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(54) Title: AUGMENTATION OF CELL THERAPY EFFICACY INCLUDING TREATMENT WITH ALPHA 1-3 FUCOSYLTRANSFERASE

(57) Abstract: Disclosed are methods, compositions of matter, and kits useful for augmentation of homing and engraftment of stem, progenitor and mature cells through modification of cellular membrane properties following ex vivo treatment. The methods, compositions, and cells may be used for the treatment of a wide variety of disorders in which augmentation of cell trafficking, homing and engraftment is desired.

## AUGMENTATION OF CELL THERAPY EFFICACY INCLUDING TREATMENT WITH ALPHA 1-3 FUCOSYLYTRANSFERASE

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/060,084, filed on June 9, 2008 entitled AUGMENTATION OF CELL THERAPY EFFICACY BY SURFACE MODIFICATION OF MOLECULES INVOLVED IN ENDOTHELIAL INTERACTION INCLUDING TREATMENT WITH ALPHA 1-3 FUCOSYLTRANSFERASE, which is incorporated herein by reference in its entirety.

### BACKGROUND

#### Field

[0002] The present embodiments relate generally to the field of cellular therapy. More specifically, some embodiments relate to methods of enhancing the natural process of cell migration through augmentation of specific glycosylation features on the surface of various cell types. More specifically, some embodiments relate to treatment of cells with fucosyltransferases in order to enhance the interaction between blood-borne stem cells, progenitor cells and endothelial cells facilitating entry into biological niches and tissues where they may function on a number of different levels for therapeutic and restorative intervention.

#### Description of the Related Art

[0003] Cell therapy offers immense possibilities for treatment of a wide variety of medical conditions. Currently cell therapy is practiced in numerous embodiments, for example, bone marrow transplantation for treatment of hematopoietic and non-hematopoietic malignancies. The successful establishment of procedures for transplantation of donor cells into recipients whose function is malignant (leukemia) or altered (stroke, limb ischemia, etc),

or insufficient (due to chemotherapy, radiotherapy, or congenital abnormality) constitutes a major medical breakthrough in the therapeutic management of these conditions.

[0004] One limiting factor of any cellular therapy is the need for blood-borne or directly injected cells to migrate to the targeted tissue in order to maximize their therapeutic potential. With regard to hematopoietic stem cells as a particular example, it is known that only a small percentage of these cells when administered systemically home to the bone marrow microenvironment. This migration is regulated in part by adhesive factors present on the luminal surface of endothelial cells which constitute the microvascular lining of the bone marrow and in part by chemotactic gradients secreted at a constant rate by bone marrow stromal cells. In addition, for the treatment of myocardial infarction or stroke, only a small fraction of injected stem cells actually home and enter the area of tissue damage. Thus, there exists a need to administer a high number of stem cells, sometimes prohibitively too high to be obtained in an autologous and even allogeneic setting.

#### SUMMARY

[0005] The ability of cells such as leukocytes to interact with the endothelium has been known for decades. It has also been known that various glycosylation patterns are critical for cells such as leukocytes to “roll” on the endothelium prior to extravasation. What is desirable is the identification of novel methods that enhance cell trafficking and engraftment to areas of need in a simple and clinically applicable manner.

[0006] Accordingly, provided herein are methods of enhancing homing and engraftment of therapeutically-administered cells in a patient. It should be noted that the term “patient” is meant to broadly include any animal. For example, the animal can be a mammal, a bird, a fish, a reptile, a fish, an insect or any other animal. Some non-limiting examples of mammals may include humans and other primates, equines such as horses, bovines such as cows, mice, rats, rabbits, Guinea Pigs, pigs, and the like. It is also worth noting that the compositions and methods can be used with or applied to individual cells (for example *ex vivo* treatment or modification), to insect cells, etc. Also provided are cells that have been modified to enhance homing and engraftment. The embodiments provided herein are based in part on the surprising finding that by modification of molecules involved in the

cell-endothelium interaction, it is possible to enhance the homing and subsequent efficacy of cellular therapy.

**[0007]** One embodiment provides a method of enhancing homing and engraftment of a therapeutically-administered cell in a patient in need of treatment with a cell population; providing cells that may have been contacted with an agent that modifies at least one surface molecule on the cells, resulting in a population of modified cells; and providing or administering the population of modified cells to a patient in need thereof. In certain aspects, the cell surface molecule may be modified so as to result in an alteration of cell charge.

**[0008]** In one embodiment, a method of enhancing homing and engraftment of a cell, may comprise providing one or more cells selected from stem cells, progenitor cells, neutrophils, macrophages and T-cells. The stem or progenitor cells may be embryonic stem cells, adult stem cells, expanded stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, neuronal stem cells, cardiomyocyte stem cells, placental stem cells, endothelial progenitor cells, circulating and mobilized peripheral blood stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells. One or more cells may have been contacted with an agent that modifies at least one surface molecule on the cell that may result in enhanced selectin-mediated binding. This may result in a population of modified cells. These cells may be provided to animals. Such animals may include birds, reptiles, fish, insects, and mammals including but not limited to humans, equines such as horses, bovines such as cows, dogs, mice, rats, pigs, guinea pigs, rabbits and like.

**[0009]** In certain aspects of the above embodiments, the cell surface molecule may be modified by treatment with an enzyme and appropriate substrate(s) under conditions sufficient for causing an alteration of cell surface charge. In certain aspects, the enzyme may be a glycosidase, glycosyltransferase, a fucosyltransferase, a neuraminidase, an acetylglucosaminyltransferase, or any glycosyltransferase capable of increasing the number or affinity of cell surface selectin binding components. In certain aspects, the enzyme may be

alpha 1,3-fucosyltransferase I, alpha 1,3-fucosyltransferase III, alpha 1,3-fucosyltransferase IV, alpha 1,3-fucosyltransferase V, alpha 1,3-fucosyltransferase VI, alpha 1,3-fucosyltransferase VII, or alpha 1,3-fucosyltransferase IX.

**[0010]** In another aspect of the above embodiment, the cell may be treated with a reagent or reagents that link a binding unit to the cell surface. The binding unit may consist of a particle as well as a ligand of natural or non-natural sugars shown to possess binding affinity for receptors present on endothelial cells similar to that seen with natural sugars. The added binding unit may increase the functionalization of the cell.

**[0011]** In certain further aspects of the above embodiment, the cell may be treated with a single or plurality of molecules having ability to cause alpha 1-3 fucosylation of glycan determinants. In certain aspects, the molecule may be an alpha 1-3 fucosyltransferase mixed together with a concentration of a fucose carrier under conditions sufficient to provide enhanced alpha 1-3 fucosylation of glycan determinants. In certain aspects, the fucose carrier may be guanosine diphosphate fucose. In certain aspects, the alpha 1-3 fucosyltransferase may be alpha 1-3 fucosyltransferase VI. In other aspects, the alpha 1-3 fucosyltransferase may be alpha 1-3 fucosyltransferase VII. In other aspects, the alpha 1-3 fucosyltransferase may be alpha 1-3 fucosyltransferase IV.

**[0012]** In certain aspects of the above embodiment, prior to the providing or administering, the population of modified cells has been further contacted for a period of time insufficient for cell division to occur with a CD26 peptidase inhibitor in an amount effective to inhibit CD26 peptidase activity and effective to increase the migratory response to CXCL12. PCT Application No. PCT/US2009/\_\_\_\_\_, filed on June 9, 2009, entitled METHODS FOR ENHANCING CELL THERAPY EFFICACY INCLUDING TREATMENT WITH CD26 PEPTIDASE INHIBITORS, which is incorporated herein by reference in its entirety, discloses and describes methods and compositions, any of which can be used with the technology of this application in any combination.

**[0013]** In certain aspects, prior to providing modified cells, a recipient may be contacted for a period of time and with sufficient dosing of a CD26 peptidase inhibitor in an amount effective to inhibit recipient CD26 peptidase activity effective to increase the migratory response of donor cells to chemottractant agents such as stromal cell-derived factor.

**[0014]** In certain aspects, the cell population may comprise or consist essentially of a population of stem cells, both embryonic and adult and expanded cell populations. In certain aspects, the stem cells may be embryonic stem cells, cord blood stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, hematopoietic stem cells, mesenchymal stem cells, neuronal stem cells, cardiomyocyte stem cells, circulating and immobilized peripheral blood stem cells, endothelial progenitor cells, monocyte-derived stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells. In certain aspects, the embryonic stem cells may be totipotent. In certain aspects, the stem cell may be hematopoietic, mesenchymal, neural or cardiomyocyte stem cells. In certain aspects the hematopoietic stem cells may be further defined and differentiated as CD38-, lin- or ALDH-bright cells.

**[0015]** In certain aspects, the cell population may comprise or consist essentially of a population of committed progenitor cells or differentiated cells. In certain aspects, the cell population may be a mature blood cell population. In certain aspects, the mature blood cell may be neutrophils, macrophages, T-cells, activated T-cells, helper T cells, cytolytic T-cells, memory T-cells, regulatory T-cells or reprogrammed cells. In certain aspects, the T-cells may be from a heterogeneous population of T-cells.

