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(54) **Title:** MESENCHYMAL STEM CELLS WITH ENHANCED EFFICACY

(57) **Abstract:** Disclosed are protocols, isolation means, and compositions of matter useful for identifying mesenchymal stem cells possessing enhanced clinical activity. In one embodiment, markers associated with said enhanced mesenchymal stem cell activity are utilized to identify donors whose mesenchymal stem cells possess superior efficacy compared to mesenchymal stem cells from donors who lack said markers associated with said enhanced efficacy. In one embodiment, said markers are utilized to select for mesenchymal stem cells possessing enhanced efficacy from in vitro cultures. In another embodiment, surfaces markers associated with said markers associated with enhanced efficacy are utilized to positively select for cells possessing enhanced efficacy. In another embodiment, the invention teaches markers whose expression is correlated with negative efficacy. Said markers can be utilized to exclude mesenchymal stem cell donors, or in vitro generated and/or isolated mesenchymal stem cells prior to clinical use.



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MESENCHYMAL STEM CELLS WITH ENHANCED EFFICACY**Cross-Reference to Related Applications**

[001] The present invention claims priority to U.S. Provisional Application No. 62/309,308, filed March 16, 2016, which is hereby incorporated in its entirety including all tables, figures, and claims.

Field of the Invention

[002] The invention pertains to the area of stem cell therapeutics, more specifically, the invention pertains to the area of mesenchymal stem cell therapeutics, more specifically, the invention pertains to means of selecting stem cells possessing enhanced efficacy, furthermore the invention pertains to the area of stem cell efficacy markers, and furthermore the invention pertains to augmenting mesenchymal stem cell efficacy by inhibiting the expression of proteins found in higher concentrations in cells without enhanced clinical activity.

Background

[003] According to the definition by the U.S. Food and Drug Administration (FDA), somatic cell therapy (or cell therapy) is the prevention, treatment, cure, diagnosis, or mitigation of diseases or injuries in humans by the administration of autologous, allogeneic or xenogeneic cells that have been manipulated or altered ex vivo. Generally, said manipulation and alteration include the propagation, expansion, selection, and/or pharmacological treatment of the cells. The goal of cell therapy is to repair, replace or restore damaged tissues or organs. Cell therapy may provide extensive applications in modern medicine. For example, in Nov. 10, 2011, the U.S. FDA granted marketing approval to the New York Blood Center's allogeneic cord-blood product, HEMACORD, the first FDA-licensed hematopoietic progenitor cell therapy. HEMACORD is indicated for hematopoietic progenitor cell (HPC) transplantation procedures in patients with inherited, acquired, or myeloablative-treatment-related diseases that affect the hematopoietic system. Once the HPCs are infused into patients, the cells migrate to the bone marrow where they divide and mature. When the mature cells move into the

bloodstream they can partially or fully restore the number and function of many blood cells, including immune function.

[004] Mesenchymal stem cell therapeutics has entered the clinical arena in the treatment of various degenerative conditions including cardiovascular, neurological, and immunological. Regulatory approval of mesenchymal stem cell based products has been achieved in several jurisdictions, particularly of mesenchymal stem cells. Mesenchymal stem cells are classically defined as adherent cells possessing ability to differentiate into osteoblasts, adipocytes and chondrocytes and possessing the surface markers CD73, CD90, and CD105, while lacking the markers CD14, CD34, and CD45.

[005] There are many factors affecting the therapeutic efficacy of mesenchymal stem cell therapy, in particular selection of donors for generation of mesenchymal stem cell therapy appears to be a major factor in whether such therapy will be efficacious. Although criteria such as young donor age and absence of chronic disease, is utilized by some as a means of selecting donors with enhanced efficacy, to date, no consistent means of selecting donors based on markers exists that has been validated in a clinical setting. In view of the foregoing, there exists a need in the art for providing and/or developing an alternative strategy that; a) allows for selection of mesenchymal stem cells with enhanced efficacy; and b) allows for selection of donors whose MSC possess enhanced efficacy.

Description of the Invention

[006] The invention teaches means of selecting mesenchymal stem cells (MSC) for enhanced efficacy based on expression, or lack of expression of certain proteins. In one particular embodiment, MSC are generated from a series of MSC donors, with each donor representing a lot of MSC. Said lots are screened for enhanced efficacy based on expression of markers selected from a group comprising of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein, Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding

protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1. Furthermore, within the practice of the invention MSC donor lots are selected for reduced expression of markers Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1. In one preferred embodiment MSC donor lots are selected for both enhanced expression of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein and reduced expression of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural

cell adhesion molecule L1 as compared to comparator donor lots. MSC sources are known in the literature and can be derived from tissue, or bodily fluids.

[007] Differentiation is the process by which an unspecialized ("uncommitted") or less specialized cell acquires the features of a specialized cell, such as a nerve cell or a muscle cell, for example. A differentiated cell is one that has taken on a more specialized ("committed") position within the lineage of a cell. The term committed, when applied to the process of differentiation, refers to a cell that has proceeded in the differentiation pathway to a point where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type or revert to a less differentiated cell type. De-differentiation refers to the process by which a cell reverts to a less specialized (or committed) position within the lineage of a cell. As used herein, the lineage of a cell defines the heredity of the cell, i.e. which cells it came from and what cells it can give rise to. The lineage of a cell places the cell within a hereditary scheme of development and differentiation.

[008] In a broad sense, a progenitor cell is a cell that has the capacity to create progeny that are more differentiated than itself, and yet retains the capacity to replenish the pool of progenitors. By that definition, stem cells themselves are also progenitor cells, as are the more immediate precursors to terminally differentiated cells. When referring to the cells of the present invention, as described in greater detail below, this broad definition of progenitor cell may be used. In a narrower sense, a progenitor cell is often defined as a cell that is intermediate in the differentiation pathway, i.e., it arises from a stem cell and is intermediate in the production of a mature cell type or subset of cell types. This type of progenitor cell is generally not able to self-renew. Accordingly, if this type of cell is referred to herein, it will be referred to as a non-renewing progenitor cell or as an intermediate progenitor or precursor cell.

