein the method further comprises the steps of freezing and thawing the expanded stem cell sample after step (c) and before step (d).

22. The method of any one of claims 1-21, wherein the patient has pancytopenia or neutropenia.

23. The method of claim 22, wherein the pancytopenia or neutropenia is caused by an intensive chemotherapy regimen, a myeloablative regimen for hematopoietic cell transplantation, or exposure to acute ionizing radiation.

24. A method of producing a bank of frozen, expanded human cord blood stem cells comprising the following steps in the order stated:

(a) expanding, *ex vivo*, human cord blood stem cells present in a population enriched for hematopoietic stem cells or hematopoietic stem and progenitor cells obtained from a pool of umbilical cord blood and/or placental blood, which pool is obtained from two or more different humans at birth, to produce an expanded human cord blood stem cell sample;

(b) freezing the expanded human cord blood stem cell sample to produce a frozen expanded human cord blood stem cell sample;

(c) storing the frozen expanded human cord blood stem cell sample; and

(d)   repeating steps (a)-(c) at least 50 times to produce a bank of at least 50
stored, frozen expanded human cord blood stem cell samples.

25.   The method of claim 24, wherein the method further comprises a step of
assigning each frozen expanded human cord blood stem cell sample an identifier that
distinguishes the frozen expanded human cord blood stem cell sample from the other
frozen expanded stem cell samples.

26.   The method of claim 24 or 25, wherein the method further comprises a
step of storing each identifier in one or more computer databases, wherein said stored
identifier is associated with information on the physical location where the frozen
expanded human cord blood stem cell sample corresponding to the identifier is stored in
said bank.

27.   A blood bank comprising at least 50 units of frozen pools of expanded
human cord blood stem cell samples, wherein each pool comprises two or more different
expanded human cord blood stem cell samples, each different sample in the pool being
derived from the umbilical cord blood and/or placental blood of a different human at
birth, wherein the different samples in each pool collectively do not mismatch at more
than 2 of the HLA antigens or alleles typed in each samples in each pool.

28.   A computer-implemented method for selecting a frozen expanded human
cord blood stem cell sample for use in providing hematopoietic function to a human
patient in need thereof, comprising the following steps performed by a suitably
programmed computer:
        (a)   selecting an identifier from a plurality of at least 50 identifiers stored in a
computer database, each identifier identifying a frozen, stored pool of expanded human
cord blood stem cell samples, wherein each pool comprises two or more different
expanded human cord blood stem cell samples, each different sample in the pool being
derived from the umbilical cord blood and/or placental blood of a different human at
birth, such that the samples in the pool identified by the selected identifier collectively
do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the
patient, wherein the selecting is to identify a pool of expanded human cord blood stem

cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and

(b)   outputting or displaying the selected identifier.

29.   The computer-implemented method of claim 28, wherein the outputting or displaying step further outputs or displays information on the physical location of each pool of expanded human cord blood stem cell samples identified by the identifier.

30.   The computer-implemented method of claim 28 or 29, wherein the method further comprises implementing robotic retrieval of each identified pool of expanded human cord blood stem cell samples.

31.   A computer program product for use in conjunction with a computer system, the computer program product comprising a computer readable storage medium and a computer program mechanism embedded therein, the computer program mechanism comprising:

(a)   executable instructions for selecting an identifier from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a frozen, stored pool of expanded human cord blood stem cell samples, wherein each pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the samples in the pool identified by the selected identifier collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein the selecting is to identify a pool of expanded human cord blood stem cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and

(b)   executable instructions for outputting or displaying the selected identifier.

32.   An apparatus comprising:
a processor;
a memory, coupled to the processor, the memory storing a module, the module comprising:

(a)   executable instructions for selecting an identifier from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a pool of

expanded human cord blood stem cell samples, wherein each pool comprises two or more different expanded human cord blood stem cell samples, each different sample in the pool being derived from the umbilical cord blood and/or placental blood of a different human at birth, wherein the samples in the pool identified by the selected

5   identifier collectively do not mismatch the patient at more than 2 of the HLA antigens or alleles typed in the patient, wherein the selecting is to identify a pool of expanded human cord blood stem cell samples for administration of the pool, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and

(b)   executable instructions for outputting or displaying the selected identifier.

10   33.   The method of any one of claims 4-18, wherein the selecting further comprises rejecting pools containing samples that do not contain at least 75 million CD34$^+$ cells.

34.   The method of any one of claims 4-18 and 33, wherein the selecting further comprises rejecting pools containing samples that contain more than 500,000

15   CD3$^+$ cells per kilogram patient weight.

35.   The computer-implemented method of any one of claims 28-30, wherein the selecting further comprises rejecting pools that do not contain at least 75 million CD34$^+$ cells.

36.   The computer-implemented method of any one of claims 28-30 and 35,

20   wherein the selecting further comprises rejecting pools that contain more than 500,000 CD3$^+$ cells per kilogram patient weight.

37.   The computer-implemented method of any one of claims 28-30, 35 and 36, wherein the selecting further comprises accepting pools of samples, wherein the samples in the pool collectively do not mismatch at more than 2 HLA antigens or alleles

25   typed in the patient.

38.   The computer program product of claim 31, wherein the executable instructions for selecting further comprises instructions for rejecting identifiers that identify pools that do not contain more than 75 million CD34$^+$ cells.

39.   The computer program product of claim 31 or 38, wherein the executable instructions for selecting further comprises instructions for rejecting identifiers that identify pools that contain more than 500,000 CD3$^+$ cells per kilogram patient weight.

40.   The computer program product of any one of claims 31, 38 and 39, wherein the executable instructions for selecting further comprises accepting identifiers that identify pools of samples wherein the samples in the pool collectively do not mismatch at more than 2 of the HLA antigens or alleles typed in the patient.

41.   The apparatus of claim 32, wherein the executable instructions for selecting further comprises instructions for rejecting identifiers that identify pools that do not contain more than 75 million CD34$^+$ cells.

42.   The apparatus of claim 32 or 41, wherein the executable instructions for selecting further comprises instructions for rejecting identifiers that identify pools that contain more than 500,000 CD3$^+$ cells per kilogram patient weight.

43.   The apparatus of any one of claims 32, 41 and 42, wherein the executable instructions for selecting further comprises accepting identifiers that identify pools of samples wherein the samples in the pool collectively do not mismatch at more than 2 HLA antigens or alleles typed in the patient.

44.   The computer-implemented method of any one of claims 28-30 and 35-37, wherein said selecting comprises rejecting identifiers that identify pools of samples that collectively mismatch at more than 2 of the HLA antigens or alleles typed in the patient.

45.   The computer program product of any one of claims 31 and 38-40, wherein the executable instructions for selecting further comprises instructions for rejecting identifiers that identify pools of samples that collectively mismatch at more than 2 of the HLA antigens or alleles typed in the patient.

46.   The apparatus of any one of claims 32 and 41-43, wherein the executable instructions for selecting further comprises instructions for rejecting identifiers that

identify pools of samples that collectively mismatch at more than 2 of the HLA antigens or alleles typed in the patient.

47.   The method of claim 1, wherein the method further comprises before said administering step, a step of pooling said two or more different expanded human cord blood stem cells samples to form said pool.

48.   The method of claim 47, wherein the method further comprises before said pooling step, a step of selecting said two or more expanded human cord blood stem cell samples to form said pool.