**[0016]** In certain aspects, the patient in need of treatment with a cell population suffers from a malignant or non-malignant blood disorder such as an acute leukemia, a chronic leukemia, a myelodysplastic syndrome, a stem cell disorder, a myeloproliferative disorder, a lymphoproliferative disorder, a phagocyte disorder, a histiocytic disorder, a lysosomal storage disease, an age related disorder, an arterial or blood vessel or cardiovascular disorder, an enzyme deficiency disorder, a congenital immune system disorder, an inherited erythrocyte abnormality, an inherited platelet abnormality, a plasma cell disorder, a tumor or an autoimmune disease. In certain aspects, the patient in need of treatment with a cell population may suffer from peripheral arterial diseases, ischemic limb injury, diabetes, heart disease, bone disease, liver disease, muscular dystrophy, Alzheimer's disease, ALS, multiple sclerosis, Parkinson's disease, spinal cord injury, stroke or infertility.

[0017] In certain aspects, the population of modified cells may be administered intravenously, intraarterially, intramuscularly, subcutaneously, transdermally, intratracheally, intraperitoneally, intrathecally intracranially, intravitreally, or directly into the microvascular compartment of bone or into spinal fluid. In certain aspects, the population of modified cells may be administered in or proximal to a site of injury. In certain aspects, the homing and engraftment may take place within the bone marrow of the patient in need thereof.

[0018] In another embodiment, a composition may comprise an isolated population of cells modified for enhanced selectin-mediated binding. The isolated population of cells may be neutrophils, macrophages, T-cells, subpopulation of T-cells, or stem or progenitor cells selected from a group consisting of: embryonic stem cells, adult stem cells, expanded stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, neuronal stem cells, cardiomyocyte stem cells, endothelial progenitor cells, circulating and mobilized peripheral blood stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, or reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells and a pharmaceutically-acceptable carrier

[0019] In certain aspects, the isolated population may comprise a cell surface modification. In certain aspects, the cell surface molecule may be modified by treatment with an enzyme and appropriate substrate(s) under conditions sufficient for causing an alteration of cell surface charge.

[0020] In certain aspects, the enzyme is selected from a group comprising of: a glycosidase, a glycosyltransferase, a fucosyltransferase, a neuraminidase, and an acetylglucosaminyltransferase or any other glycotransferases capable of increasing cell surface selectin binding components. In certain aspects, the enzyme is selected from a group comprising of alpha 1,3-fucosyltransferase I, alpha 1,3-fucosyltransferase III, alpha 1,3-fucosyltransferase IV, alpha 1,3-fucosyltransferase V, alpha 1,3-fucosyltransferase VI, alpha 1,3-fucosyltransferase VII and alpha 1,3-fucosyltransferase IX.

[0021] In certain aspects, the cell may be treated with a single or plurality of molecules having ability to cause alpha 1-3 fucosylation of glycan determinants. In certain

aspects, the molecule may be an alpha-1-3 fucosyltransferase mixed together with a concentration of a fucose carrier under conditions sufficient to provide enhanced alpha 1-3 fucosylation of glycan determinants. In certain aspects, the fucose carrier may be guanosine diphosphate fucose. In certain aspects, the alpha 1-3 fucosyltransferase may be alpha 1-3 fucosyltransferase VI. In certain aspects, the alpha 1-3 fucosyltransferase may be alpha 1-3 fucosyltransferase VII. In certain aspects the alpha 1-3 fucosyltransferase may be alpha 1-3 fucosyltransferase IV. In certain aspects the molecule may be a non-naturally occurring enzyme having the ability to add a glycan determinant or a non-natural sugar that mimics the activity of fucose or other sugars that enhance the selectin binding process.

**[0022]** In certain aspects, the cell population may comprise or consist essentially of a population of stem cells both embryonic and adult. In certain aspects, the stem cells may be embryonic stem cells, cord blood stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, hematopoietic stem cells, mesenchymal stem cells, neuronal stem cells, cardiomyocyte stem cells, circulating and mobilized peripheral blood stem cells, endothelial progenitor cells, monocyte-derived stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells. In certain aspects, the embryonic stem cells may be totipotent. In certain aspects, the stem cell may be hematopoietic, mesenchymal, neural or cardiomyocyte stem cells.

**[0023]** In certain aspects, the cell may be a mature blood cell. In certain aspects, the mature blood cell may be a neutrophil, macrophage, or T-cell. In certain aspects, the T-cells may be from a heterogeneous population of T-cells or from an ex vivo expanded cell population.

**[0024]** Another embodiment provides a method of enhancing homing and engraftment of a cell, comprising providing one or more cells selected from stem cells, progenitor cells, neutrophils, macrophages and T-cells. The stem or progenitor cells may be embryonic stem cells, adult stem cells, expanded stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, neuronal stem cells, cardiomyocyte stem cells, endothelial progenitor cells, circulating and immobilized peripheral blood stem cells, muscle

stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells. One or more cells may be contacted with an agent that modifies at least one surface molecule on the cell(s) to result in enhanced selectin-mediated binding, resulting in a population of modified cells.

**[0025]** Also provided herein is a method of fucosylation of cells so as to increase the ability of the cells to traffic, home and engraft into an area of biological need. The cells may be mature fully differentiated cells whose homing to specific targets is desired, such as islets, hepatocytes, or neutrophils or cells may be progenitor cells capable of differentiating into functional cells such as hepatic, renal, cardiac, or islet progenitors, or alternatively, the cells may be stem cells with multilineage differentiation ability such as embryonic stem cells, cord blood stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, neuronal stem cells, circulating and mobilized peripheral blood stem cells, mesenchymal stem cells, endothelial stem cells, cardiomyocyte stem cells, germinal stem cells, committed endothelial progenitor cells, committed progenitor cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, chemically, biologically, or electronically reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population of stem cells.

**[0026]** Other aspects relate to enhancing the ability of cells to modulate the immune system through enabling the cells to function with augmented efficiency at trafficking and homing. In another aspect, cells useful for immune therapy are “reprogrammed” ex vivo with endowment of distinct immunological properties. Surface modification may be performed before reprogramming, during reprogramming or after reprogramming. Reprogramming may be performed so as to increase immune stimulatory properties of the immune cells, or may be performed to allow the immune cells to suppress other immune cells. Reprogramming may be performed during expansion of cells, or to cells that have already been expanded.

[0027] In certain aspects, the cells can be fucosylated so as to enhance ability to home. Fucosylation may be performed on specific molecules present on the cells, or may be performed globally in a non-specific manner. Cells may be fucosylated through culture with an enzyme such as a fucosyltransferase capable of transferring fucose groups such as a fucosyltransferase. In certain aspects, the fucosyltransferase may be an alpha 1,3 fucosyltransferase. In another aspect the enzyme is selected from a group comprising of alpha 1,3-fucosyltransferase IX, alpha 1,3-fucosyltransferase III, alpha 1,3-fucosyltransferase IV, alpha 1,3-fucosyltransferase V, alpha 1,3-fucosyltransferase VI and alpha 1,3-fucosyltransferase VII. Appropriate culture conditions and substrates are also provided within the scope of the embodiments in order to allow proper fucosylation to occur. The conditions may include addition of substrates such as GDP-fucose or other similar compounds that provide a source of fucose.

[0028] In certain aspects, cells may be treated with a single or plurality of agents in order to augment expression of proteins involved in migration. Since the proteins involved in migration, when expressed de-novo, are not properly fucosylated, the addition of exogenous fucose groups increases ability of the de-novo expressed proteins to interact with endothelium and properly home. Specific molecules may include histone deacetylase inhibitors or DNA methyltransferase inhibitors.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Figure 1 the effect of pretreatment of HNSCs with FTVI on level of fucosylation. The expression of CLA was used to determine the levels of fucosylation. The CLA expression by hNSCs was determined by FACS analysis with untreated cells (A) or following pre-incubation with the fucosylation mix (GDP-Fucose, manganese and FTVI conditioned medium) (B). Isotype-matched IgG was used as the negative control. Fluorescence intensity (FL2) of samples was evaluated by FACScanto. The results of one experiment are shown.

#### DETAILED DESCRIPTION

[0030] Some embodiments relate generally to compositions for and methods of enhancing homing and engraftment of a therapeutically-administered cell in a patient. Also, some embodiments relate to cells that have been modified to enhance homing and engraftment. The embodiments provided herein are based in part on the surprising finding that by modification of molecules involved in the cell-endothelium interaction, it is possible to enhance the homing and subsequent efficacy of cellular therapy.

[0031] The process by which cells exit the systemic circulation and enter distinct biological niches is a complex coordinated process that involves numerous molecules. The process of cellular exit is a bi-directional communication between the vascular endothelium cells and the circulating stem and progenitor cells. This process has been best characterized in the description of leukocyte exit from systemic circulation. An important family of molecules involved critically and initially in the process of cellular trafficking are the selectins. These molecules are type 1 transmembrane proteins that contain what is known as C-type lectin domains. Lectins are proteins that bind sugars. Most commonly known lectins include concanavalin-A and phytohemagglutinin. The C-type lectin domains on the selectins reside at the N-terminal of the selectins and interact with a wide array of glycoprotein ligands. Since the lectin domains bind sugar moieties, it is important that proper placement of sugars onto the proteins such that interaction of the selectins occurs. Since placement of sugars (glycosylation) usually occurs as a post-translational event, the mere genetic manipulation of cells is not sufficient usually to alter ability of cells to interact with selectins. The exception to this is, of course, genetic manipulation in the sense of transfecting cells with enzymes that are involved in the addition of sugars.

[0032] Provided herein are means of endowing enhanced trafficking/homing capabilities onto cells for use in cell therapy, said means consisting of modification of various glycosylation patterns on cells in order to augment ability to “roll”, ‘tether’, and ‘adhere’ on the endothelium. The concept of cells rolling, tethering, and adhering on the endothelium is commonly known in the art and means of augmentation of this rolling process have been described strictly in the areas of hematopoietic cells, as well as tumor cells.