[009] As used herein, the phrase differentiates into a mesodermal, ectodermal or endodermal lineage refers to a cell that becomes committed to a specific mesodermal, ectodermal or endodermal lineage, respectively. Examples of cells that differentiate into a mesodermal lineage or give rise to specific mesodermal cells include, but are not limited to, cells that are adipogenic, chondrogenic, cardiogenic, dermatogenic, hematopoietic, hemangiogenic, myogenic, nephrogenic, urogenitogenic, osteogenic, pericardiogenic, or stromal. Examples of cells that differentiate into ectodermal lineage include, but are not limited to epidermal cells, neurogenic cells, and neurogliagenic cells. Examples of cells

that differentiate into endodermal lineage include, but are not limited to, pluripotent cells, hepatogenic cells, cells that give rise to the lining of the intestine, and cells that give rise to pancreogenic and splanchnogenic cells.

[0010] The cells of the present invention are generally referred to as umbilicus-derived cells (or UDCs). They also may sometimes be referred to more generally herein as postpartum-derived cells or postpartum cells (PPDCs). In addition, the cells may be described as being stem or progenitor cells, the latter term being used in the broad sense. The term derived is used to indicate that the cells have been obtained from their biological source and grown or otherwise manipulated in vitro (e.g., cultured in a growth medium to expand the population and/or to produce a cell line). The in vitro manipulations of umbilical stem cells and the unique features of the umbilicus-derived cells of the present invention are described in detail below.

[0011] Various terms are used to describe cells in culture. Cell culture refers generally to cells taken from a living organism and grown under controlled condition ("in culture" or "cultured"). A primary cell culture is a culture of cells, tissues, or organs taken directly from an organism(s) before the first subculture. Cells are expanded in culture when they are placed in a growth medium under conditions that facilitate cell growth and/or division, resulting in a larger population of the cells. When cells are expanded in culture, the rate of cell proliferation is sometimes measured by the amount of time needed for the cells to double in number. This is referred to as doubling time.

[0012] A cell line is a population of cells formed by one or more subcultivations of a primary cell culture. Each round of subculturing is referred to as a passage. When cells are subcultured, they are referred to as having been passaged. A specific population of cells, or a cell line, is sometimes referred to or characterized by the number of times it has been passaged. For example, a cultured cell population that has been passaged ten times may be referred to as a P10 culture. The primary culture, i.e., the first culture following the isolation of cells from tissue, is designated P0. Following the first subculture, the cells are described as a secondary culture (P1 or passage 1). After the second subculture, the cells become a tertiary culture (P2 or passage 2), and so on. It will be understood by those of skill in the art that there may be many population doublings during the period of passaging; therefore the number of population doublings of a culture is greater than the passage number. The expansion of cells (i.e., the number of population doublings) during the period between passaging depends on many factors, including but not limited to the seeding density, substrate, medium, growth conditions, and time between passaging.

[0013] A conditioned medium is a medium in which a specific cell or population of cells has been cultured, and then removed. When cells are cultured in a medium, they may secrete cellular factors that can provide trophic support to other cells. Such trophic factors include, but are not limited to hormones, cytokines, extracellular matrix (ECM), proteins, vesicles, antibodies, and granules. The medium containing the cellular factors is the conditioned medium.

[0014] Generally, a trophic factor is defined as a substance that promotes or at least supports, survival, growth, proliferation and/or maturation of a cell, or stimulates increased activity of a cell.

[0015] When referring to cultured vertebrate cells, the term senescence (also replicative senescence or cellular senescence) refers to a property attributable to finite cell cultures; namely, their inability to grow beyond a finite number of population doublings (sometimes referred to as Hayflick's limit). Although cellular senescence was first described using fibroblast-like cells, most normal human cell types that can be grown successfully in culture undergo cellular senescence. The in vitro lifespan of different cell types varies, but the maximum lifespan is typically fewer than 100 population doublings (this is the number of doublings for all the cells in the culture to become senescent and thus render the culture unable to divide). Senescence does not depend on chronological time, but rather is measured by the number of cell divisions, or population doublings, the culture has undergone. Thus, cells made quiescent by removing essential growth factors are able to resume growth and division when the growth factors are re-introduced, and thereafter carry out the same number of doublings as equivalent cells grown, continuously. Similarly, when cells are frozen in liquid nitrogen after various numbers of population doublings and then thawed and cultured, they undergo substantially the same number of doublings as cells maintained unfrozen in culture. Senescent cells are not dead or dying cells; they are actually resistant to programmed cell death (apoptosis), and have been maintained in their nondividing state for as long as three years. These cells are very much alive and metabolically active, but they do not divide. The nondividing state of senescent cells has not yet been found to be reversible by any biological, chemical, or viral agent.

[0016] As used herein, the term Growth Medium generally refers to a medium sufficient for the culturing of umbilicus-derived cells. In particular, one presently preferred medium for the culturing of the cells of the invention herein comprises Dulbecco's Modified Essential Media (also abbreviated DMEM herein). Particularly

preferred is DMEM-low glucose (also DMEM-LG herein) (Invitrogen, Carlsbad, Calif.). The DMEM-low glucose is preferably supplemented with 15% (v/v) fetal bovine serum (e.g. defined fetal bovine serum, Hyclone, Logan Utah), antibiotics/antimycotics (preferably penicillin (100 Units/milliliter), streptomycin (100 milligrams/milliliter), and amphotericin B (0.25 micrograms/milliliter), (Invitrogen, Carlsbad, Calif.)), and 0.001% (v/v) 2-mercaptoethanol (Sigma, St. Louis Mo.). In some cases different growth media are used, or different supplementations are provided, and these are normally indicated in the text as supplementations to Growth Medium.

[0017] Also relating to the present invention, the term standard growth conditions, as used herein refers to culturing of cells at 37.degree. C., in a standard atmosphere comprising 5% CO.sub.2. Relative humidity is maintained at about 100%. While foregoing the conditions are useful for culturing, it is to be understood that such conditions are capable of being varied by the skilled artisan who will appreciate the options available in the art for culturing cells, for example, varying the temperature, CO.sub.2, relative humidity, oxygen, growth medium, and the like.