49.   The method of claim 48, wherein each said different sample is frozen at the time of said selecting, and the method further comprises, after said selecting and prior to said pooling, a step of thawing the selected samples.

50.   The method of claim 49, wherein said selecting comprises sequentially accepting samples for pooling such that the samples collectively do not mismatch at more than 2 HLA antigens or alleles typed in the patient.

51.   A computer-implemented method for selecting frozen expanded human cord blood stem cell samples for use in providing hematopoietic function to a human patient in need thereof, comprising the following steps performed by a suitably programmed computer:

        (a)   selecting a plurality of identifiers from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a frozen stored expanded human cord blood stem cell sample derived from the umbilical cord blood and/or placental blood of one or more different humans at birth, wherein the selected identifiers identify frozen expanded human cord blood stem cell samples to be thawed and pooled for administration of the expanded human cord blood stem cell sample, or an aliquot thereof, identified by said identifiers to a human patient in need thereof; and

        (b)   outputting or displaying the selected identifiers.

52.   A computer program product for use in conjunction with a computer system, the computer program product comprising a computer readable storage medium

and a computer program mechanism embedded therein, the computer program mechanism comprising:

(a)   executable instructions for selecting a plurality of identifiers from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a frozen stored expanded human cord blood stem cell sample derived from the umbilical cord blood and/or placental blood of one or more humans at birth, wherein the selected identifiers identify frozen stored expanded human cord blood stem cell samples to be thawed and pooled for administration of the expanded human cord blood stem cell sample, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and

(b)   executable instructions for outputting or displaying the selected identifiers.

53.   An apparatus comprising:

a processor;

a memory, coupled to the processor, the memory storing a module, the module comprising:

(a)   executable instructions for selecting a plurality of identifiers from a plurality of at least 50 identifiers stored in a computer database, each identifier identifying a frozen stored expanded human cord blood stem cell sample derived from the umbilical cord blood and/or placental blood of one or more humans at birth, wherein the selected identifiers identify frozen stored expanded human cord blood stem cell samples for administration of the expanded human cord blood stem cell sample, or an aliquot thereof, identified by said identifier to a human patient in need thereof; and

(b)   executable instructions for outputting or displaying the selected identifiers.

54. The method of claim 1, wherein 6 HLA antigens or alleles are typed in the patient.

Fig. 1

SCID Repopulating Cell Frequency

Figure 2a

Human Engraftment

Figure 2b

Overall Human Engraftment
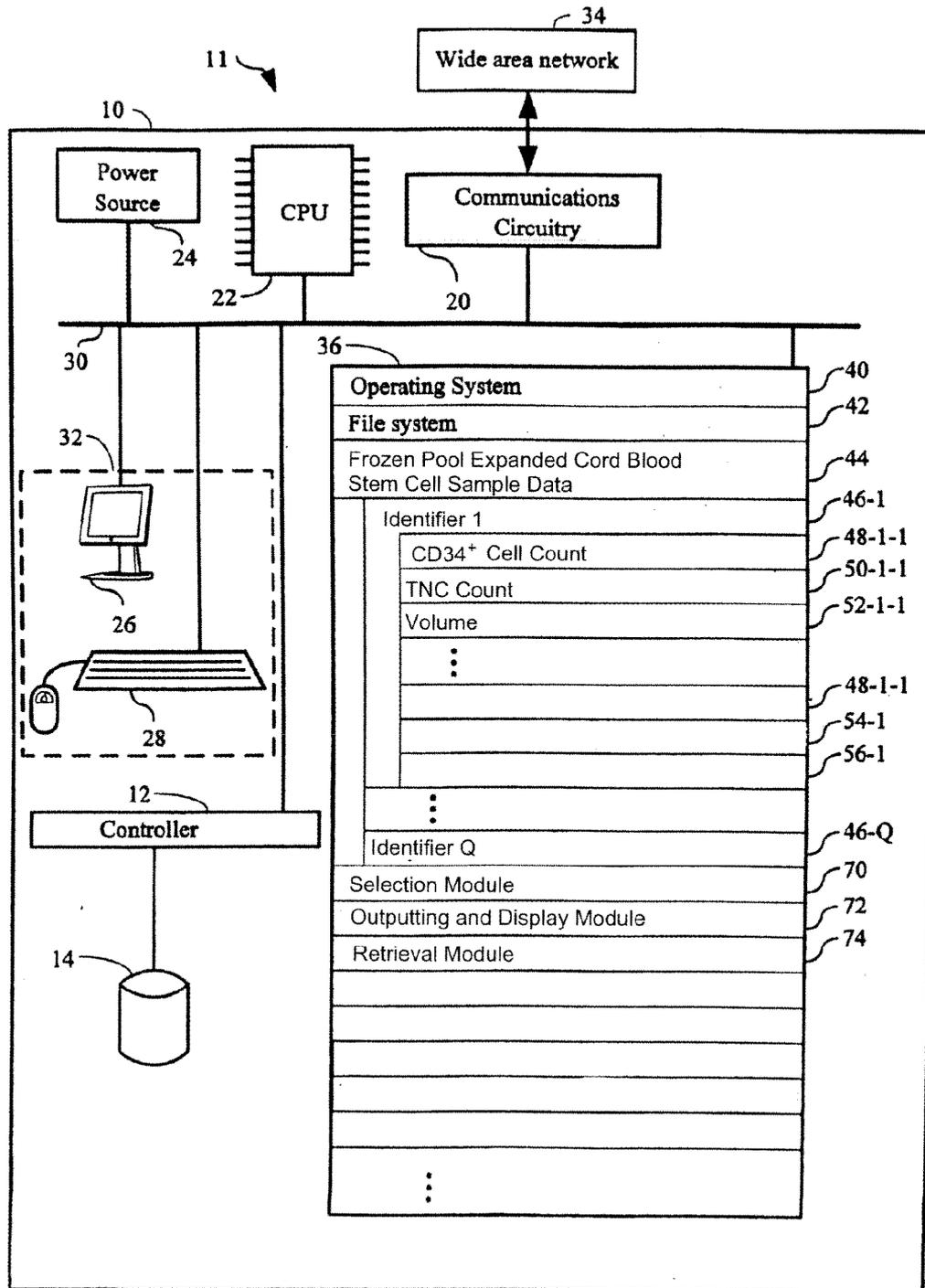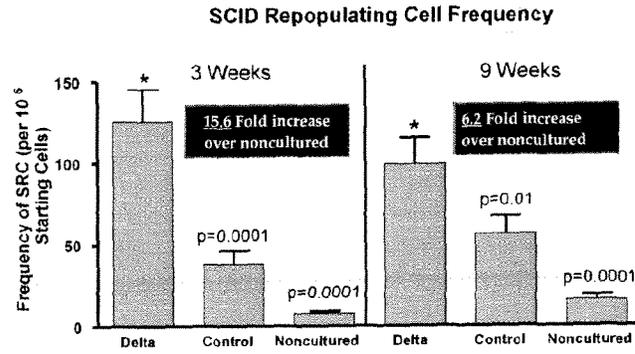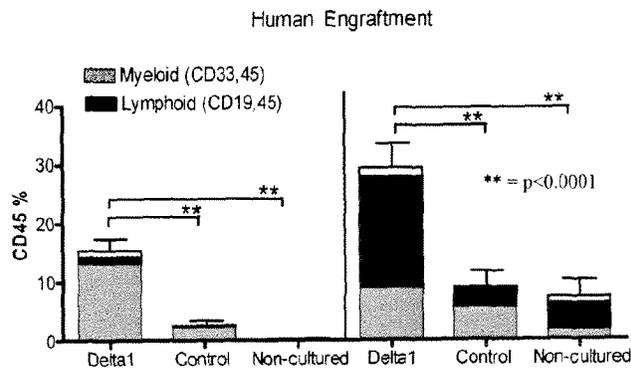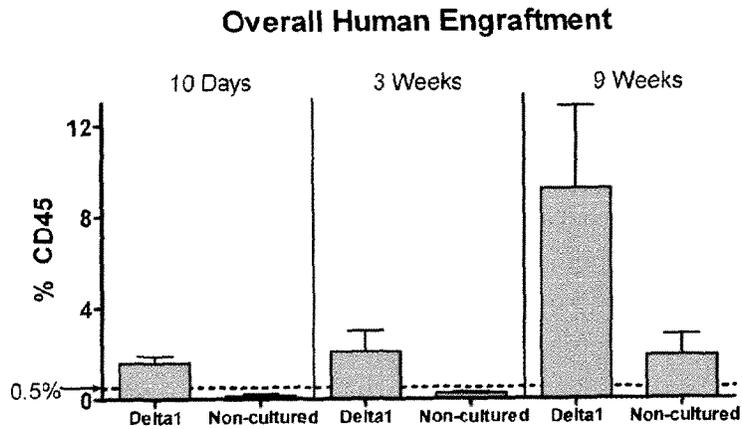
Figure 3