[0033] Previous studies with hematopoietic cells have demonstrated that it is possible to alter glycosylation and fucosylation patterns on the surface of cells by treating of

cells with enzymes such as fucosyltransferases. In addition to establishing the ability to modify the surface of cells, the functional consequences of this modification has been documented. Specifically, several reports (Xia et al., Blood 2004 104:3091 – 3096; Hidalgo, et al., J. Clin. Invest. 2002 110:559 – 569, each of which is hereby incorporated by reference in its entirety) demonstrated that fucosylation of cord blood hematopoietic cells enhances binding of P and E selectin, enhances ex vivo binding to P and E-selectin-coated plates under physiological shear stress conditions and enhances homing and engraftment into bone marrow of NOD-SCID mice. (See also U.S. Patent No. 7,332,334 and U.S. Pub. No. 2006/0228340 to Xia and McEver, each of which is hereby incorporated by reference in its entirety). These findings in hematopoietic cells have been further described by others, including Sackstein et al. (U.S. 2003/0040607 and U.S. 2008/0044383, each of which is incorporated by reference in its entirety). Each reference listed in this paragraph is incorporated herein by reference in its entirety.

**[0034]** However another report demonstrated that while early (minutes to hours following iv injection) adhesion of cord blood hematopoietic cells is increased after ex vivo fucosylation, no increase in bone marrow homing at 16 – 24 hrs was observed (Hidalgo and Frenette, Blood 2005 105:567 – 575, which is incorporated herein by reference in its entirety).

**[0035]** It has been suggested by Sackstein et al. that mesenchymal stem cells, which do not express E-selectin ligands, can be glycosylated enzymatically in an effort to enhance migration of these cells to the bone marrow (Sackstein et al. Nature Medicine 2008 14:181-187; which is incorporated herein by reference in its entirety).

**[0036]** Thus, embodiments presented herein are based in part on the novel observation that fucosylation of ligands increases binding to tissue and can be used to enhance migration of various types of cells, enumerated herein, to an area of need. Specifically, some embodiments provided herein relate in part to the surprising finding that augmentation of the tethering and rolling process through various means is useful for enhancing functional capabilities of a wide variety of non-hematopoietic cells and stem cells. Accordingly, the methods and compositions provided herein may be useful for treatment of a wide variety of medical conditions which are amenable to cell therapy.

Therapeutic Methods

[0037] In accordance with the above, provided herein are methods of enhancing homing and engraftment of a therapeutically-administered cell in a patient. Specifically, provided herein are methods of modification of sugar residues, both natural and non-natural, on the surface of cells used for cellular therapy so as to enhance their interaction with members of the selectin family, thereby enhancing trafficking of the cells administered systemically to an area of need.

[0038] Also provided are cells that have been modified to enhance homing and engraftment. The embodiments provided herein are based in part on the surprising finding that by modification of molecules involved in the cell-endothelium interaction, it is possible to enhance the homing and subsequent efficacy of cellular therapy.

[0039] Examples of cells include, without being limited thereto, neutrophils, macrophages and T-cells, wherein the stem or progenitor cells are selected from a group consisting of: embryonic stem cells, adult stem cells, expanded stem cells, placental stem cells, bone marrow stem cells, hematopoietic stem cells, mesenchymal stem cells, amniotic fluid stem cells, neuronal stem cells, cardiomyocyte stem cells, endothelial progenitor cells, circulating and mobilized peripheral blood stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells. In some aspects, one or more of the cell types mentioned can be specifically excluded from the methods or compositions described herein. As one example, in some aspects mesenchymal or hematopoietic stem cells can be excluded.

[0040] In one embodiment is provided a method of enhancing homing and engraftment of a therapeutically-administered cell in a patient comprising selecting a patient in need of treatment with a cell population; providing cells that have been contacted with an agent that modifies at least one surface molecule on the cells, resulting in a population of modified cells; and providing or administering the population of modified cells to a patient in

need thereof. In certain aspects, the cell surface molecule is modified so as to result in an alteration of cell charge.

#### Modification Enzymes

**[0041]** In certain aspects of the above embodiments, the cell surface molecule is modified by treatment with an enzyme and appropriate substrate(s) under conditions sufficient for causing an alteration of cell surface charge. Enzymes which modify cell surface molecules are known in the art. Such enzymes include a purified glycosyltransferase polypeptide. Glycosyltransferases include for example, fucosyltransferase, galactosyltransferase, sialyltransferase and N-acetylglucosaminotransferase. The fucosyltransferase can be, for example, an alpha 1,3 fucosyltransferase such as an alpha 1,3-fucosyltransferase I, alpha 1,3-fucosyltransferase III, alpha 1,3-fucosyltransferase IV, alpha 1,3-fucosyltransferase V, alpha 1,3-fucosyltransferase VI, alpha 1,3-fucosyltransferase VII, and alpha 1,3-fucosyltransferase IX. It should be noted that in some embodiments, one or more of the enzymes listed herein can be specifically excluded. For example, in some aspects, FTVI can be specifically excluded from the methods and compositions described herein.

**[0042]** In certain aspects, the cell surface molecule is modified in the presence of a sugar donor suitable for the specific glycosyltransferase. Sugar donors for glycosyltransferases are known in the art. For example, when the glycosyltransferase is a fucosyltransferase, the donor is GDP-fucose. Whereas, when the glycosyltransferase is a sialyltransferase, the donor is CMP-sialic acid. In some instances the sugar can be a non-natural sugar added by a natural or modified glycosyltransferase.

**[0043]** The glycosyltransferases are biologically active. By biologically active is meant that the glycosyltransferases are capable of transferring a sugar molecule from a donor to acceptor. For example, the glycosyltransferase is capable of transferring 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 2.5, 5, 10 or more  $\mu$ moles of sugar per minute at pH 6.5 at 37°C.

**[0044]** Physiologically acceptable solution is any solution that does not cause cell damage, e.g. death. For example, the viability of the cell or cell particle is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more after treatment according to the methods presented

herein. Suitable physiologically acceptable solutions include for example, Hank's Balanced Salt Solution (HBSS), Dulbecco's Modified Eagle Medium (DMEM), a Good's buffer (see N. E. Good, G. D. Winget, W. Winter, T. N. Conolly, S. Izawa and R. M. M. Singh, *Biochemistry* 5, 467 (1966); N. E. Good, S. Izawa, *Methods Enzymol.* 24, 62 (1972) such as a HEPES buffer, a 2-Morpholinoethanesulfonic acid (MES) buffer, or phosphate buffered saline (PBS). Each reference listed in this paragraph is incorporated herein by reference in its entirety.

[0045] Thus, in certain embodiments provided herein, cells can be treated *ex vivo* with a kit that contains some or all of the agents, such as enzyme, buffer, cofactors and substrate necessary to achieve fucosylation of the cell surface glycoproteins that mediate adhesive interactions between the circulating cells following iv administration and endothelial cells at targeted tissue sites. In another embodiment provided herein, cells such as cord blood can be pretreated with the fucosylation kit prior to freezing or storage.

[0046] In certain aspects, the syn-anti, and or  $\alpha$ - $\beta$  substitution and orientation of the polysaccharide are modulated to provide the desired effect. Cell surface oligosaccharides are highly diversified in their structures and are associated with a variety of cell functions. In an inflammatory response, for example, neutrophils or leukocytes bind to injured tissues where the adhesion process occurs. This process has been found to be mediated by the tetrasaccharide sialyl Lewis X on neutrophils or leukocytes and the receptor ELAM-1 (endothelial leukocyte adhesion molecule 1), a glycoprotein of the selectin family. Several sialyl lewis analogues and mimetics have been analyzed, in part, to understand affects of syn versus anti sugar conformers. Similar studies have examined fucosyl and galactosyl conformers, diastereomers, epimers and chiral analogues to examine adhesion and inhibitory properties. See Ichikawa Y *et al*; *J. Am. Chem. Soc.* 1992, 114, 9283-9298; Nelson, Richard M. *et al.* *J. Clin. Invest.* 1993, 1157-1166; Chun-Cheng Lin *et al.*, *J. Am. Chem. Soc.* 1996, 118, 6826-6840; Clarke Julia L. *J. Am. Chem. Soc.* 1996, 118, 6826-6840; Chikara Ohyama, *et al.*, *The EMBO Journal* Vol.18 No.6 pp.1516-1525, 1999; each of which is incorporated herein by reference in its entirety. Each reference listed in this paragraph is incorporated herein by reference in its entirety.

[0047] The introduction of fucose onto surface glycans of the cells of interest can be accomplished by enzymatic transfer from a donor substrate utilizing an alpha 1-3-fucosyltransferase (FT) by a process well known to someone skilled in the art. For this transfer to occur the cells at varying concentrations can be exposed to an incubation buffer containing a number of ingredients each of which can be optimized for efficient transfer of the fucose. The selection of buffer can come from a number of available buffers with Hanks balanced salt solution (HBSS) serving as the primary example. The substrate, guanosine diphosphate-fucose (GDP-fucose), at 1 mM can be mixed with the FT added at sufficient activity, expressed as Units/mL, to achieve maximal transfer of fucose to the cells of interest. In addition, MnCl<sub>2</sub> at a final concentration of 0 – 10 mM can be added, if needed, depending on the cell population to further accelerate the enzymatic transfer reaction. The temperature and time of incubation can also be optimized for maximal transfer of fucose under practical application conditions with minimum toxicity to the cells of interest but is generally conducted at 37°C for 40 minutes.