[0018] “Mesenchymal stem cell” or “MSC” in some embodiments refers to cells that are (1) adherent to plastic, (2) express CD73, CD90, and CD105 antigens, while being CD14, CD34, CD45, and HLA-DR negative, and (3) possess ability to differentiate to osteogenic, chondrogenic and adipogenic lineage. Other cells possessing mesenchymal-like properties are included within the definition of “mesenchymal stem cell”, with the condition that said cells possess at least one of the following: a) regenerative activity; b) production of growth factors; c) ability to induce a healing response, either directly, or through elicitation of endogenous host repair mechanisms. As used herein, “mesenchymal stromal cell” or mesenchymal stem cell can be used interchangeably. Said MSC can be derived from any tissue including, but not limited to, bone marrow, adipose tissue, amniotic fluid, endometrium, trophoblast-derived tissues, cord blood, Wharton jelly, placenta, amniotic tissue, derived from pluripotent stem cells, and tooth. In some definitions of “MSC”, said cells include cells that are CD34 positive upon initial isolation from tissue but are similar to cells described about phenotypically and functionally. As used herein, “MSC” may include cells that are isolated from tissues using cell surface markers selected from the list comprised of NGF-R, PDGF-R, EGF-R, IGF-R, CD29, CD49a, CD56, CD63, CD73, CD105, CD106, CD140b, CD146, CD271, MSCA-1, SSEA4, STRO-1 and STRO-3 or any combination thereof, and satisfy the ISCT criteria either before or after expansion. Furthermore, as used herein, in some

contexts, “MSC” includes cells described in the literature as bone marrow stromal stem cells (BMSSC), marrow-isolated adult multipotent inducible cells (MIAMI) cells, multipotent adult progenitor cells (MAPC), mesenchymal adult stem cells (MASCS), MultiStem[®], Prochymal[®], remestemcel-L, Mesenchymal Precursor Cells (MPCs), Dental Pulp Stem Cells (DPSCs), PLX cells, PLX-PAD, AlloStem[®], Astrostem[®], Ixmyelocel-T, MSC-NTF, NurOwn[™], Stemedyne[™]-MSC, Stempeucel[®], StempeucelCLI, StempeucelOA, HiQCell, Hearticellgram-AMI, Revascor[®], Cardiorel[®], Cartistem[®], Pneumostem[®], Promostem[®], Homeo-GH, AC607, PDA001, SB623, CX601, AC607, Endometrial Regenerative Cells (ERC), adipose-derived stem and regenerative cells (ADRCs).

[0019] “Enhanced MSC” refers to MSC or MSC-like cells that are selected for higher expression of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein and lower expression of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1. In a preferred embodiment “Enhanced MSC” are MSC or MSC-like cells that possess higher levels of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein and lower levels of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta

type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1 as compared to other MSC in a culture population, or as compared to MSC derived from different donors.

[0020] Oct-4 (oct-3 in humans) is a transcription factor expressed in the pregastrulation embryo, early cleavage stage embryo, cells of the inner cell mass of the blastocyst, and embryonic carcinoma ("EC") cells (Nichols, J. et al. (1998) *Cell* 95: 379-91), and is down-regulated when cells are induced to differentiate. The oct-4 gene (oct-3 in humans) is transcribed into at least two splice variants in humans, oct-3A and oct-3B. The oct-3B splice variant is found in many differentiated cells whereas the oct-3A splice variant (also previously designated oct-3/4) is reported to be specific for the undifferentiated embryonic stem cell. See Shimosaki et al. (2003) *Development* 130: 2505-12. Expression of oct-3/4 plays an important role in determining early steps in embryogenesis and differentiation. Oct-3/4, in combination with rox-1, causes transcriptional activation of the Zn-finger protein rex-1, which is also required for maintaining ES cells in an undifferentiated state (Rosfjord, E. and Rizzino, A. (1997) *Biochem Biophys Res Commun* 203: 1795-802; Ben-Shushan, E. et al. (1998) *Mol Cell Biol* 18: 1866-78).

[0021] The term "neoplasm" generally denotes disorders involving the clonal proliferation of cells. Neoplasms may be benign, which is to say, not progressive and non-recurrent, and, if so, generally are not life-threatening. Neoplasms also may be malignant, which is to say, that they progressively get worse, spread, and, as a rule, are life threatening and often fatal.

[0022] Inflammatory conditions is an inclusive term and includes, for example: (1) tissue damage due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock intestinal ischemia, spinal cord injury, and traumatic brain injury; (2) inflammatory disorders, e.g., burns, endotoxemia and septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis; anaphylactic shock, severe asthma, angioedema,

Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis; (3) transplant rejection, e.g., hyperacute xenograft rejection; (4) pregnancy related diseases such as recurrent fetal loss and pre-eclampsia, and (5) adverse drug reactions, e.g., drug allergy, IL-2 induced vascular leakage syndrome and radiographic contrast media allergy. Complement-mediated inflammation associated with autoimmune disorders including, but not limited to, myasthenia gravis, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjogren's syndrome, and Takayasu's arteritis, may also be detected with the methods described herein.

[0023] Neurodegenerative condition (or disorder) is an inclusive term encompassing acute and chronic conditions, disorders or diseases of the central or peripheral nervous system. A neurodegenerative condition may be age-related, or it may result from injury or trauma, or it may be related to a specific disease or disorder. Acute neurodegenerative conditions include, but are not limited to, conditions associated with neuronal cell death or compromise including cerebrovascular insufficiency, e.g. due to stroke, focal or diffuse brain trauma, diffuse brain damage, spinal cord injury or peripheral nerve trauma, e.g., resulting from physical or chemical burns, deep cuts or limb severance. Examples of acute neurodegenerative disorders are: cerebral ischemia or infarction including embolic occlusion and thrombotic occlusion, reperfusion following acute ischemia, perinatal hypoxic-ischemic injury, cardiac arrest, as well as intracranial hemorrhage of any type (such as epidural, subdural, subarachnoid and intracerebral), and intracranial and intravertebral lesions (such as contusion, penetration, shear, compression and laceration), as well as whiplash and shaken infant syndrome. Chronic neurodegenerative conditions include, but are not limited to, Alzheimer's disease, Pick's disease, diffuse Lewy body disease, progressive supranuclear palsy (Steel-Richardson syndrome), multisystem degeneration (Shy-Drager syndrome), chronic epileptic conditions associated with neurodegeneration, motor neuron diseases including amyotrophic lateral sclerosis, degenerative ataxias, cortical basal degeneration, ALS-Parkinson's-Dementia complex of Guam, subacute sclerosing panencephalitis, Huntington's disease, Parkinson's disease, synucleinopathies (including multiple system atrophy), primary progressive aphasia, striatonigral degeneration, Machado-Joseph disease/spinocerebellar ataxia type 3 and

olivopontocerebellar degenerations, Gilles De La Tourette's disease, bulbar and pseudobulbar palsy, spinal and spinobulbar muscular atrophy (Kennedy's disease), primary lateral sclerosis, familial spastic paraplegia, Werdnig-Hoffmann disease, Kugelberg-Welander disease, Tay-Sach's disease, Sandhoff disease, familial spastic disease, Wohlfart-Kugelberg-Welander disease, spastic paraparesis, progressive multifocal leukoencephalopathy, familial dysautonomia (Riley-Day syndrome), and prion diseases (including, but not limited to Creutzfeldt-Jakob, Gerstmann-Straussler-Scheinker disease, Kuru and fatal familial insomnia), demyelination diseases and disorders including multiple sclerosis and hereditary diseases such as leukodystrophies.