**Cultured vs
Cultured and Cryopreserved**
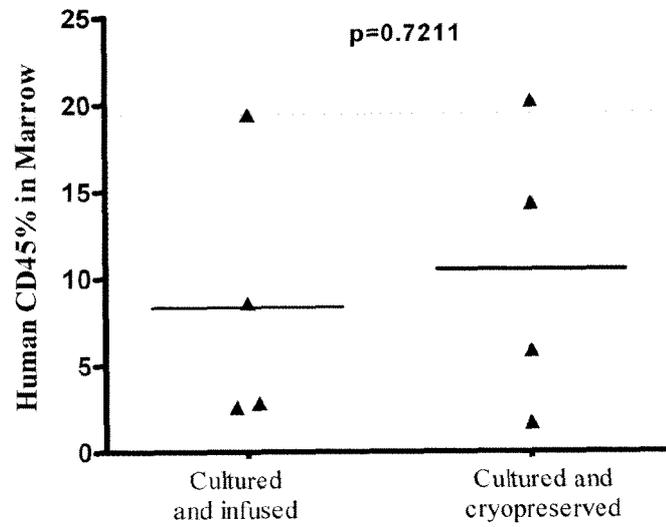
Figure 4a

**Engraftment of Cryopreserved
Expanded Cord Blood Progenitor Cells**
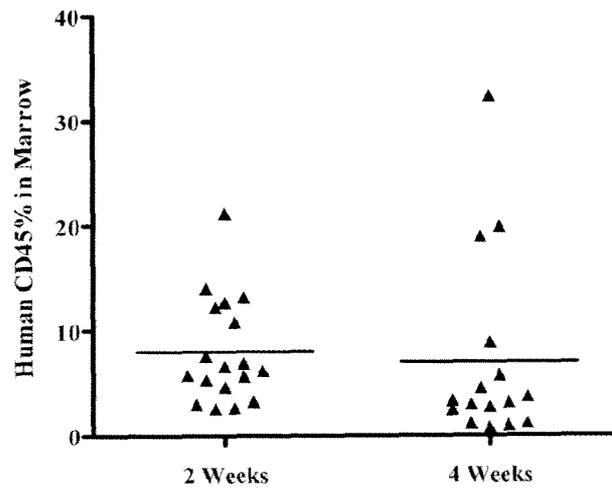
Figure 4b

Cryopreserved Expanded CB Progenitors:
Methods of thaw and infuse
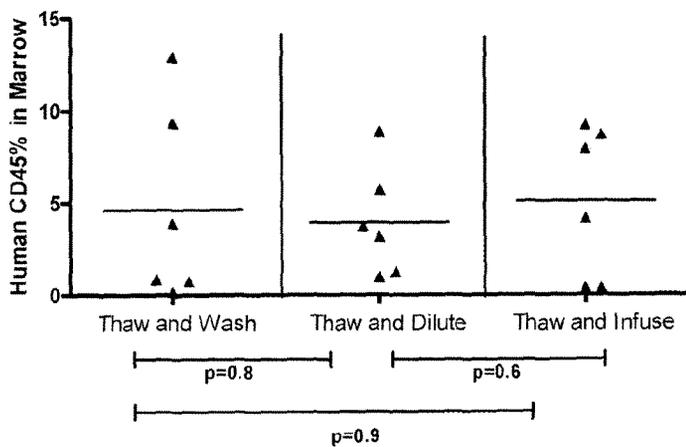
Figure 4c

ENGRAFTMENT IN PERIPHERAL BLOOD
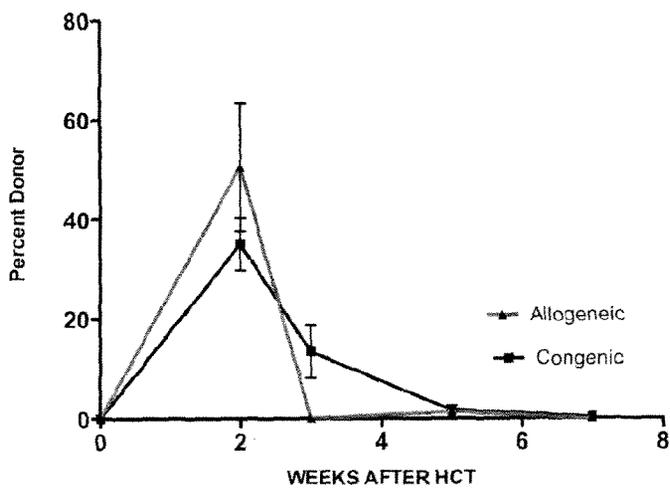
Figure 5
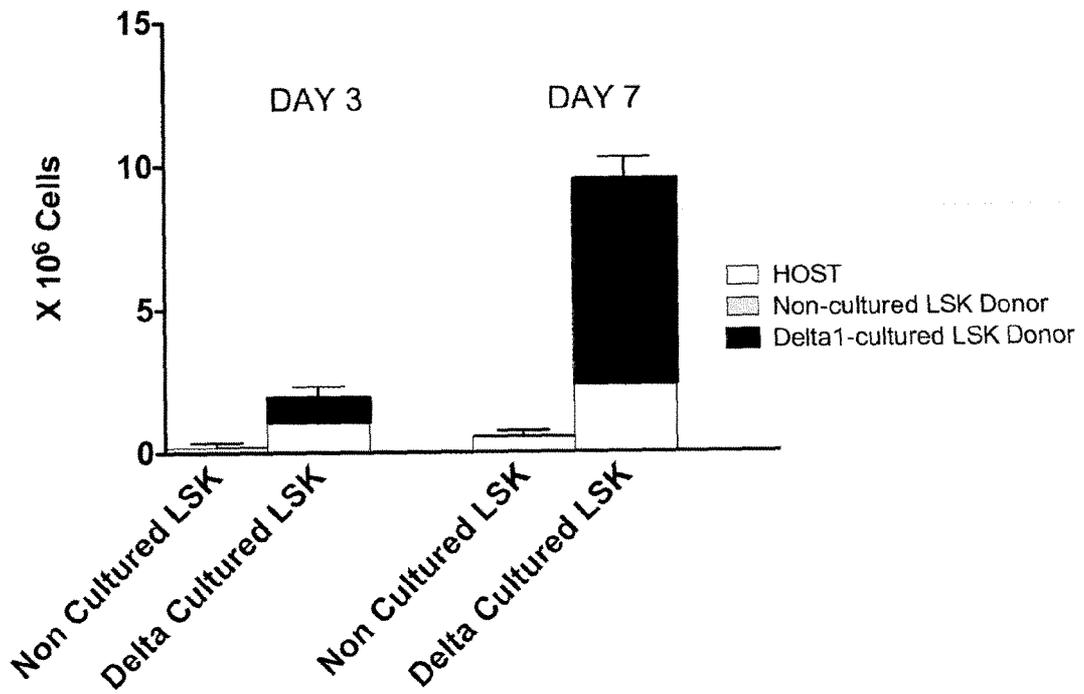
Figure 6