[0048] Confirmation of fucosylated epitopes on the cells of interest as means of confirming maximal levels of fucosylation can be verified by Flow Cytometry utilizing agents and procedures well know to someone skilled in the art. For example, sialyl LewisX is a fucosylation epitope found on both P and E-selectins. By incubation of the FT-treated cells with anti-sLeX mAb HECA 452 (IgM) followed by treatment with FITC-conjugated fragment to the IgM, the sLeX epitopes on the cell surface can be visualized using standard Flow Cytometry procedures.

#### Combination Treatment with CD26 Peptidase Inhibitors

[0049] In certain aspects of the above embodiment, prior to the administering, the population of modified cells has been further contacted for a period of time insufficient for cell division to occur with a CD26 peptidase inhibitor in an amount effective to inhibit CD26 peptidase activity and effective to increase the migratory response to CXCL12.

[0050] Exemplary methods of treating stem cells with CD26 (dipeptidylpeptidase) inhibitors are described in Christopherson et al. (U.S. Pub No. 2004/0247574, incorporated by reference in its entirety). In certain aspects, the CD26 inhibitor is selected from the group

consisting of Diprotin A (Ile-Pro-Ile), Valine-Pyrrolidide, sitagliptin, vildagliptin, saxagliptin, alogliptin or any other class of compounds shown to exhibit potent inhibition of either purified, soluble or cell surface (CD26) dipeptidylpeptidase. PCT Application No. PCT/US2009/\_\_\_\_\_, filed on June 9, 2009, entitled METHODS FOR ENHANCING CELL THERAPY EFFICACY INCLUDING TREATMENT WITH CD26 PEPTIDASE INHIBITORS, which is incorporated herein by reference in its entirety, discloses and describes methods and compositions, any of which can be used with the technology of this application in any combination.

**[0051]** In certain aspects, the cell population is contacted with said CD26 inhibitor for about 5 minutes to about 12 hours or conditions suitable for sufficient inhibition of cell surface CD26 leading to an enhanced migratory response to chemotactic factors such as stromal cell-derived factor. In certain aspects, the cell population is contacted with said CD26 inhibitor for about 5 minutes to about 12 hours. In certain aspects, the cell population is contacted with said CD26 inhibitor for less than 6 hours. In certain aspects, the cell population is contacted with said CD26 inhibitor for less than 2 hours. In certain aspects, the cell population is contacted with said CD26 inhibitor for less than 1 hour.

**[0052]** In certain aspects, the inhibitor is administered in a concentration of less than about 1 nM, about 1  $\mu$ M, about 5  $\mu$ M, about 10  $\mu$ M, about 50  $\mu$ M, about 100  $\mu$ M, about 1 mM or about 5 mM. In certain aspects, the inhibitor is administered in a concentration of no less than about 5 mM.

**[0053]** In certain aspects, at least 1 donor cell is treated. In selected embodiments, at least  $1 \times 10^2$ ,  $1 \times 10^3$ ,  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$  donor cells per mL are treated.

**[0054]** In certain aspects the recipient can be treated with CD26 inhibitor simultaneous with injection of cell surface modified cells. In certain aspects the recipient can be treated with CD26 inhibitor prior to injection of cell surface modified cells. In certain aspects the recipient is pretreated with single or multiple doses of the CD26 inhibitor either simultaneous or prior to cell injection to achieve sustained inhibition of either or both administered cells and recipient CD26 activity leading to enhanced homing of administered cells.

### Therapeutic Cell Populations

[0055] Stem Cells. In certain aspects, the cell population comprises or consists essentially of a population of stem cells. In certain aspects, the stem cells are selected from a group consisting of: embryonic stem cells, cord blood stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, hematopoietic stem cells, mesenchymal stem cells, neuronal stem cells, cardiomyocyte stem cells, circulating and immobilized peripheral blood stem cells, mesenchymal stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells or transdifferentiated cells. In certain aspects, the embryonic stem cells are totipotent.

[0056] As used herein, a "mesenchymal cell" means a cell forming a mesenchymal tissue, such as osteoblast, chondrocyte, myoblast, adipocyte, stroma cell, tendon cell, and the like, a mesenchymal stem cell capable of differentiating into these cells, and its pre-mesenchymal stem cell. Mesenchymal cells generated during the embryo development, mesenchymal cells within an animal body, and mesenchymal cells differentiated and generated from pluripotent stem cells in vitro or in vivo are all encompassed in the term "mesenchymal cell."

[0057] As used herein, a "mesenchymal stem cell" means a mesenchymal cell possessing the ability of differentiating into mesenchymal cells of one or more types and the ability of self-replication. The mesenchymal stem cell differentiated from a pluripotent stem cell in vitro is positive for PDGFR $\alpha$  and negative for FLK1. Mesenchymal stem cells are able to differentiate into osteoblasts, chondrocytes, myoblasts, adipocytes, stroma cells, tendon cells, and the like, as with mesodermal cells.

[0058] As used herein, a "pre-mesenchymal stem cell" means a mesenchymal cell possessing the ability of differentiating into mesenchymal stem cells of one or more types and the ability of self-replication. The pre-mesenchymal stem cell differentiated from a pluripotent stem cell in vitro expresses Sox1, a neuroectodermal marker. The pre-mesenchymal stem cell

is able to differentiate into a mesenchymal stem cell which is PDGFR $\alpha$ -positive and FLK1-negative.

[0059] As used herein, a "neural stem cell" refers to a multipotent cell obtained from the central nervous system that can be caused to differentiate into cells that possess one or more biological activities of a neuronal cell type. Neural stem cells differentiate into neurons, astrocytes, and oligodendrocytes after plating onto substrates which stimulate adhesion and differentiation, for example poly-L-ornithine or laminin. In addition, these multipotent CNS stem cells proliferate and expand in response to epidermal growth factor ("EGF") and basic fibroblast growth factor ("bFGF") and differentiate into neurons, astrocytes, oligodendrocytes, and muscle stem cells.

#### Committed Progenitor Cells and Differentiated Cells.

[0060] In certain aspects, the cell population comprises or consists essentially of a population of committed progenitor cells or differentiated cells or transdifferentiated cells. In certain aspects, the cell population is a mature blood cell population. In certain aspects, the mature blood cell is selected from the group consisting of: neutrophils, macrophages and T-cells. In certain aspects, the T-cells are from a heterogeneous population of T-cells.

#### Patients in Need of Treatment with Modified Cell Populations

[0061] In certain aspects, the patient in need of treatment with a cell population suffers from a condition selected from the group consisting of: an acute leukemia, a chronic leukemia, a myelodysplastic syndrome, a stem cell disorder, a myeloproliferative disorder, a lymphoproliferative disorder, a phagocyte disorder, a histiocytic disorder, a lysosomal storage disease, a congenital immune system disorder, an inherited erythrocyte abnormality, an inherited platelet abnormality, a plasma cell disorder, a tumor and an autoimmune disease. In certain aspects, the patient in need of treatment with a cell population suffers from a condition selected from the group consisting of: peripheral arterial diseases, ischemic limb injury, diabetes, heart disease, liver disease, bone disease, muscular dystrophy, Alzheimer's disease, ALS, multiple sclerosis, Parkinson's disease, spinal cord injury, stroke and infertility. Further examples of the above-described conditions are set forth in Table I below.

Table I

<p><b><u>Acute Leukemias</u></b>  Acute Biphenotypic Leukemia  Acute Lymphocytic Leukemia (ALL)  Acute Myelogenous Leukemia (AML)  Acute Undifferentiated Leukemia</p>	<p><b><u>Chronic Leukemias</u></b>  Chronic Lymphocytic Leukemia (CLL)  Chronic Myelogenous Leukemia (CML)  Juvenile Chronic Myelogenous Leukemia (JCML)  Juvenile Myelomonocytic Leukemia (JMML)</p>
<p><b><u>Myelodysplastic Syndromes</u></b>  Amyloidosis  Chronic Myelomonocytic Leukemia (CMML)  Refractory Anemia (RA)  Refractory Anemia with Excess Blasts (RAEB)  Refractory Anemia with Excess Blasts in Transformation (RAEB-T)  Refractory Anemia with Ringed Sideroblasts (RARS)</p>	<p><b><u>Stem Cell Disorders</u></b>  Aplastic Anemia (Severe)  Congenital Cytopenia  Dyskeratosis Congenita  Fanconi Anemia  Paroxysmal Nocturnal Hemoglobinuria (PNH)</p>
<p><b><u>Myeloproliferative Disorders</u></b>  Acute Myelofibrosis  Agnogenic Myeloid Metaplasia (Myelofibrosis)  Essential Thrombocythemia  Polycythemia Vera</p>	<p><b><u>Lymphoproliferative Disorders</u></b>  Hodgkin's Disease  Non-Hodgkin's Lymphoma  Prolymphocytic Leukemia</p>
<p><b><u>Phagocyte Disorders</u></b>  Chediak-Higashi Syndrome  Chronic Granulomatous Disease  Neutrophil Actin Deficiency  Reticular Dysgenesis</p>	<p><b><u>Histiocytic Disorders</u></b>  Familial Erythrophagocytic  Lymphohistiocytosis  Hemophagocytosis  Histiocytosis-X  Langerhans' Cell Histiocytosis</p>
<p><b><u>Lysosomal Storage Diseases</u></b>  Adrenoleukodystrophy  Alpha Mannosidosis  Gaucher's Disease  Hunter's Syndrome (MPS-II)  Hurler's Syndrome (MPS-IH)  Krabbe Disease  Maroteaux-Lamy Syndrome (MPS-VI)  Metachromatic Leukodystrophy  Morquio Syndrome (MPS-IV)</p>	<p><b><u>Congenital (Inherited) Immune System Disorders</u></b>  Absence of T and B Cells SCID  Absence of T Cells, Normal B Cell SCID  Ataxia-Telangiectasia  Bare Lymphocyte Syndrome  Common Variable Immunodeficiency  DiGeorge Syndrome  Kostmann Syndrome  Leukocyte Adhesion Deficiency</p>