[0024] Mesenchymal stem cells ("MSC") were originally derived from the embryonal mesoderm and subsequently have been isolated from adult bone marrow and other adult tissues. They can be differentiated to form muscle, bone, cartilage, fat, marrow stroma, and tendon. Mesoderm also differentiates into visceral mesoderm which can give rise to cardiac muscle, smooth muscle, or blood islands consisting of endothelium and hematopoietic progenitor cells. The differentiation potential of the mesenchymal stem cells that have been described thus far is limited to cells of mesenchymal origin, including the best characterized mesenchymal stem cell (See Pittenger, et al. Science (1999) 284: 143-147 and U.S. Pat. No. 5,827,740 (SH2.sup.+ SH4.sup.+ CD29.sup.+ CD44.sup.+ CD71.sup.+ CD90.sup.+ CD106.sup.+ CD120a.sup.+ CD124.sup.+ CD14.sup.- CD34.sup.- CD45.sup.-)). The invention teaches the use of various mesenchymal stem cells

[0025] In one embodiment MSC donor lots are generated from umbilical cord tissue. Means of generating umbilical cord tissue MSC have been previously published and are incorporated by reference [1-7]. The term "umbilical tissue derived cells (UTC)" refers, for example, to cells as described in U.S. Pat. No. 7,510,873, U.S. Pat. No. 7,413,734, U.S. Pat. No. 7,524,489, and U.S. Pat. No. 7,560,276. The UTC can be of any mammalian origin e.g. human, rat, primate, porcine and the like. In one embodiment of the invention, the UTC are derived from human umbilicus. umbilicus-derived cells, which relative to a human cell that is a fibroblast, a mesenchymal stem cell, or an iliac crest bone marrow cell, have reduced expression of genes for one or more of: short stature homeobox 2; heat shock 27 kDa protein 2; chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1); elastin (supravalvular aortic stenosis, Williams-Beuren syndrome); Homo sapiens mRNA; cDNA DKFZp586M2022 (from clone DKFZp586M2022); mesenchyme homeobox 2 (growth arrest-specific homeobox); sine oculis homeobox

homolog 1 (*Drosophila*); crystallin, alpha B; disheveled associated activator of morphogenesis 2; DKFZP586B2420 protein; similar to neuralin 1; tetranectin (plasminogen binding protein); src homology three (SH3) and cysteine rich domain; cholesterol 25-hydroxylase; runt-related transcription factor 3; interleukin 11 receptor, alpha; procollagen C-endopeptidase enhancer; frizzled homolog 7 (*Drosophila*); hypothetical gene BC008967; collagen, type VIII, alpha 1; tenascin C (hexabrachion); iroquois homeobox protein 5; hephaestin; integrin, beta 8; synaptic vesicle glycoprotein 2; neuroblastoma, suppression of tumorigenicity 1; insulin-like growth factor binding protein 2, 36kDa; Homo sapiens cDNA FLJ12280 fis, clone MAMMA1001744; cytokine receptor-like factor 1; potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4; integrin, beta 7; transcriptional co-activator with PDZ-binding motif (TAZ); sine oculis homeobox homolog 2 (*Drosophila*); KIAA1034 protein; vesicle-associated membrane protein 5 (myobrevin); EGF-containing fibulin-like extracellular matrix protein 1; early growth response 3; distal-less homeobox 5; hypothetical protein FLJ20373; aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II); biglycan; transcriptional co-activator with PDZ-binding motif (TAZ); fibronectin 1; proenkephalin; integrin, beta-like 1 (with EGF-like repeat domains); Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1968422; EphA3; KIAA0367 protein; natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C); hypothetical protein FLJ14054; Homo sapiens mRNA; cDNA DKFZp564B222 (from clone DKFZp564B222); BCL2/adenovirus E1B 19 kDa interacting protein 3-like; AE binding protein 1; and cytochrome c oxidase subunit VIIa polypeptide 1 (muscle). In addition, these isolated human umbilicus-derived cells express a gene for each of interleukin 8; reticulon 1; chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha); chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2); chemokine (C-X-C motif) ligand 3; and tumor necrosis factor, alpha-induced protein 3, wherein the expression is increased relative to that of a human cell which is a fibroblast, a mesenchymal stem cell, an iliac crest bone marrow cell, or placenta-derived cell. The cells are capable of self-renewal and expansion in culture, and have the potential to differentiate into cells of other phenotypes.

[0026] Methods of deriving cord tissue mesenchymal stem cells from human umbilical tissue are provided. The cells are capable of self-renewal and expansion in culture, and have the potential to differentiate into cells of other phenotypes. The method comprises (a) obtaining human umbilical tissue; (b) removing substantially all of blood to

yield a substantially blood-free umbilical tissue, (c) dissociating the tissue by mechanical or enzymatic treatment, or both, (d) resuspending the tissue in a culture medium, and (e) providing growth conditions which allow for the growth of a human umbilicus-derived cell capable of self-renewal and expansion in culture and having the potential to differentiate into cells of other phenotypes.

[0027] Tissue can be obtained from any completed pregnancy, term or less than term, whether delivered vaginally, or through other routes, for example surgical Cesarean section. Obtaining tissue from tissue banks is also considered within the scope of the present invention.

[0028] The tissue is rendered substantially free of blood by any means known in the art. For example, the blood can be physically removed by washing, rinsing, and diluting and the like, before or after bulk blood removal for example by suctioning or draining. Other means of obtaining a tissue substantially free of blood cells might include enzymatic or chemical treatment.

[0029] Dissociation of the umbilical tissues can be accomplished by any of the various techniques known in the art, including by mechanical disruption, for example, tissue can be aseptically cut with scissors, or a scalpel, or such tissue can be otherwise minced, blended, ground, or homogenized in any manner that is compatible with recovering intact or viable cells from human tissue.