Competitive Repopulation Assay in a Mismatched Setting

Freshly Sorted Ly5a Lin-Sca-1+c-kit+ (H-2b, CD45.1)

Delta1ext-IgG cultured at 2.5 and 5 µg/ml

Cultured for 14 days in serum with 4GF

Ig-G cultured LSK

Harvest at day 14

Transplanted into irradiated Balb-c mice (H-2d, CD45.2)
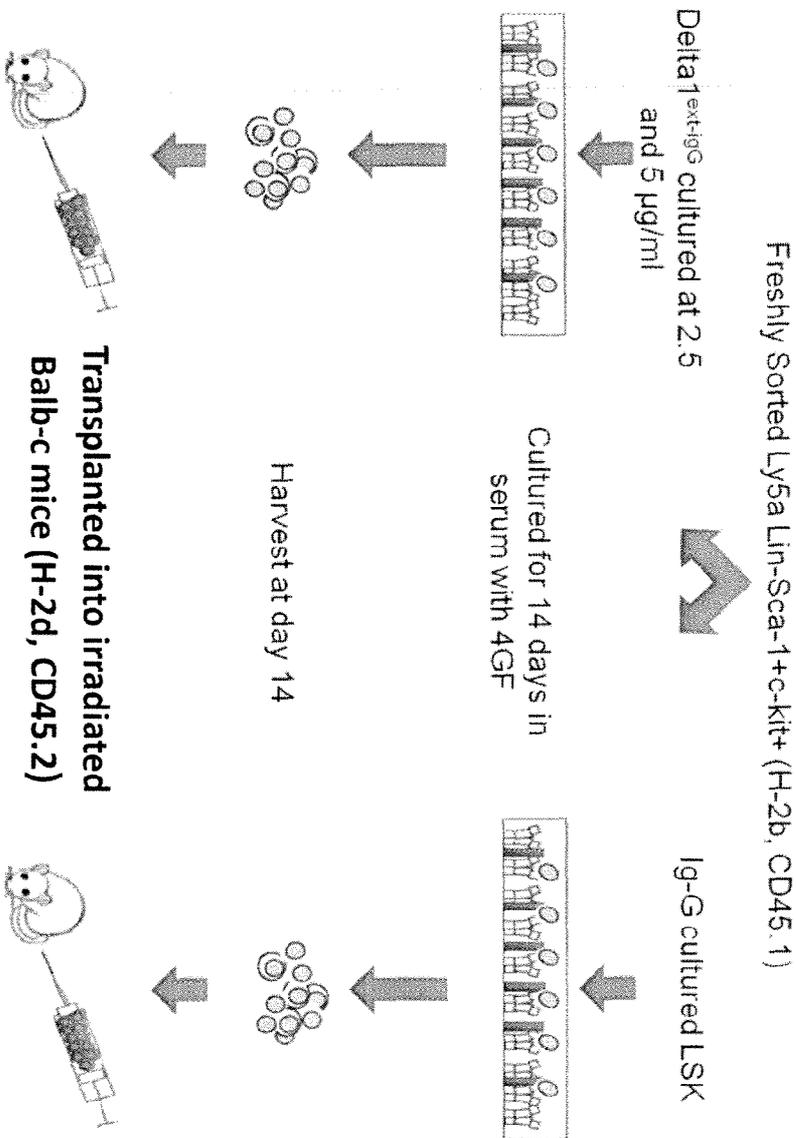
Figure 7

Efficacy – Murine Model

- Initial studies confirming that effective engraftment is achieved when expanded murine progenitors are infused in a mismatched setting after a single dose of radiation

Balb-c engraftment in bone marrow
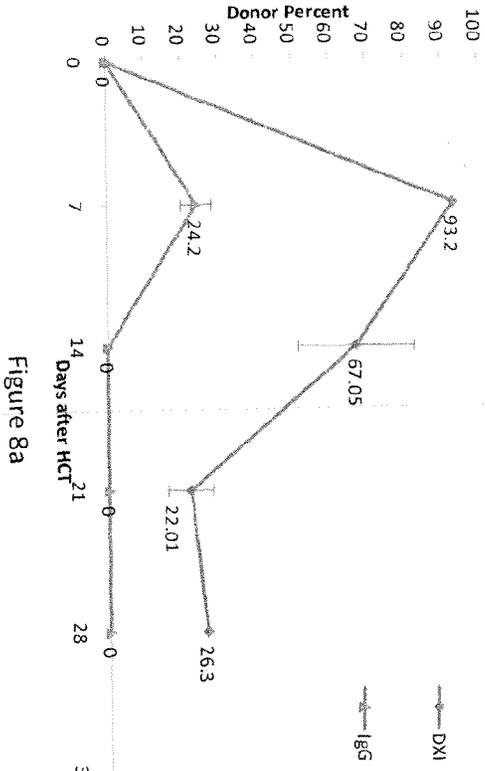
Figure 8a

Balb-c engraftment in peripheral blood
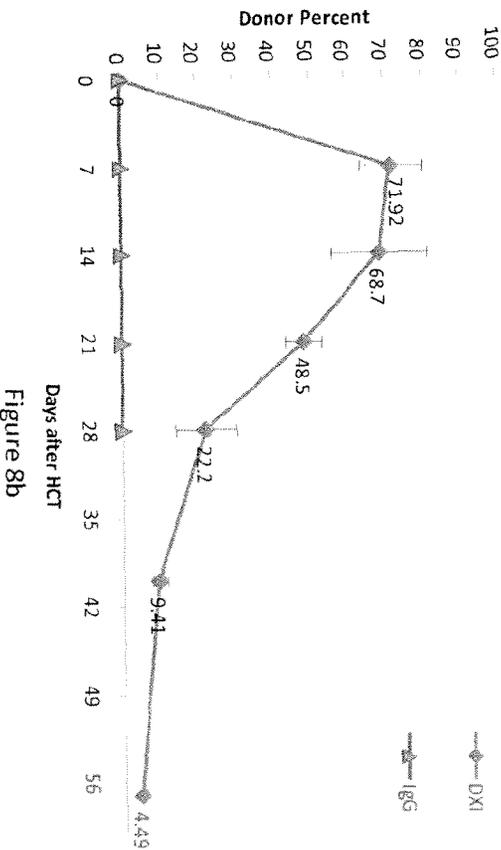
Figure 8b