<p>Mucopolipidosis II (I-cell Disease)  Mucopolysaccharidoses (MPS)  Niemann-Pick Disease  Sanfilippo Syndrome (MPS-III)  Scheie Syndrome (MPS-IS)  Sly Syndrome, Beta-Glucuronidase Deficiency (MPS-VII)  Wolman Disease</p>	<p>Omenn's Syndrome  Severe Combined Immunodeficiency (SCID)  SCID with Adenosine Deaminase Deficiency  Wiskott-Aldrich Syndrome  X-Linked Lymphoproliferative Disorder</p>
<p><b><u>Inherited Erythrocyte Abnormalities</u></b>  Beta Thalassemia Major  Blackfan-Diamond Anemia  Pure Red Cell Aplasia  Sickle Cell Disease</p>	<p><b><u>Other Inherited Disorders</u></b>  Cartilage-Hair Hypoplasia  Ceroid Lipofuscinosis  Congenital Erythropoietic Porphyria  Glanzmann Thrombasthenia  Lesch-Nyhan Syndrome  Osteopetrosis  Sandhoff Disease</p>
<p><b><u>Inherited Platelet Abnormalities</u></b>  Amegakaryocytosis / Congenital  Thrombocytopenia</p>	<p><b><u>Plasma Cell Disorders</u></b>  Multiple Myeloma  Plasma Cell Leukemia  Waldenstrom's Macroglobulinemia</p>
<p><b><u>Other Malignancies</u></b>  Brain Tumors  Ewing Sarcoma  Neuroblastoma  Ovarian Cancer  Renal Cell Carcinoma  Small-Cell Lung Cancer  Testicular Cancer</p>	<p><b><u>Autoimmune Diseases</u></b>  Multiple Sclerosis  Rheumatoid Arthritis  Systemic Lupus Erythematosus  Diabetes Mellitus  Inflammatory Bowel Diseases</p>
<p><b><u>Other Applications</u></b>  Bone Marrow Transplants  Heart Disease (myocardial infarction), either alone or in combination with enhancing agents such as erythropoietin  Liver Disease  Muscular Dystrophy  Alzheimer's Disease  Parkinson's Disease  Spinal Cord Injury  Stroke, either alone or in combination with enhancing agents</p>	

<p>such as erythropoietin  Peripheral Vascular Disease  Head trauma  Ex vivo and In vivo expanded stem and progenitor cell populations  In vitro fertilization application and enhancement  Hematopoietic Rescue Situations (Intense Chemo/Radiation)  Stem cells and progenitor cells derived from various tissues sources  Application in humans and animals  Limb regeneration, alone or in combination with enhancing agents</p>	
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**[0062]** Cellular therapy is also desirable for treatment of diseases in which the immune system is sought to be enhanced. One particular form of cell therapy involves the expansion of T cells that possess specificity for a distinct antigen, for example a tumor antigen. In other types of cell therapy, T cells are generated, and reprogrammed ex vivo for ability to kill a plurality of cells that express a plurality of markers. Examples of such cell therapy include expansion of autologous T cells with IL-2, stimulation with tumor cell lysates, and reintroduction of said cells into the patient.

**[0063]** On the other hand, cell therapy may be performed in situations where suppression of an immune response is desired. In such situations expansion of cells such as CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells is desirable since these cells are capable of inhibiting immune responses in an antigen-specific manner. Methods for expansion of these cells are commonly known and include use of cytokines such as TGF- $\beta$ .

**[0064]** One issue in bone marrow homing is that the receptors on endothelial cells for the glycosylated ligands of circulating cells are constitutively expressed. These receptors, such as P and E selectins, induce numerous activities after interacting with cells, including causing apoptosis or proliferative arrest (Winkler et al., Blood 2004 103: 1685 – 1692). Accordingly, the administration of hematopoietic cells and their subsequent homing to the bone marrow is dependent on molecules that are constitutively expressed.

[0065] For the purpose of a broader application of this approach for regenerative medicine in which cells are administered for non-hematopoietic purposes, the trafficking/homing of cells to the targeted location is much more complex and involves ligands that are not constitutively expressed, but expressed as a result of inflammation or tissue damage. For example, administration of stem cells for the purpose of treating myocardial infarction depends on homing of these cells to areas bathed in cytokine locally released which not only induces expression of E selectin and P selectin on the endothelium but also mediates chemoattraction to the site. This tissue localized upregulation of receptors and chemoattractant agents allows for homing of stem cells into areas of injury.

#### Routes of Administration

[0066] Administration of the modified cells is performed in agreement with standard practices that are known to one skilled in the art. Several embodiments are possible. For example, routes of administration may include parenteral, e.g., intravenous, intradermal, microvascular bed of bone marrow, subcutaneous, oral (e.g., ingestion or inhalation), transdermal (topical), transmucosal, and rectal administration. In certain particular aspects, the population of modified cells is administered from a route selected from a group consisting of: intravenously, intraarterially, intramuscularly, subcutaneously, transdermally, intratracheally, intraperitoneally, intravitreally, via direct injection, into bone compartments or into spinal fluid. In some aspects the cells, compositions or other materials can be used with a scaffolding support. In certain aspects, the population of modified cells is administered in or proximal to a site of injury. In certain aspects, the homing and engraftment takes place within the bone marrow of the patient in need thereof. In certain aspects the cells are administered by multiple routes and sites either simultaneously or sequentially.

[0067] In another embodiment, the methods, compositions, cells and other materials can be useful for enhancing functional capabilities in a wide variety of not only hematological disorders but also non-hematological disorders. Specifically, the methods, compositions, cells and other materials may be useful for the treatment of medical conditions which are amenable to cell therapy. More specifically, the methods, compositions, cells and

other materials may be useful for the treatment of acute leukemias, chronic leukemias, myelodysplastic syndromes, stem cell disorders, myeloproliferative disorders, lymphoproliferative disorders, phagocyte disorders, histiocytic disorders, lysosomal storage diseases, congenital immune system disorders, inherited erythrocyte abnormalities, other inherited disorders, inherited platelet abnormalities, plasma cell disorder, various malignancies such as brain tumors or Ewing sarcoma, Autoimmune Diseases, and other applications such as bone marrow transplants, diabetes, heart disease, liver disease, hematopoietic rescue situations following intense chemo/radiation, limb ischemia and limb regeneration (including cartilage regeneration, skin regeneration, blood vessel regeneration, etc), cartilage regeneration, skin regeneration, blood vessel regeneration, etc.

**[0068]** Furthermore, presented herein is the finding that the general increased adhesion of cells that have been fucosylated *ex vivo* can be utilized for augmented binding to localized niche areas in absence of chemotactic gradient such as in the context of portal vein injection or pulmonary artery injection.

**[0069]** For P-selectin binding cells can be incubated with anti-CD34+-PE and with P-selectin isolated from human platelets. P-selectin binding can be detected with FITC-labeled S12, a non-blocking mAb to human P-selectin. For E-selectin binding cells can be incubated with E-selectin/IgM after Fc receptor blocking. E-selectin can then be detected with FITC-labeled goat anti-human IgM polyclonal antibodies. Visualization of binding can be achieved using FACS analysis. Incubation for both P and E-selectin can be carried out at 4°C for 20 min.

**[0070]** To confirm a functional consequence of fucosylation following treatment with fucosyltransferase the cells can be examined for adhesion to either E-selectin or P-selectin under physiological shear forces using an in vitro flow chamber rolling assay system. P-selectin isolated from human platelets can be immobilized on plates in a parallel-plate flow chamber. A P-selectin site density of about 145 sites/ $\mu\text{m}^2$  can be used and measured by binding of  $^{125}\text{I}$ -labeled anti-P-selectin mAb S12. For E-selectin soluble human E-selectin can also be immobilized on plates in a parallel-plate flow chamber at a density of 200 sites/ $\mu\text{m}^2$  as measured by binding of  $^{125}\text{I}$ -labeled anti-human E-selectin mAb ES1. Sham-treated or FTVI-treated cells (in Hanks' balanced salt solution and 0.5% human albumin) can

be perfused over P- or E-selectin coated plates at a wall shear stress of 1 dyn/cm<sup>2</sup>. The accumulated number of rolling cells can be measured with the aid of a videomicroscopy system coupled to an image analysis system. Specificity of interaction of cells with the coated plates can be confirmed with the inclusion of specific inhibitors to the binding and examination of rolling on plates coated only with human serum albumin.

## EXAMPLES

### EXAMPLE 1

#### PARAMETERS FOR MAXIMAL FTVI ACTIVITY IN CORD BLOOD

[0071] Enzymatic-mediated fucosylation ( $\alpha$  1-3-linked fucose addition to cell-surface glycans) has shown both phenotypic and functional changes in MNC and CD34+ cell populations.