[0030] In a presently preferred embodiment, the isolation procedure also utilizes an enzymatic digestion process. Many enzymes are known in the art to be useful for the isolation of individual cells from complex tissue matrices to facilitate growth in culture. As discussed above, a broad range of digestive enzymes for use in cell isolation from tissue is available to the skilled artisan. Ranging from weakly digestive (e.g. deoxyribonucleases and the neutral protease, dispase) to strongly digestive (e.g. papain and trypsin), such enzymes are available commercially. A nonexhaustive list of enzymes compatible herewith includes mucolytic enzyme activities, metalloproteases, neutral proteases, serine proteases (such as trypsin, chymotrypsin, or elastase), and deoxyribonucleases. Presently preferred are enzyme activities selected from metalloproteases, neutral proteases and mucolytic activities. For example, collagenases are known to be useful for isolating various cells from tissues. Deoxyribonucleases can digest single-stranded DNA and can minimize cell-clumping during isolation. Enzymes can be used alone or in combination. Serine protease are preferably used in a sequence following the use of other enzymes as they may degrade the other enzymes being used.

The temperature and time of contact with serine proteases must be monitored. Serine proteases may be inhibited with alpha 2 microglobulin in serum and therefore the medium used for digestion is preferably serum-free. EDTA and DNase are commonly used and may improve yields or efficiencies. Preferred methods involve enzymatic treatment with for example collagenase and dispase, or collagenase, dispase, and hyaluronidase, and such methods are provided wherein in certain preferred embodiments, a mixture of collagenase and the neutral protease dispase are used in the dissociating step. More preferred are those methods which employ digestion in the presence of at least one collagenase from *Clostridium histolyticum*, and either of the protease activities, dispase and thermolysin. Still more preferred are methods employing digestion with both collagenase and dispase enzyme activities. Also preferred are methods which include digestion with a hyaluronidase activity in addition to collagenase and dispase activities. The skilled artisan will appreciate that many such enzyme treatments are known in the art for isolating cells from various tissue sources. For example, the LIBERASE BLENDZYME (Roche) series of enzyme combinations of collagenase and neutral protease are very useful and may be used in the instant methods. Other sources of enzymes are known, and the skilled artisan may also obtain such enzymes directly from their natural sources. The skilled artisan is also well-equipped to assess new, or additional enzymes or enzyme combinations for their utility in isolating the cells of the invention. Preferred enzyme treatments are 0.5, 1, 1.5, or 2 hours long or longer. In other preferred embodiments, the tissue is incubated at 37.degree. C. during the enzyme treatment of the dissociation step. Diluting the digest may also improve yields of cells as cells may be trapped within a viscous digest.

[0031] While the use of enzyme activities is presently preferred, it is not required for isolation methods as provided herein. Methods based on mechanical separation alone may be successful in isolating the instant cells from the umbilicus as discussed above.

[0032] The cells can be resuspended after the tissue is dissociated into any culture medium as discussed herein above. Cells may be resuspended following a centrifugation step to separate out the cells from tissue or other debris. Resuspension may involve mechanical methods of resuspending, or simply the addition of culture medium to the cells.

[0033] Providing the growth conditions allows for a wide range of options as to culture medium, supplements, atmospheric conditions, and relative humidity for the cells. A preferred temperature is 37.degree. C., however the temperature may range from about

35.degree. C. to 39.degree. C. depending on the other culture conditions and desired use of the cells or culture.

[0034] Presently preferred are methods which provide cells which require no exogenous growth factors, except as are available in the supplemental serum provided with the Growth Medium. Also provided herein are methods of deriving umbilical cells capable of expansion in the absence of particular growth factors. The methods are similar to the method above, however they require that the particular growth factors (for which the cells have no requirement) be absent in the culture medium in which the cells are ultimately resuspended and grown in. In this sense, the method is selective for those cells capable of division in the absence of the particular growth factors. Preferred cells in some embodiments are capable of growth and expansion in chemically-defined growth media with no serum added. In such cases, the cells may require certain growth factors, which can be added to the medium to support and sustain the cells. Presently preferred factors to be added for growth on serum-free media include one or more of FGF, EGF, IGF, and PDGF. In more preferred embodiments, two, three or all four of the factors are added to serum free or chemically defined media. In other embodiments, LIF is added to serum-free medium to support or improve growth of the cells.

[0035] Also provided are methods wherein the cells can expand in the presence of from about 5% to about 20% oxygen in their atmosphere. Methods to obtain cells that require L-valine require that cells be cultured in the presence of L-valine. After a cell is obtained, its need for L-valine can be tested and confirmed by growing on D-valine containing medium that lacks the L-isomer.

[0036] Methods are provided wherein the cells can undergo at least 25, 30, 35, or 40 doublings prior to reaching a senescent state. Methods for deriving cells capable of doubling to reach 10×10^4 cells or more are provided. Preferred are those methods which derive cells that can double sufficiently to produce at least about 10×10^4 , 10×10^5 , 10×10^6 , or 10×10^7 or more cells when seeded at from about 10×10^3 to about 10×10^6 cells/cm² in culture. Preferably these cell numbers are produced within 80, 70, or 60 days or less. In one embodiment, cord tissue mesenchymal stem cells are isolated and expanded, and possess one or more markers selected from a group comprising of CD10, CD13, CD44, CD73, CD90, CD141, PDGFr-alpha, or HLA-A,B,C. In addition, the cells do not produce one or more of CD31, CD34, CD45, CD117, CD141, or HLA-DR,DP, DQ.

[0037] In one embodiment, bone marrow MSC lots are generated, means of generating BM MSC are known in the literature and examples are incorporated by reference.