- LD$_{100}$ radiation dose was chosen for these experiments to:
  - evaluate whether expanded progenitors can prolong survival at a lethal dose of radiation
  - to determine the maximum cell dose tolerated leading to improved survival
  - to generate survival curves
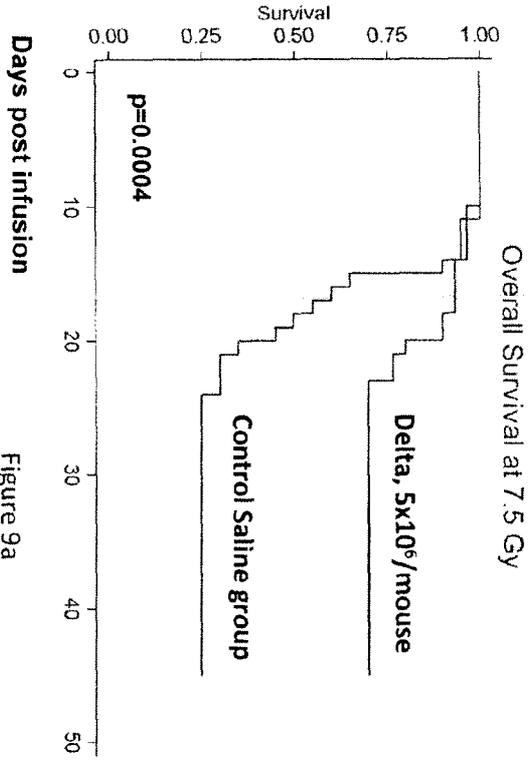
Figure 9a
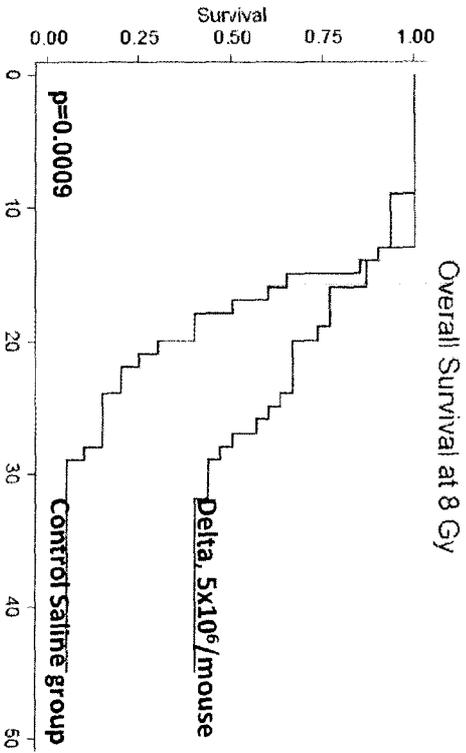
Figure 9b
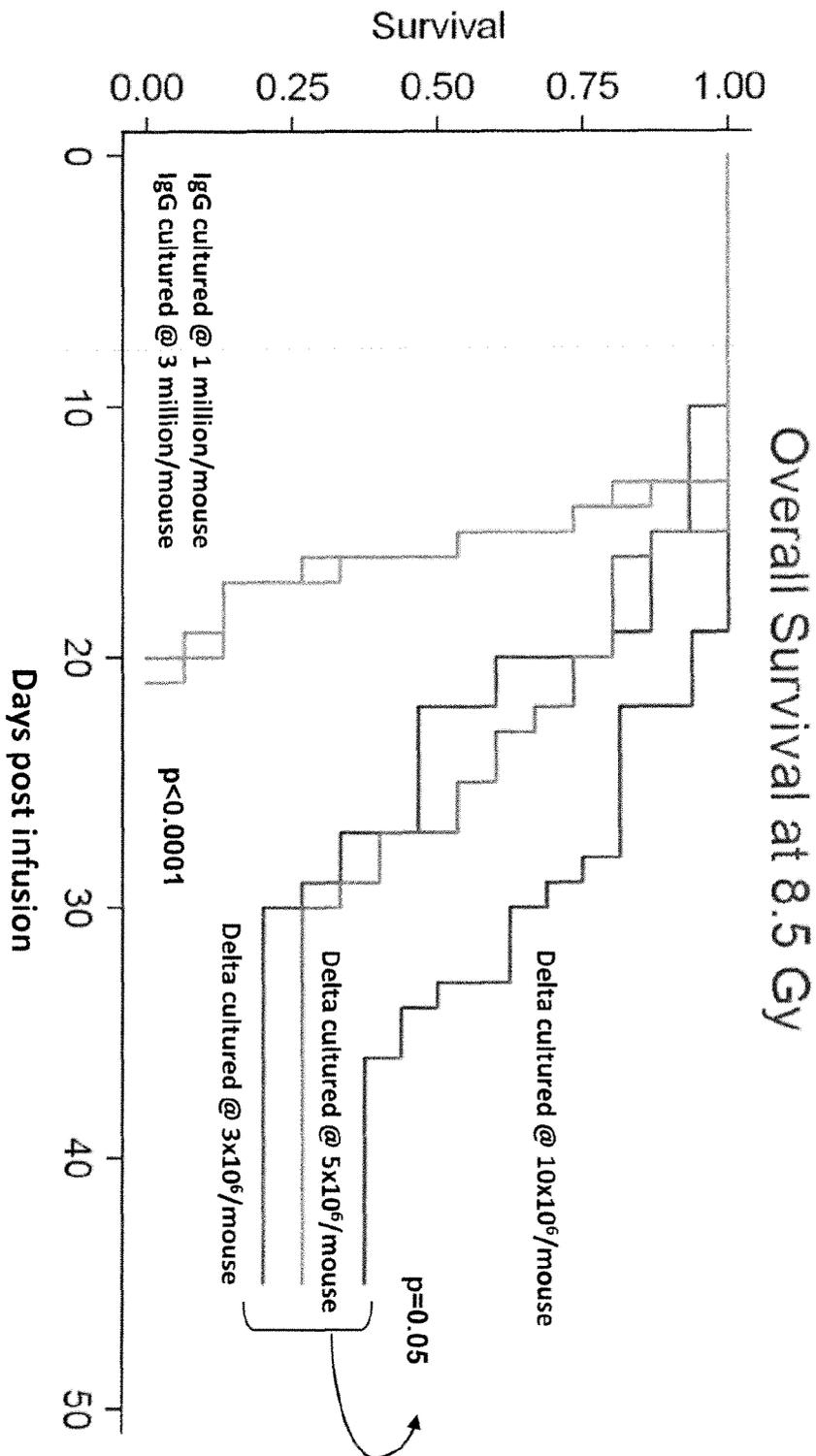
Figure 10

Level of Donor Engraftment is Enhanced with Increasing Dose of Radiation

Balb-c engraftment in bone marrow
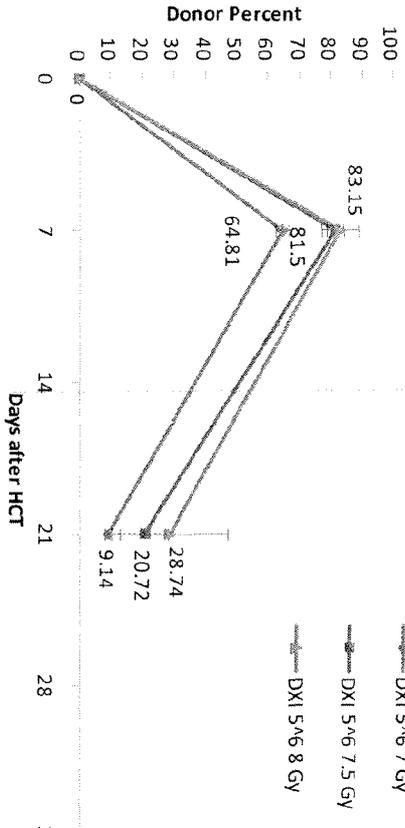
Figure 11a