[0072] These *in vitro* studies are structured to examine the various components integral to the enzymatic-mediated fucosylation using a cell preparation procedure routinely practiced in the clinic. A frozen thawed human cord blood mononuclear cell population is washed by a procedure that involves a 1 to 10 dilution with chilled 10% Dextran-40/5% HSA solution, placing this diluted solution in a pre-cooled (2- 6°C) centrifuge for 5 – 10 min followed by mild centrifugation at approximately 550 g for 20 min. The supernatant is discarded while the pellet is resuspended in Hank's balanced salt solution (HBSS) containing 1% HSA at a target cell concentration ranging from  $0.5 \times 10^6$  to  $1 \times 10^9$  per ml. This cell population suspended in fucosyltransferase VI (FTVI) reaction media constitutes the core preparation used to examine a range of various parameters in order to identify the optimal conditions for maximal activity of FTVI within this cell population. The parameters examined are Mn<sup>++</sup> (over final concentration ranging from 0.0 to 10 mM), GDP-fucose (over final concentration ranging from 0.3 to 10 mM), time course analysis with varying periods of incubation (ranging from 15 min to 60 min), FTVI (over a 20 fold range of enzyme concentration), temperature [10°C, 25°C (considered as room temperature) and 37°C] and cell concentration (over final concentration ranging from  $0.5 \times 10^6$  to  $1 \times 10^9$  per ml). Additional activities within these optimization efforts include assessing the stability of the fucosylated

product with an examination of HECA-452 binding at 30, 60, 120, 180 and 240 min post termination of reaction and assessing the extent of fucosylation of additional cell populations. The cell preparation in reaction mix is incubated for 30 min (except for time course study) with occasional gently mixing. The solutions are then diluted with cold HBSS 1% HSA, filtered through 70 micron cell screen and subjected to chilled centrifugation for 12 min. The supernatant is discarded, pellet loosened and resuspended for either injection or analysis by FACS using a procedure that is familiar to someone skilled in the art. For FACS analysis, aliquots of the cells, at approximately  $5 \times 10^5$ , are centrifuged at 500 x g for 13 minutes at 4°C. The supernatant is discarded, pellet loosened for the addition of flow stain cocktail containing the appropriate stain(s) or control cocktail. The mixture is stored in the dark for 30 – 40 min with occasional mixing. Each tube is diluted with 3 ml of cold flow wash buffer followed by centrifugation at 500 x g for 12 min at 4°C. The supernatant is discarded, pellet loosened with the addition of approximately 200 µl of cold flow buffer or flow fix buffer. Aliquots of each sample are examined by FACS for percentage of double positives for CD34+ vs HECA-452 which is the primary outcome measure for determining maximal expression of FTVI activity in the cell mix. Also generated as an outcome measure for assessment of enzyme activity is the mean fluorescence intensity

## EXAMPLE 2

### ENHANCED ENGRAFTMENT IN THE BONE MARROW USING A COMBINATION APPROACH CONSISTING OF MAXIMAL FUCOSYLATION OF CELL PREPARATION PLUS EXPOSURE TO A CD26 INHIBITOR

**[0073]** First, maximal fucosylation of cells *in vitro* is accomplished. To accomplish this, washed mononuclear cells (MNCs) are resuspended in Hank's Balanced Salt Solution (HBSS) at a concentration of  $0.5 \times 10^6$  –  $1 \times 10^9$  per ml and then incubated with a fucosylation mix consisting at final concentration of 5 mM GDP-fucose, purified human recombinant  $\alpha$ 1-3 fucosyltransferase VI at a predetermined Units per ml (for maximal fucosylation), and 1 - 10 mM  $MnCl_2$  in HBSS. This mix is incubated for 30 min at 37°C or room temperature in a humidified atmosphere containing 5%  $CO_2$ . The period of incubation could take longer or shorter depending on the incubation temperature chosen. Following

completion of the fucosylation and to achieve inhibition of CD26 (dipeptidylpeptidase, DPPIV) as part of the combination approach, this preparation of fucosylated mononuclear cells (MNCs) is either washed first or directly incubated with a potent DPPIV inhibitor for 5 - 15 min at room temperature at a concentration sufficient to achieve complete or nearly complete inhibition of DPPIV. Following incubation the cell suspension is volume adjusted with HBSS or another clinically compatible solution to obtain the appropriate concentration of cells in preparation for iv injection. As an alternative scenario for a combination approach, the patients are subjected to systemic administration of the DPPIV inhibitor at a dose sufficient to achieve a sustained level in the body for significant inhibition of bone marrow and circulating plasma DPPIV activity. Subsequent to systemic pretreatment, MNCs exposed to conditions for maximal fucosylation are then injected into the patient. The DPPIV inhibitor can be added simultaneous with the injection of the fucosylated cell preparation or shortly before. The patients are prepared for this combined approach by subjecting them to conditions for myeloablation or even partial myeloablation (mini) prior to injection of the fucosylated and inhibitor treated cells. The rate of recovery and extent of chimerism is assessed with an examination of serially collected blood samples in addition to an examination of cells obtained from the bone marrow. A multilineage analysis of the rate of recovery and extent of engraftment and chimerism is accomplished using cell surface markers specific for cell types in addition to an examination of mature hematopoietic cells in the blood stream. These markers and cells are detected using preparation techniques and FACS analysis procedures that are familiar to one skilled in the art.

### EXAMPLE 3

#### *EX VIVO* FUCOSYLATION OF MESENCHYMAL STEM CELLS

**[0074]** The introduction of fucose onto surface glycans of mesenchymal stem cells (MSC) is accomplished by enzymatic transfer from a donor substrate utilizing an alpha 1-3-fucosyltransferase (FT). For this transfer to occur the cells at varying concentrations are exposed to an incubation buffer containing a number of ingredients each of which has been optimized for efficient transfer of the fucose, and performed in Hanks balanced salt solution (HBSS). The substrate, guanosine diphosphate-fucose (GDP-fucose), at 1 mM is mixed with

the FT added at sufficient activity, in order to achieve maximal transfer of fucose to MSCs. In addition,  $MnCl_2$  at a final concentration of 0 – 10 mM is added, as needed, to further accelerate the enzymatic transfer reaction. The incubation is performed at 37°C for 40 minutes with minimum toxicity to the cells.

**[0075]** Confirmation of fucosylated epitopes on the cells of interest as means of confirming maximal levels of fucosylation is verified by Flow Cytometry in order to detect sialyl LewisX (sLeX), a fucosylation epitope found on both P and E-selectins. The FT-treated cells are incubated with anti-sLeX mAb HECA 452 (IgM), followed by treatment with FITC-conjugated fragment to the IgM. Finally, the sLeX epitopes on the cell surface are visualized using standard Flow Cytometry procedures.

**[0076]** To measure P-selectin binding, cells are incubated with anti-CD34+PE and with P-selectin isolated from human platelets. P-selectin binding is detected with FITC-labeled S12, a non-blocking mAb to human P-selectin. To measure E-selectin binding, cells are incubated with E-selectin/IgM after Fc receptor blocking. E-selectin is then detected with FITC-labeled goat anti-human IgM polyclonal antibodies. Visualization of binding is achieved using FACS analysis. Incubation for both P and E-selectin is carried out at 4°C for 20 min.

**[0077]** To confirm a functional consequence of fucosylation following treatment with FT the cells are examined for adhesion to either E-selectin or P-selectin under physiological shear forces using an in vitro flow chamber rolling assay system. P-selectin isolated from human platelets is immobilized on plates in a parallel-plate flow chamber. A P-selectin site density of about 145 sites/ $\mu m^2$  is used and measured by binding of  $^{125}I$ -labeled anti-P-selectin mAb S12. For E-selectin soluble human E-selectin is also immobilized on plates in a parallel-plate flow chamber at a density of 200 sites/ $\mu m^2$  as measured by binding of  $^{125}I$ -labeled anti-human E-selectin mAb ES1. Sham-treated or FTVI-treated cells (in Hanks' balanced salt solution and 0.5% human albumin) is perfused over P- or E-selectin coated plates at a wall shear stress of 1 dyn/cm<sup>2</sup>. The accumulated number of rolling cells is measured with the aid of a videomicroscopy system coupled to an image analysis system. Specificity of interaction of cells with the coated plates is then confirmed with the inclusion

of specific inhibitors to the binding and examination of rolling on plates coated only with human serum albumin.

#### EXAMPLE 4

##### ADMINISTRATION OF MODIFIED STEM CELLS TO BONE MARROW TRANSPLANT PATIENTS

**[0078]** Patients in need of a bone marrow transplant are subjected to either myeloablative or non-myeloablative conditions. Stem cells obtained from one of a number of different sources are incubated *ex vivo* with fucosyltransferase + GDP-fucose at sufficient concentrations and for a sufficient period of time to result in maximal formation of fucosylated product, such as sialyl Lewis X, on the cell surface. Following treatment, the cell preparation is washed or directly injected into the patient. Effectiveness of this application in the patient is determined with accelerated appearance over time of neutrophils and platelets in the blood stream compared to patients injected with control untreated stem cells.