[0038] In one embodiment BM-MSC are generated as follows

1. 500 mL Isolation Buffer is prepared (PBS+2% FBS+2 mM EDTA) using sterile components or filtering Isolation Buffer through a 0.2 micron filter. Once made, the Isolation Buffer was stored at 2-8.degree. C.
2. The total number of nucleated cells in the BM sample is counted by taking 10 .mu.L BM and diluting it 1/50-1/100 with 3% Acetic Acid with Methylene Blue (STEMCELL Catalog #07060). Cells are counted using a hemacytometer.
3. 50 mL Isolation Buffer is warmed to room temperature for 20 minutes prior to use and bone marrow was diluted 5/14 final dilution with room temperature Isolation Buffer (e.g. 25 mL BM was diluted with 45 mL Isolation Buffer for a total volume of 70 mL).
4. In three 50 mL conical tubes (BD Catalog #352070), 17 mL Ficoll-Paque.TM. PLUS (Catalog #07907/07957) is pipetted into each tube. About 23 mL of the diluted BM from step 3 was carefully layered on top of the Ficoll-Paque.TM. PLUS in each tube.
5. The tubes are centrifuged at room temperature (15-25.degree. C.) for 30 minutes at 300.times.g in a bench top centrifuge with the brake off.
6. The upper plasma layer is removed and discarded without disturbing the plasma:Ficoll-Paque.TM. PLUS interface. The mononuclear cells located at the interface layer are carefully removed and placed in a new 50 mL conical tube. Mononuclear cells are resuspended with 40 mL cold (2-8.degree. C.) Isolation Buffer and mixed gently by pipetting.
7. Cells were centrifuged at 300.times.g for 10 minutes at room temperature in a bench top centrifuge with the brake on. The supernatant is removed and the cell pellet resuspended in 1-2 mL cold Isolation Buffer.
8. Cells were diluted 1/50 in 3% Acetic Acid with Methylene Blue and the total number of nucleated cells counted using a hemacytometer.
9. Cells are diluted in Complete Human MesenCult.RTM.-Proliferation medium (STEMCELL catalog #05411) at a final concentration of 1.times.10.sup.6 cells/mL.

10. BM-derived cells were ready for expansion and CFU-F assays in the presence of GW2580, which can then be used for specific applications.

[0039] Said BM-MSC are derived in lots and cells from said lots are assayed for presence of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein, Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1. Lots containing higher expression of proteins Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein are chosen for utilization. Furthermore lots expressing lower levels of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1 are chosen for utilization. In a preferred embodiment, lots expressing higher concentrations of Thymidylate synthase, Cytochrome c, Carbohydrate

sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein and lower concentrations of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1 are utilized.

[0040] In one embodiment of the invention MSC are selected for expression of enhanced levels of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein. In another embodiment MSC are selected for reduced levels of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1, in another preferred embodiment MSC are selected for both enhanced levels of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein and reduced levels of Mitogen-activated

protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1 as compared to other MSC in a culture, or as compared to MSC from other donors. Said MSC are used for therapeutic activity and are referred to as "enhanced MSC."

[0041] In one embodiment, MSC are generated according to protocols previously utilized for treatment of patients utilizing bone marrow derived MSC. Specifically, bone marrow is aspirated (10-30 ml) under local anesthesia (with or without sedation) from the posterior iliac crest, collected into sodium heparin containing tubes and transferred to a Good Manufacturing Practices (GMP) clean room. Bone marrow cells are washed with a washing solution such as Dulbecco's phosphate-buffered saline (DPBS), RPMI, or PBS supplemented with autologous patient plasma and layered on to 25 ml of Percoll (1.073 g/ml) at a concentration of approximately $1-2 \times 10^7$ cells/ml. Subsequently the cells are centrifuged at 900 g for approximately 30 min or a time period sufficient to achieve separation of mononuclear cells from debris and erythrocytes. Said cells are then washed with PBS and plated at a density of approximately 1×10^6 cells per ml in 175 cm² tissue culture flasks in DMEM with 10% FCS with flasks subsequently being loaded with a minimum of 30 million bone marrow mononuclear cells. The MSCs are allowed to adhere for 72 h followed by media changes every 3-4 days. Adherent cells are removed with 0.05% trypsin-EDTA and replated at a density of 1×10^6 per 175 cm². Said bone marrow MSC may be administered intravenously, or in a preferred embodiment, intrathecally in a patient suffering radiation associated neurodegenerative manifestations. Although doses may be determined by one of skill in the art, and are dependent on various patient characteristics, intravenous administration may be performed at concentrations ranging from 1-10 million MSC per kilogram, with a preferred dose of approximately 2-5 million cells per kilogram.

[0042] In some embodiments of the invention MSC are transferred to possess enhanced neuromodulatory and neuroprotective properties. Said transfection may be accomplished by use of lentiviral vectors, said means to perform lentiviral mediated transfection are well-known in the art and discussed in the following references [8-14]. Some specific examples of lentiviral based transfection of genes into MSC include transfection of SDF-1 to promote stem cell homing, particularly hematopoietic stem cells [15], , GDNF to treat Parkinson's in an animal model [16], HGF to accelerate remyelination in a brain injury model [17], akt to protect against pathological cardiac remodeling and cardiomyocyte death [18], TRAIL to induce apoptosis of tumor cells [19-22], PGE-1 synthase for cardioprotection [23], NUR77 to enhance migration [24], BDNF to reduce ocular nerve damage in response to hypertension [25], HIF-1 alpha to stimulate osteogenesis [26], dominant negative CCL2 to reduce lung fibrosis [27], interferon beta to reduce tumor progression [28], HLA-G to enhance immune suppressive activity [29], hTERT to induce differentiation along the hepatocyte lineage [30], cytosine deaminase [31], OCT-4 to reduce senescence [32, 33], BAMBI to reduce TGF expression and protumor effects [34], HO-1 for radioprotection [35], LIGHT to induce antitumor activity [36], miR-126 to enhance angiogenesis [37, 38], bcl-2 to induce generation of nucleus pulposus cells [39], telomerase to induce neurogenesis [40], CXCR4 to accelerate hematopoietic recovery [41] and reduce unwanted immunity [42], wnt11 to promote regenerative cytokine production [43], and the HGF antagonist NK4 to reduce cancer [44].

[0043] Cell cultures are tested for sterility weekly, endotoxin by limulus amebocyte lysate test, and mycoplasma by DNA-fluorochrome stain.