Balb-c engraftment in peripheral blood
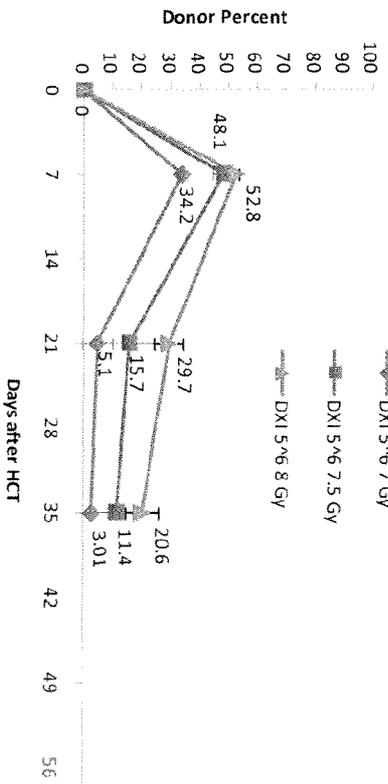
Figure 11b
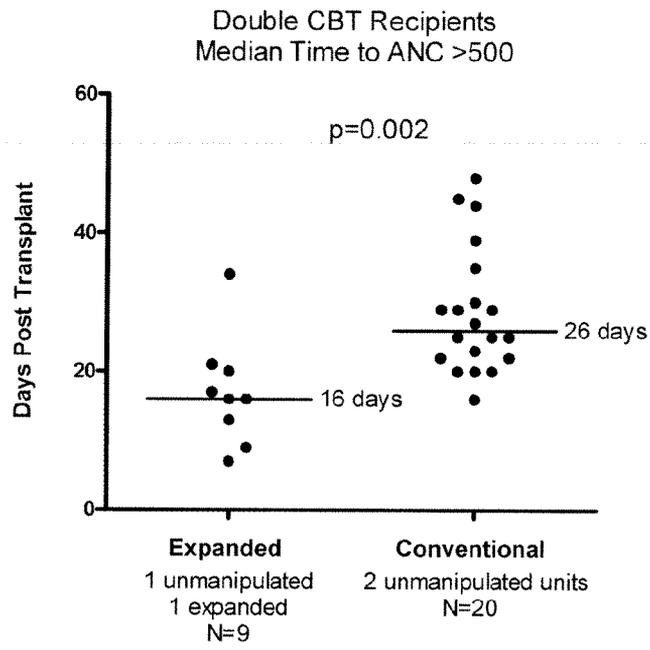
Figure 12
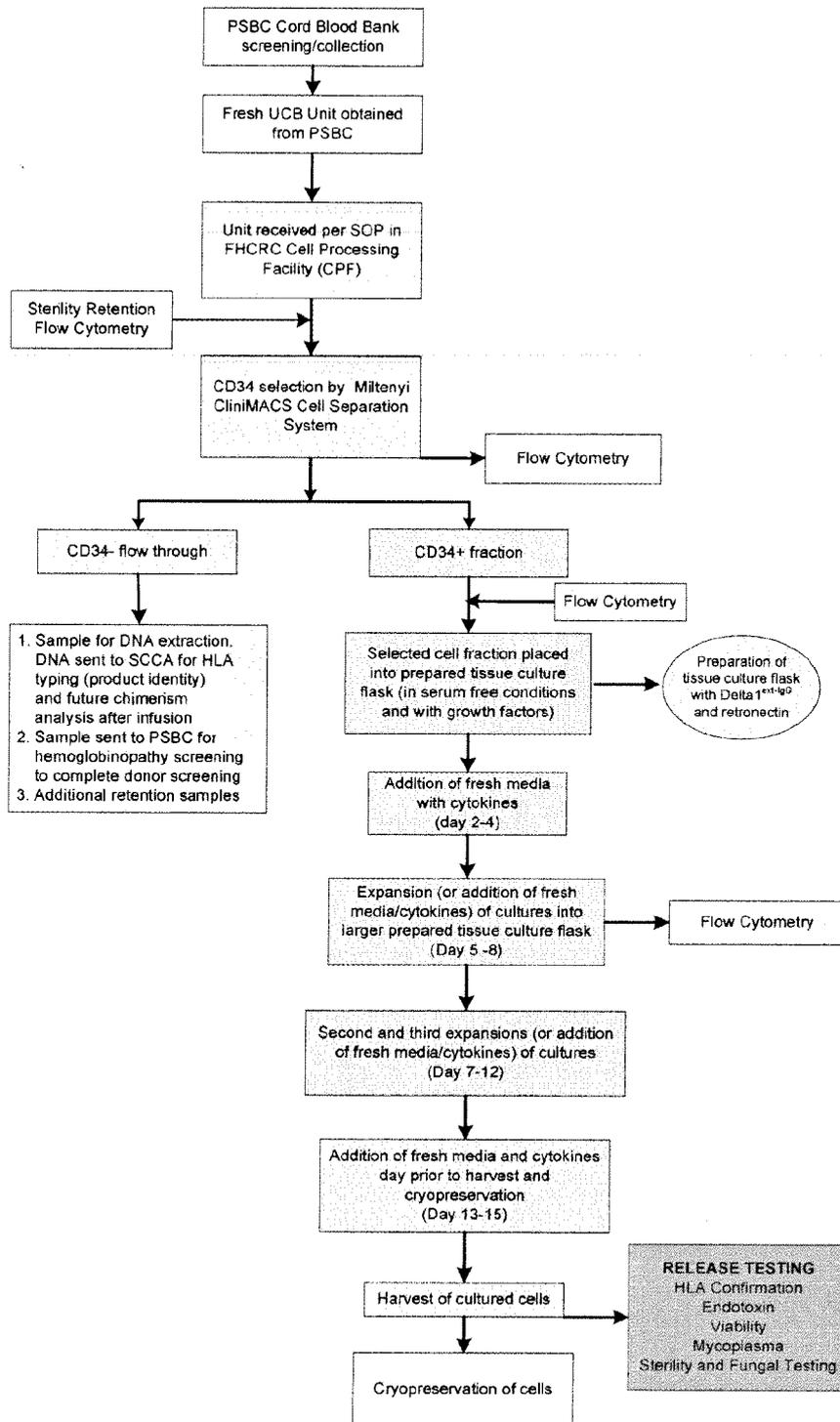
Figure 13