#### EXAMPLE 5

##### MODIFICATION OF NEURAL STEM CELLS

**[0079]** A cell population consisting of neural stem cells is treated with conditions so as to endow increased surface ligands for enhanced interaction with endothelium. Cells are modified with the addition of alpha 1-3-linked fucose to cell-surface glycans by *ex vivo* treatment of cells with the enzyme alpha 1-2 fucosyltransferase VI. Specifically, cells are treated with 1 mM GDP fucose, 20 mU/mL {alpha}1-3 fucosyltransferase VI, and 10 mM MnCl<sub>2</sub> in 0.5 mL HBSS containing 1% human serum albumin (HSA) for 30 minutes at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> under conditions that cause minimum toxicity to CD34+ cells as tested by propidium iodide staining measured by flow cytometry. Other modifications of this treatment procedure may be performed based on the knowledge of one skilled in the art. Said treated neural stem cells are subsequently assessed for fucosylation using flow cytometric methodology. The cells are placed on ice for 5 min followed by washing with PBS (1 ml). To detect the presence of new fucosylated units on cell surface the cell preparation is treated with the 1<sup>st</sup> antibody, anti-CLA 1:200 dilution in

blocking buffer (400ul), and then incubated for one hour at room temp or overnight at 4°. Cells are then washed three times with PBS. The secondary antibody (anti-rat-IgM-PE, 1:200) in blocking buffer (400ul) is then added. The preparation is incubated for 2 hrs at room temperature then rinsed with PBS. The result of this treatment and analysis is shown in Fig 1.

## EXAMPLE 6

### MODIFICATION OF IMMUNE MODULATORY CELLS

**[0080]** A cell population consisting of cells with immune modulatory potential are treated with certain conditions so as to endow increased surface ligands for endothelium. Cells are modified with alpha 1-3-linked fucose to cell-surface glycans by treatment of cells with the enzyme alpha 1-2 fucosyltransferase VI. Specifically, cells are diluted to a concentration of 10(7) per ml and treated with 1 mM GDP fucose (EMD Biosciences, San Diego, CA), 20 mU/mL {alpha}1-3 fucosyltransferase VI (FTVI; EMD Biosciences), and 10 mM MnCl<sub>2</sub> in 0.5 mL HBSS containing 1% human serum albumin (HSA) for 30 minutes at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> under conditions that cause minimum toxicity to CD34+ cells as tested by propidium iodide staining measured by flow cytometry. Other modifications of this treatment procedure may be performed based on the knowledge of one skilled in the art. Said treated immune cells are subsequently assessed for fucosylation status using either a flow cytometric methodology (assessment of HECA-453 binding) or functional methodology (assessment of rolling on endothelium). Cells are subsequently administered to a patient for immune modulation.

## EXAMPLE 7

### AUGMENTING EFFICACY OF TUMOR INFILTRATING LYMPHOCYTES AFTER EX VIVO EXPANSION

**[0081]** Tumor infiltrating lymphocytes are collected as described by Zhou et al *The Journal of Immunology*, 2005, 175: 7046-7052. Briefly, explants of small (2 mm<sup>3</sup>) tumor fragments or 1 x 10<sup>6</sup> viable cells of tumor tissue digests are used to initiate TIL culture in 2 ml of RPMI 1640-based medium (Invitrogen Life Technologies) containing 10% human

serum and 6000 IU/ml IL-2 (Chiron). After 2–4 wk of culture, usually several million TIL cells are obtained and screened by IFN secretion assay for recognition of tumor cells. Antitumor TIL cultures were further expanded in AIM V medium (Invitrogen Life Technologies) supplemented with irradiated allogeneic feeder cells, anti-CD3 Ab (Ortho Biotech), and 6000 IU/ml IL-2. This expansion protocol typically resulted in 1000-fold expansions of cells by the time of administration 14–15 days after initiation of the expansions. Subsequent to expansion cells are harvested, centrifuged, diluted to a concentration of  $10^7$  per ml and treated with 1 mM GDP fucose (EMD Biosciences, San Diego, CA), 20 mU/mL alpha 1-3 fucosyltransferase VI (FTVI; EMD Biosciences), and 10 mM MnCl<sub>2</sub> in 0.5 mL HBSS containing 1% human serum albumin (HSA) for 30 minutes at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> under conditions that cause minimum toxicity to cells as tested by propidium iodide staining measured by flow cytometry. Cells are administered on a weekly basis at a concentration of at least about 1 million cells but in some situations up to 100 million over a period of 60-120 minutes. After 4 cycles of therapy tumor regression is noted.

**[0082]** One skilled in the art will appreciate that these methods, compositions, and cells are and may be adapted to carry out the objectives and obtain the ends and advantages mentioned, as well as those inherent therein. The methods, procedures, and devices described herein are presently representative of preferred embodiments and are exemplary and are not intended as limitations on the scope of the technology. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the technology and are defined by the scope of the disclosure. It will be apparent to one skilled in the art that varying substitutions and modifications can be used or substituted into any of the embodiments described herein may be made without departing from the scope and spirit of the described technology. Examples of such substitutions are non-natural enzymes and sugars. Those skilled in the art recognize that the aspects and embodiments set forth herein may be practiced separate from each other or in conjunction with each other. Therefore, combinations of separate embodiments are within the scope of the technology as disclosed

herein. All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the technology pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

**[0083]** The embodiments illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms “comprising,” “consisting essentially of” and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions indicates the exclusion of equivalents of the features shown and described or portions thereof. It is recognized that various modifications are possible within the scope of the embodiments disclosed. Thus, it should be understood that although the present technology has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this technology as defined by the disclosure.

WHAT IS CLAIMED IS:

1. A method of enhancing homing and engraftment of a cell, comprising:
  - a) providing one or more cells selected from stem cells, progenitor cells, neutrophils, macrophages and T-cells, wherein the stem or progenitor cells are selected from a group consisting of: embryonic stem cells, adult stem cells, expanded stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, neuronal stem cells, cardiomyocyte stem cells, placental stem cells, endothelial progenitor cells, circulating and mobilized peripheral blood stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells, wherein the one or more cells have been contacted with an agent that modifies at least one surface molecule on said cell to result in enhanced selectin-mediated binding, resulting in a population of modified cells; and
  - b) providing said population of modified cells to an animal in need thereof.
2. The method of claim 1, wherein said enzyme is selected from a group comprising of: a glycosidase, a fucosyltransferase, a neuraminidase, an acetylglucosaminyltransferase, and any glycosyltransferases capable of increasing the number or affinity of cell surface selectin binding components .
3. The method of claim 2, wherein said enzyme is selected from a group comprising of alpha 1,3-fucosyltransferase I, alpha 1,3-fucosyltransferase III, alpha 1,3-fucosyltransferase IV, alpha 1,3-fucosyltransferase V, alpha 1,3-fucosyltransferase VI, alpha 1,3-fucosyltransferase VII, and alpha 1,3-fucosyltransferase IX.
4. The method of claim 1, wherein said one or more cells is treated with a single or plurality of molecules having ability to cause alpha 1-3 fucosylation of glycan determinants.
5. The method of claim 4, wherein said molecule is an alpha 1-3 fucosyltransferase mixed together with a concentration of a fucose carrier under conditions sufficient to provide enhanced alpha 1-3 fucosylation of glycan determinants.

6. The method of claim 5, wherein said fucose carrier is guanosine diphosphate fucose.
7. The method of claim 5, wherein said alpha 1-3 fucosyltransferase is alpha 1-3 fucosyltransferase VI.
8. The method of claim 5, wherein said alpha 1-3 fucosyltransferase is alpha 1-3 fucosyltransferase VII.
9. The method of claim 5, wherein said alpha 1-3 fucosyltransferase is alpha 1-3 fucosyltransferase IV.
10. The method of claim 1, wherein, prior to said providing of modified cells, said population of modified donor cells has been further contacted for a period of time insufficient for cell division to occur with a CD26 peptidase inhibitor in an amount effective to inhibit CD26 peptidase activity and effective to increase the migratory response to CXCL12.
11. The method of claim 1, wherein, prior to said providing of modified cells, a recipient has been contacted for a period of time and with sufficient dosing of a CD26 peptidase inhibitor in an amount effective to inhibit recipient CD26 peptidase activity effective to increase the migratory response of donor cells to chemotractant agents such as stromal cell-derived factor.
12. The method of any of claims 1-11, wherein said cell population comprises or consists essentially of a population of stem cells or progenitor cells.
13. The method of claim 12, wherein said embryonic stem cells are totipotent.
14. The method of claim 12, wherein the cell population is a neural stem cell population.
15. The method of claim 12, wherein the cell population is a cardiomyocyte stem cell population.
16. The method of claim 12, wherein the cell population is endothelial progenitor cells
17. The method of any of claim 12, wherein said cell population comprises or consists essentially of a population of committed progenitor cells or differentiated cells.
18. The method of claim 17, wherein said mature blood cell is selected from the group consisting of: neutrophils, macrophages and subpopulations of T-cells.

19. The method of claim 17, wherein said T-cells are from a heterogeneous population of T-cells.

20. The method of claim 1, wherein said patient in need of treatment with a cell population suffers from a condition selected from the group consisting of: an acute leukemia, a chronic leukemia, a myelodysplastic syndrome, a stem cell disorder, a myeloproliferative disorder, a lymphoproliferative disorder, a phagocyte disorder, a histiocytic disorder, a liposomal storage disease, a congenital immune system disorder, an inherited erythrocyte abnormality, an inherited platelet abnormality, a plasma cell disorder, a tumor and an autoimmune disease.

21. The method of claim 1, wherein said patient in need of treatment with a cell population suffers from a condition selected from the group consisting of: peripheral arterial diseases, ischemic limb injury, diabetes, heart disease, liver disease, bone disease, muscular dystrophy, Alzheimer's disease, ALS, multiple sclerosis, Parkinson's disease, spinal cord injury, stroke, head trauma and infertility.