[0044] In order to determine the quality of MSC cultures, flow cytometry is performed on all cultures for surface expression of SH-2, SH-3, SH-4 MSC markers and lack of contaminating CD14- and CD-45 positive cells. Cells were detached with 0.05% trypsin-EDTA , washed with DPBS + 2% bovine albumin, fixed in 1% paraformaldehyde, blocked in 10% serum, incubated separately with primary SH-2, SH-3 and SH-4 antibodies followed by PE-conjugated anti-mouse IgG(H+L) antibody . Confluent MSC in 175 cm² flasks are washed with Tyrode's salt solution, incubated with medium 199 (M199) for 60 min, and detached with 0.05% trypsin-EDTA (Gibco). Cells from 10 flasks were detached at a time and MSCs were resuspended in 40 ml of M199 + 1% human serum albumin (HSA; American Red Cross, Washington DC, USA). MSCs harvested from each 10-flask set were stored for up to 4 h at 4°C and combined at the end

of the harvest. A total of $2-10 \times 10^6$ MSC/kg were resuspended in M199 + 1% HSA and centrifuged at 460 g for 10 min at 20°C. Cell pellets were resuspended in fresh M199 + 1% HSA media and centrifuged at 460 g for 10 min at 20°C for three additional times. Total harvest time was 2-4 h based on MSC yield per flask and the target dose. Harvested MSC were cryopreserved in Cryocyte (Baxter, Deerfield, IL, USA) freezing bags using a rate controlled freezer at a final concentration of 10% DMSO (Research Industries, Salt Lake City, UT, USA) and 5% HSA. On the day of infusion cryopreserved units were thawed at the bedside in a 37°C water bath and transferred into 60 ml syringes within 5 min and infused intravenously into patients over 10-15 min. Patients are premedicated with 325-650 mg acetaminophen and 12.5-25 mg of diphenhydramine orally. Blood pressure, pulse, respiratory rate, temperature and oxygen saturation are monitored at the time of infusion and every 15 min thereafter for 3 h followed by every 2 h for 6 h.

[0045] In one embodiment of the invention enhanced MSC are transfected with anti-apoptotic proteins to enhance in vivo longevity. The present invention includes a method of using MSC that have been cultured under conditions to express increased amounts of at least one anti-apoptotic protein as a therapy to inhibit or prevent apoptosis. In one embodiment, the MSC which are used as a therapy to inhibit or prevent apoptosis have been contacted with an apoptotic cell. The invention is based on the discovery that MSC that have been contacted with an apoptotic cell express high levels of anti-apoptotic molecules. In some instances, the MSC that have been contacted with an apoptotic cell secrete high levels of at least one anti-apoptotic protein, including but not limited to, STC-1, BCL-2, XIAP, Survivin, and Bcl-2XL. Methods of transfecting antiapoptotic genes into MSC have been previously described which can be applied to the current invention, said antiapoptotic genes that can be utilized for practice of the invention, in a nonlimiting way, include GATA-4 [45], FGF-2 [46], bcl-2 [39, 47], and HO-1 [48]. Based upon the disclosure provided herein, MSC can be obtained from any source. The MSC may be autologous with respect to the recipient (obtained from the same host) or allogeneic with respect to the recipient. In addition, the MSC may be xenogeneic to the recipient (obtained from an animal of a different species). In one embodiment of the invention MSC are pretreated with agents to induce expression of antiapoptotic genes, one example is pretreatment with exendin-4 as previously described [49]. In a further non-limiting embodiment, MSC used in the present invention can be isolated, from the bone marrow of any species of mammal, including but not limited to, human, mouse, rat, ape, gibbon, bovine. In a non-limiting embodiment, the MSC are isolated from a human,

a mouse, or a rat. In another non-limiting embodiment, the MSC are isolated from a human.

[0046] Based upon the present disclosure, MSC can be isolated and expanded in culture in vitro to obtain sufficient numbers of cells for use in the methods described herein provided that the MSC are cultured in a manner that promotes contact with a tumor endothelial cell. For example, MSC can be isolated from human bone marrow and cultured in complete medium (DMEM low glucose containing 4 mM L-glutamine, 10% FBS, and 1% penicillin/streptomycin) in hanging drops or on non-adherent dishes. The invention, however, should in no way be construed to be limited to any one method of isolating and/or to any culturing medium. Rather, any method of isolating and any culturing medium should be construed to be included in the present invention provided that the MSC are cultured in a manner that provides MSC to express increased amounts of at least one anti-apoptotic protein. Culture conditions for growth of clinical grade MSC have been described in the literature and are incorporated by reference [50-83].

[0047] Without being limited to any one or more explanatory mechanisms for the immunomodulatory, regenerative and other properties, activities, and effects of enhanced MSC, it is worth noting that they can modulate immune responses through a variety of modalities. For instance, enhanced MSC can have direct effects on a graft or host. Such direct effects are primarily a matter of direct contact between enhanced MSC and cells of the host or graft. The contact may be with structural members of the cells or with constituents in their immediate environment. Such direct mechanisms may involve direct contact, diffusion, uptake, or other processes well known to those skilled in the art. The direct activities and effects of the enhanced MSC may be limited spatially, such as to an area of local deposition or to a bodily compartment accessed by injection.

[0048] Enhanced MSC also can "home" in response to "homing" signals, such as those released at sites of injury or disease. Since homing often is mediated by signals whose natural function is to recruit cells to the sites where repairs are needed, the homing behavior can be a powerful tool for concentrating Enhanced MSC to therapeutic targets. This effect can be stimulated by specific factors, as discussed below.

[0049] Enhanced MSC may also modulate immune processes by their response to factors. This may occur additionally or alternatively to direct modulation. Such factors may include homing factors, mitogens, and other stimulatory factors. They may also include differentiation factors, and factors that trigger particular cellular processes. Among the latter are factors that cause the secretion by cells of other specific factors,

such as those that are involved in recruiting cells, such as stem cells (including Enhanced MSC), to a site of injury or disease.

[0050] Enhanced MSC may, in addition to the foregoing or alternatively thereto, secrete factors that act on endogenous cells, such as stem cells or progenitor cells. The factors may act on other cells to engender, enhance, decrease, or suppress their activities. enhanced MSC may secrete factors that act on stem, progenitor, or differentiated cells causing those cells to divide and/or differentiate. One such factor is exosomes and microvesicles produced by said enhanced MSC. Enhanced MSC that home to a site where repair is needed may secrete trophic factors that attract other cells to the site. In this way, Enhanced MSC may attract stem, progenitor, or differentiated cells to a site where they are needed. Enhanced MSC also may secrete factors that cause such cells to divide or differentiate. Secretion of such factors, including trophic factors, can contribute to the efficacy of enhanced MSC in, for instance, limiting inflammatory damage, limiting vascular permeability, improving cell survival, and engendering and/or augmenting homing of repair cells to sites of damage. Such factors also may affect T-cell proliferation directly. Such factors also may affect dendritic cells, by decreasing their phagocytic and antigen presenting activities, which also may affect T-cell activity. Furthermore such factors, or Enhanced MSC themselves, may be capable of modulating T regulatory cell numbers.