PLAN FOR INDUCTION THERAPY
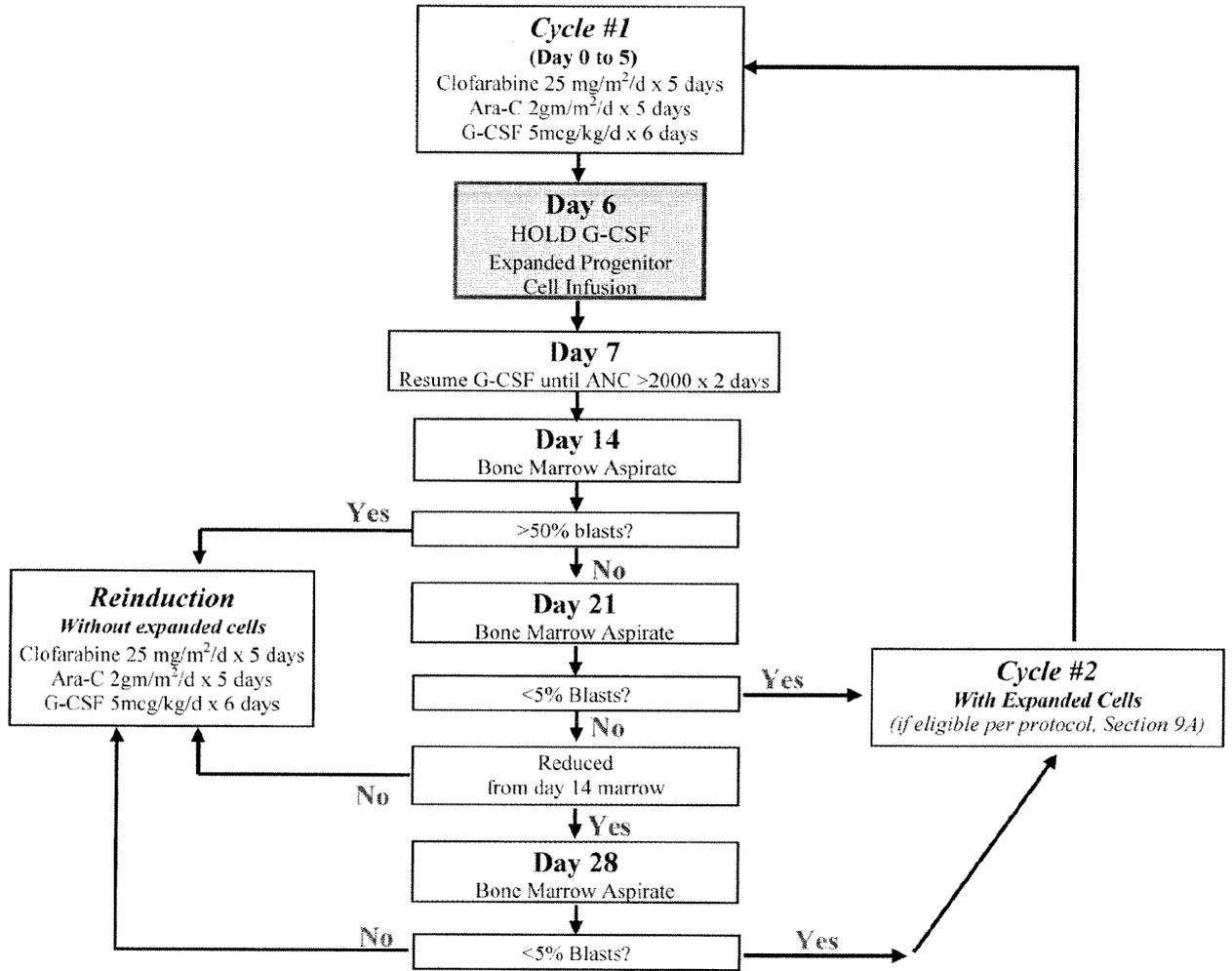
Figure 14

Patient/Cells Characteristics

Patients

Total N	9
Male/Female	2/3
Age (years)	55 (40 to 59)
Weight (kg)	87 (64 to 126)
Disease	
Relapsed	7
Primary refractory	2
Expanded cells (n=12, data n=10)	
CD34/kg (infused)	8.8 x 10⁶ (4.9 to 15.3)

Neutrophil recovery (chemo day)

Patient	ANC >500
Pt 1, cycle 1	22
Pt 1, cycle 2 **	59
Pt 2, cycle 1	18
Pt 2, cycle 2**	19
Pt 3, cycle 1	27
Pt 4, cycle 1	19
Pt 4, cycle 2 **	18
Pt 5, cycle 1	18
Pt 6, cycle 1	Blast recovery
Pt 7, cycle 1	Blast recovery
Pt 8, cycle 1	Pending
Pt 9, cycle 1	Pending

Figure 15

Expanded cell engraftment (% donor)
Day 7 post infusion of product (day 13 chemo)

Patient	CD33	CD14	CD56	CD3	Whole Blood	BM Chim
Pt 1, Cy 1	0	0	0	0	0	0
Pt 2, Cy 1	0	0	0	0	0	0
Pt 1, Cy 2*	100	100	0	0	25	11
Pt 2, Cy 1	0	0	0	0	0	0
Pt 2, Cy 2**	85	100	0	0	30	3
Pt 3, Cy 1	0	0	0	0	0	0
Pt 4, Cy 1	0	0	0	0	0	0
Pt 5, Cy 1	n/d	n/d	n/d	n/d	0	0 (60% blasts)
Pt 6, Cy 1	0	0	0	0	0	0
Pt 7, Cy 1	0	0	0	0	0	0
Pt 8, Cy 1	43	QNS	QNS	0	23	22
Pt 9, Cy 1	Too early post chemotherapy					80-90% blasts