22. The method of claim 1, wherein said population of modified cells is provided via a route selected from a group consisting of: intravenously, intraarterially, intramuscularly, subcutaneously, transdermally, intratracheally, intraperitoneally, intravitreally, into bone compartments or into spinal fluid.

23. The method of claim 1, wherein said population of modified cells is provided in or proximal to a site of injury.

24. The method of claim 1, wherein said homing and engraftment takes place within the bone marrow of said patient in need thereof.

25. A composition comprising an isolated population of cells modified for enhanced selectin-mediated binding, wherein the isolated population of cells comprises one or more of neutrophils, macrophages, T-cells, subpopulation of T-cells, or stem or progenitor cells selected from a group consisting of: embryonic stem cells, adult stem cells, expanded stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, neuronal stem cells, cardiomyocyte stem cells, endothelial progenitor cells, circulating and immobilized peripheral blood stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal

stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells and a pharmaceutically-acceptable carrier.

26. The composition of claim 25, wherein the isolated population of cells comprises a cell surface modification.

27. The composition of claim 26, wherein said cell surface molecule is modified by treatment with an enzyme and appropriate substrate(s) under conditions sufficient for causing an alteration of cell surface charge.

28. The composition claim 27, wherein said enzyme is selected from a group comprising of: a glycosidase, a fucosyltransferase, a neuraminidase, an acetylglucosaminyltransferase, and any other glycotransferases capable of increasing cell surface selectin binding components .

29. The composition of claim 28, wherein said enzyme is selected from a group comprising of alpha 1,3-fucosyltransferase I, alpha 1,3-fucosyltransferase III, alpha 1,3-fucosyltransferase IV, alpha 1,3-fucosyltransferase V, alpha 1,3-fucosyltransferase VI, alpha 1,3-fucosyltransferase VII and alpha 1,3-fucosyltransferase IX.

30. The composition of claim 29, wherein said cell is treated with a single or plurality of molecules having ability to cause alpha 1-3 fucosylation of glycan determinants.

31. The composition of claim 30, wherein said molecule is an alpha1-3 fucosyltransferase mixed together with a concentration of a fucose carrier under conditions sufficient to provide enhanced alpha 1-3 fucosylation of glycan determinants.

32. The composition of claim 31, wherein said fucose carrier is guanosine diphosphate fucose.

33. The composition of claim 31, wherein said alpha 1-3 fucosyltransferase is alpha 1-3 fucosyltransferase VI.

34. The composition of claim 31, wherein said alpha 1-3 fucosyltransferase is alpha 1-3 fucosyltransferase VII.

35. The isolated cell of claim 34, wherein said alpha 1-3 fucosyltransferase is alpha 1-3 fucosyltransferase IV.

36. The composition of any of claims 26-35, wherein said cell population comprises or consists essentially of a population of stem cells.

37. The composition of claim 36, wherein stem or progenitor cells are selected from a group consisting of: embryonic stem cells, adult stem cells, expanded stem cells, cord blood stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, hematopoietic stem cells, mesenchymal stem cells, neuronal stem cells, cardiomyocyte stem cells, endothelial progenitor cells, circulating and immobilized peripheral blood stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells.

38. The composition of claim 37, wherein said embryonic stem cells are totipotent.

39. The composition of claim 37, wherein the cell is a hematopoietic stem cell.

40. The composition of claim 37, wherein the cell is a mesenchymal stem cell.

41. The composition of claim 37, wherein the cell is a neural stem cell.

42. The composition of claim 37, wherein the cell is a cardiomyocyte stem cell.

43. The composition of any of claims 26-37, wherein the cell is a mature blood cell.

44. The composition of claim 43, wherein said mature blood cell is selected from the group consisting of: neutrophils, macrophages and T-cells.

45. The composition of claim 43, wherein said T-cells are from a heterogeneous population of T-cells.

46. The composition of claim 45, wherein the cell is from an ex vivo expanded cell population.

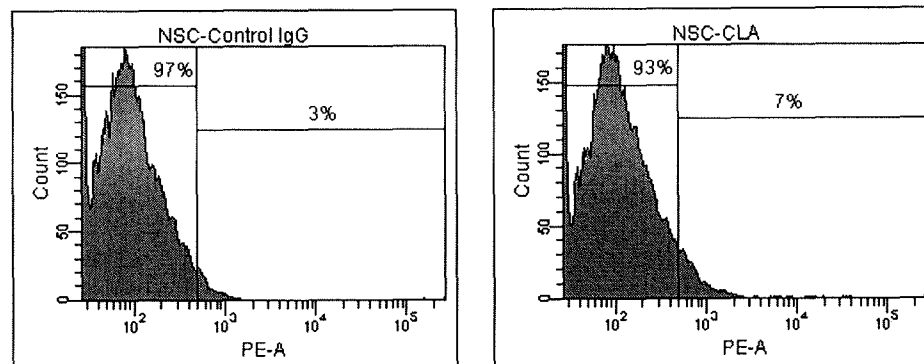
47. A method of enhancing homing and engraftment of a cell, comprising providing one or more cells selected from stem cells, progenitor cells, neutrophils, macrophages and T-cells, wherein the stem or progenitor cells are selected from a group consisting of: embryonic stem cells, adult stem cells, expanded stem cells, placental stem cells, bone marrow stem cells, amniotic fluid stem cells, neuronal stem cells, cardiomyocyte

stem cells, endothelial progenitor cells, circulating and immobilized peripheral blood stem cells, muscle stem cells, germinal stem cells, adipose tissue derived stem cells, exfoliated teeth derived stem cells, hair follicle stem cells, dermal stem cells, parthenogenically derived stem cells, reprogrammed stem cells such as induced pluripotent stem cells or somatic nuclear transfer and side population stem cells; and contacting one or more cells with an agent that modifies at least one surface molecule on said cell to result in enhanced selectin-mediated binding, resulting in a population of modified cells.

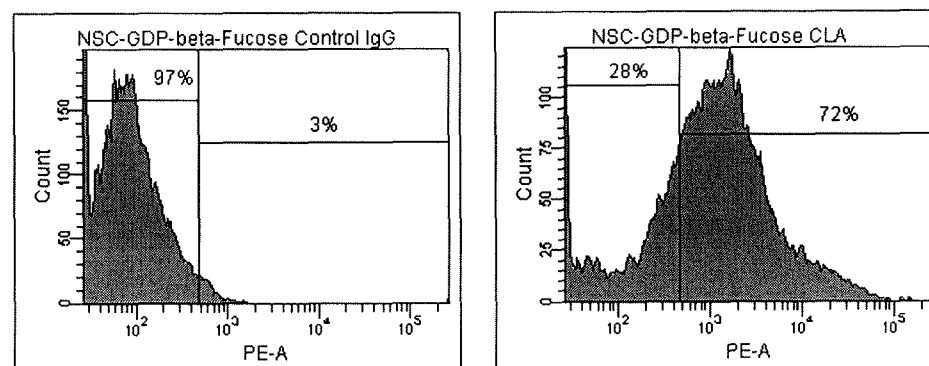
1/1

FIG 1

A



B



I. EFFECT OF PRETREATMENT OF HNSCS WITH FTVI ON LEVEL OF FUCOSYLATION. THE EXPRESSION OF CLA WAS USED TO DETERMINE THE LEVELS OF FUCOSYLATION. THE CLA EXPRESSION BY HNSCS WAS DETERMINED BY FACS ANALYSIS WITH UNTREATED CELLS (A) OR FOLLOWING PRE-INCUBATION WITH THE FUCOSYLATION MIX (GDP-FUCOSE, MANGANESE AND FTVI CONDITIONED MEDIUM) (B). ISOTYPE-MATCHED IGG WAS USED AS THE NEGATIVE CONTROL. FLUORESCENCE INTENSITY (FL2) OF SAMPLES WAS EVALUATED BY FACSCANTO. THE RESULTS OF ONE EXPERIMENT IS SHOWN.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/46800

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(8) - C12N 5/00 (2009.01)

USPC - 435/377

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)

USPC: 435/377

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 435/377, 375, 325, 363

(text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed

Search terms: fucosyltransferase, fucosylated, stem cell, transplant, selectin, administer, neural stem cell, cardiomyocyte stem cell, endothelial progenitor cell, mesenchymal stem cell, cd26, cxcl12

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2006/0228340 A1 (XIA et al.) 12 October 2006 (12.10.2006) abstract; para [0009]-[0010], [0024]-[0025], [0044], [0063], [0066], [0073], [0076]	1-9, 12-13, 20, 22, 24-39, 47 ----- 10-11, 14-19, 21, 23, 40-42
Y	US 2004/0247574 A1 (CHRISTOPHERSON et al.) 9 December 2004 (09.12.2004) claim 1; para [0006], [0012], [0034], [0079]	10-11
Y	US 2008/0044383 A1 (SACKSTEIN) 21 February 2008 (21.02.2008) para [0014], [0020], [0032]-[0034], [0042], [0045], [0049]	14-15, 17-19, 21, 23, 40-42
Y	US 2005/0048036 A1 (HEDRICK et al.) 3 March 2005 (03.03.2005) abstract; para [0052]	16
A	US 2007/0190023 A1 (BATTISTA et al.) 16 August 2007 (16.08.2007)	1-42, 47

Further documents are listed in the continuation of Box C.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 July 2009 (17.07.2009)

Date of mailing of the international search report

**29 JUL 2009**

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

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PCT OSP: 571-272-7774

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/46800

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: 43-46  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.