*Cycle 2 started day 28 of cycle 1   ** Cycle 2 started day 40 of cycle 1

Figure 16
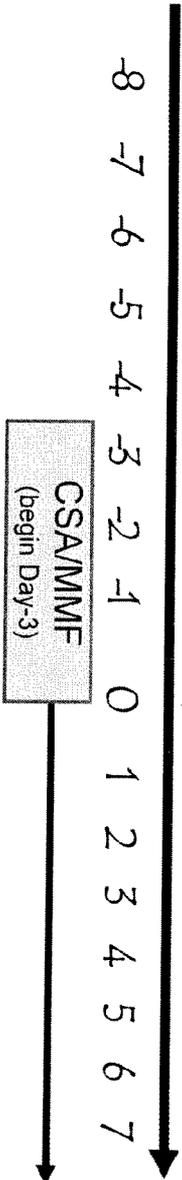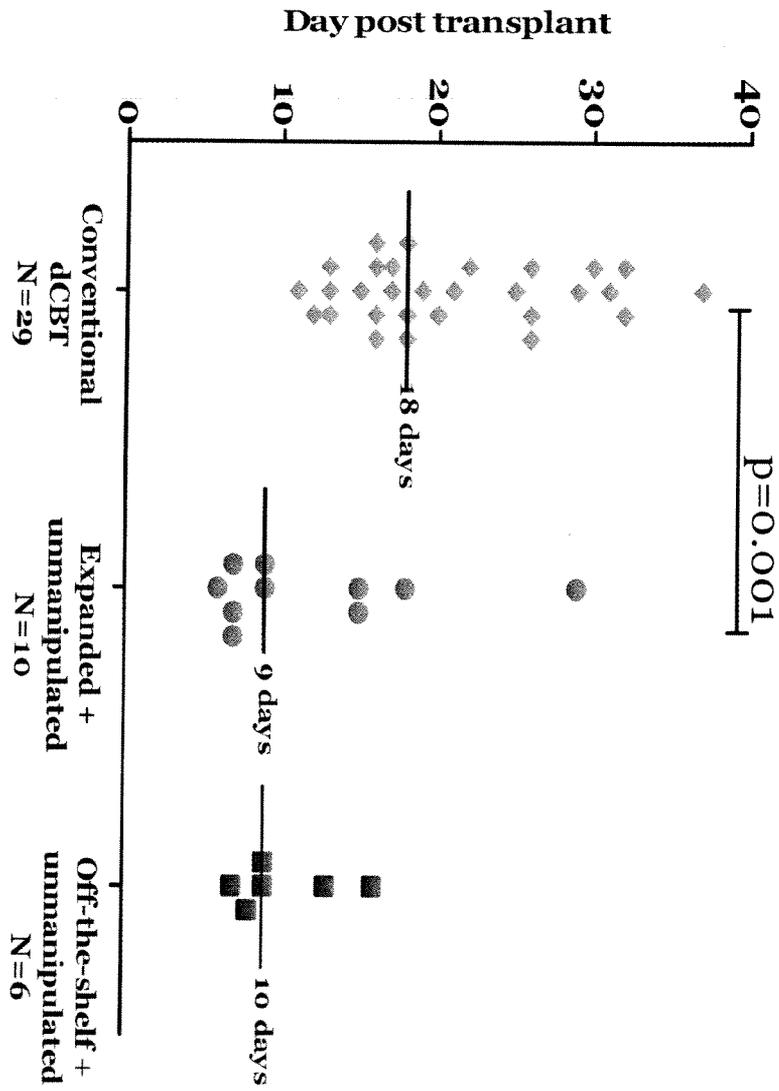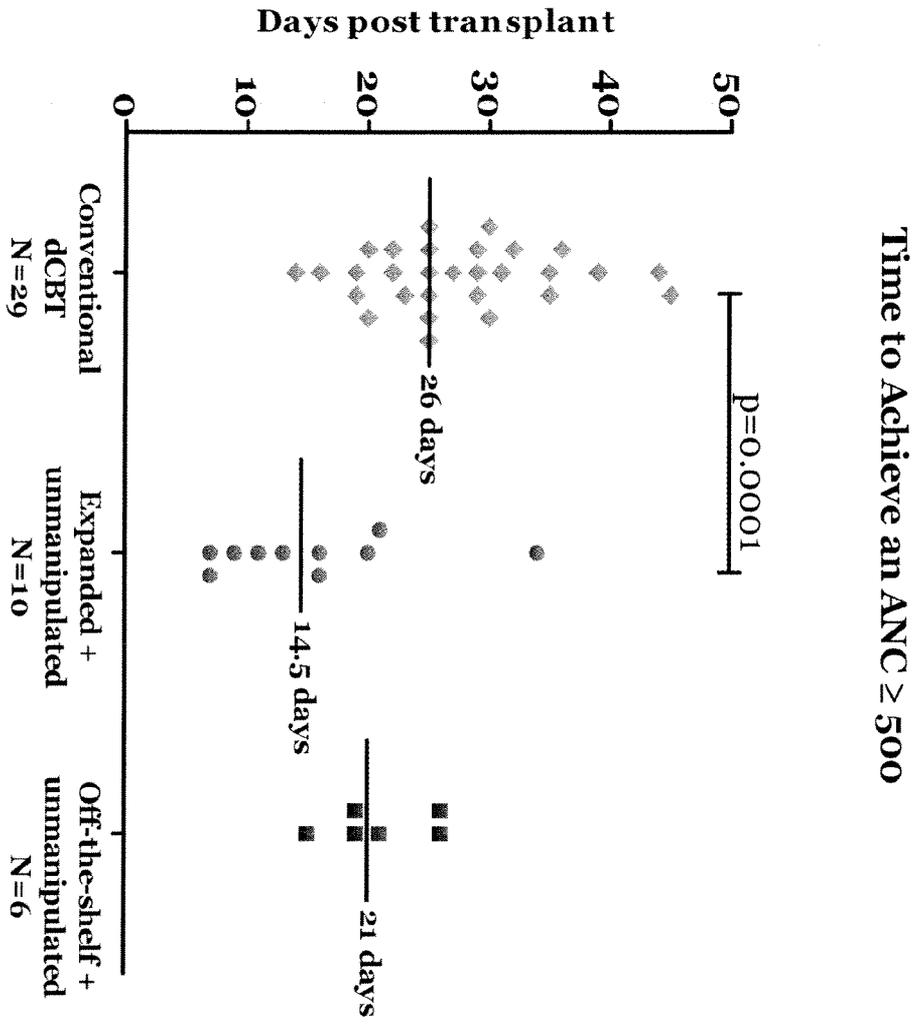
Figure 17

Figure 18

Figure 19

Day 7 Peripheral Blood DNA Chimerism Analysis

		Patient 1	Patient 2	Patient 3	Patient 4
CD33	Expanded	100%	100%	100%	100%
	Unmanipulated 1	0%	0%	0%	0%
	Unmanipulated 2	0%	0%	0%	0%
	Host	0%	0%	0%	0%
CD14	Expanded	100%	97%	100%	98%
	Unmanipulated 1	0%	3%	0%	1%
	Unmanipulated 2	0%	0%	0%	0%
	Host	0%	0%	0%	1%
CD56	Expanded	11%	5%	11%	0%
	Unmanipulated 1	79%	60%	63%	42%
	Unmanipulated 2	0%	35%	20%	56%
	Host	11%	0%	6%	2%
CD19	Expanded				
	Unmanipulated 1	QNS	QNS	QNS	QNS
	Unmanipulated 2				
	Host				
CD3	Expanded	0%	0%	0%	0%
	Unmanipulated 1	43%	81%	83%	53%
	Unmanipulated 2	34%	8%	1%	19%
	Host	23%	11%	16%	28%

Figure 20

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K35/14   A61K35/44   A61K35/50   C12N5/0797   C07K16/28
      A61P35/02   A61P7/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C12N A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DELANEY COLLEEN ET AL: "Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution", NATURE MEDICINE, vol. 16, no. 2, February 2010 (2010-02), page 232, XP009150067, ISSN: 1078-8956 cited in the application page 233, column 1 - page 234, column 2; figures; tables -----	1-40
Y	US 2004/067583 A1 (BERNSTEIN IRWIN D [US] ET AL) 8 April 2004 (2004-04-08) paragraphs [0184], [204], [255], [256], [358]; claims ----- -/--	1-40

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

*   Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2011	21/07/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Langer, Astrid

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	paragraphs [0114], [290], [316]; claims	1-40

Y	WO 03/075789 A2 (RICHARD DANIEL D [US]) 18 September 2003 (2003-09-18) claims	26-31, 34-40

A,P	REIMANN C ET AL: "Advances in adoptive immunotherapy to accelerate T-cellular immune reconstitution after HLA-incompatible hematopoietic stem cell transplantation", IMMUNOTHERAPY 2010 FUTURE MEDICINE LTD. GBR LNKD- DOI:10.2217/IMT.10.36, vol. 2, no. 4, July 2010 (2010-07), pages 481-496, XP001525876, ISSN: 1750-743X the whole document	1-40

X,P	MILANO FILIPPO ET AL: "Cryopreserved Ex-Vivo Expanded Murine Hematopoietic Progenitors Can Engraft Across MHC Barriers and Improve Survival In a Murine Model of Acute Radiation Syndrome", BLOOD, vol. 116, no. 21, November 2010 (2010-11), page 626, XP009150066, & 52ND ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); ORLANDO, FL, USA; DECEMBER 04 -07, 2010 the whole document	1-3,7-20

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			JP	2008517948	A	29-05-2008
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