[0051] By these and other mechanisms, enhanced MSC can provide beneficial immunomodulatory effects, including, but not limited to, suppression of undesirable and/or deleterious immune reactions, responses, functions, diseases, and the like. Enhanced MSC in various embodiments of the invention provide beneficial immunomodulatory properties and effects that are useful by themselves or in adjunctive therapy for precluding, preventing, lessening, decreasing, ameliorating, mitigating, treating, eliminating and/or curing deleterious immune processes and/or conditions. Such processes and conditions include, for instance, autoimmune diseases, anemias, neoplasms, HVG, GVHD, and certain inflammatory disorders. In one particular embodiment, said enhanced MSC are useful for treatment of Neurological disease, inflammatory conditions, psychiatric disorders, inborn errors of metabolisms, vascular disease, cardiac disease, renal disease, hepatic disease, pulmonary disease, ocular conditions such as uveitis, gastrointestinal disorders, orthopedic disorders, dermal disorders, neoplasias, prevention of neoplasias, hematopoietic disorders, reproductive disorders, gynecological disorders,

urological disorders, immunological disorders, olfactory disorders, and auricular disorders.

[0052] Enhanced MSC are useful in these other regards particularly in mammals. In various embodiments of the invention in this regard, Enhanced MSC are used therapeutically in human patients, often adjunctively to other therapies.

[0053] Enhanced MSC can be prepared from a variety of tissues, such as bone marrow cells, umbilical cord tissue, peripheral blood, mobilized peripheral blood, adipose tissue, menstrual blood and other tissue sources known to contain MSC. When tissue sources of MSC are used said tissue isolates from which the Enhanced MSC are isolated comprise a mixed populations of cells. Enhanced MSC constitute a very small percentage in these initial populations. They must be purified away from the other cells before they can be expanded in culture sufficiently to obtain enough cells for therapeutic applications.

[0054] In some embodiments the enhanced MSC preparations are clonally derived. In principle, the Enhanced MSC in these preparations are genetically identical to one another and, if properly prepared and maintained, are free of other cells. In some embodiments enhanced MSC preparations that are less pure than these may be used. While rare, less pure populations may arise when the initial cloning step requires more than one cell. If these are not all enhanced MSC, expansion will produce a mixed population in which enhanced MSC are only one of at least two types of cells. More often mixed populations arise when enhanced MSC are administered in admixture with one or more other types of cells.

[0055] In many embodiments the purity of enhanced MSC for administration to a subject is about 100%. In other embodiments it is 95% to 100%. In some embodiments it is 85% to 95%. Particularly in the case of admixtures with other cells, the percentage of Enhanced MSC can be 25%-30%, 30%-35%, 35%-40%, 40%-45%, 45%-50%, 60%-70%, 70%-80%, 80%-90%, or 90%-95%.

[0056] The number of enhanced MSC in a given volume can be determined by well known and routine procedures and instrumentation. The percentage of enhanced MSC in a given volume of a mixture of cells can be determined by much the same procedures. Cells can be readily counted manually or by using an automatic cell counter. Specific cells can be determined in a given volume using specific staining and visual examination and by automated methods using specific binding reagent, typically antibodies, fluorescent tags, and a fluorescence activated cell sorter.

[0057] Enhanced MSC immunomodulation may involve undifferentiated enhanced MSC. It may involve enhanced MSC that are committed to a differentiation pathway. Such immunomodulation also may involve enhanced MSC that have differentiated into a less potent stem cell with limited differentiation potential. It also may involve enhanced MSC that have differentiated into a terminally differentiated cell type. The best type or mixture of enhanced MSC will be determined by the particular circumstances of their use, and it will be a matter of routine design for those skilled in the art to determine an effective type or combination of enhanced MSC.

[0058] The choice of formulation for administering enhanced MSC for a given application will depend on a variety of factors. Prominent among these will be the species of subject, the nature of the disorder, dysfunction, or disease being treated and its state and distribution in the subject, the nature of other therapies and agents that are being administered, the optimum route for administration of the enhanced MSC, survivability of enhanced MSC via the route, the dosing regimen, and other factors that will be apparent to those skilled in the art. In particular, for instance, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, for example, liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form, such as a time release form or liquid-filled form).

[0059] For example, cell survival can be an important determinant of the efficacy of cell-based therapies. This is true for both primary and adjunctive therapies. Another concern arises when target sites are inhospitable to cell seeding and cell growth. This may impede access to the site and/or engraftment there of therapeutic Enhanced MSC. Various embodiments of the invention comprise measures to increase cell survival and/or to overcome problems posed by barriers to seeding and/or growth.

[0060] Examples of compositions comprising enhanced MSC include liquid preparations, including suspensions and preparations for intramuscular or intravenous administration (e.g., injectable administration), such as sterile suspensions or emulsions. Such compositions may comprise an admixture of Enhanced MSC with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts,

such as "REMINGTON'S PHARMACEUTICAL SCIENCE," 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

[0061] Compositions of the invention often are conveniently provided as liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues.

[0062] Various additives often will be included to enhance the stability, sterility, and isotonicity of the compositions, such as antimicrobial preservatives, antioxidants, chelating agents, and buffers, among others. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents that delay absorption, for example, aluminum monostearate, and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the cells.

[0063] Enhanced MSC solutions, suspensions, and gels normally contain a major amount of water (preferably purified, sterilized water) in addition to the cells. Minor amounts of other ingredients such as pH adjusters (e.g., a base such as NaOH), emulsifiers or dispersing agents, buffering agents, preservatives, wetting agents and jelling agents (e.g., methylcellulose) may also be present.

[0064] Typically, the compositions will be isotonic, i.e., they will have the same osmotic pressure as blood and lacrimal fluid when properly prepared for administration.

[0065] The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

[0066] Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred

because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount, which will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

[0067] A pharmaceutically acceptable preservative or cell stabilizer can be employed to increase the life of enhanced MSC compositions. If such preservatives are included, it is well within the purview of the skilled artisan to select compositions that will not affect the viability or efficacy of the enhanced MSC.

[0068] Those skilled in the art will recognize that the components of the compositions should be chemically inert. This will present no problem to those skilled in chemical and pharmaceutical principles. Problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation) using information provided by the disclosure, the documents cited herein, and generally available in the art.

[0069] Sterile injectable solutions can be prepared by incorporating the cells utilized in practicing the present invention in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired.

[0070] In some embodiments, enhanced MSC are formulated in a unit dosage injectable form, such as a solution, suspension, or emulsion. Pharmaceutical formulations suitable for injection of Enhanced MSC typically are sterile aqueous solutions and dispersions. Carriers for injectable formulations can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), and suitable mixtures thereof.

[0071] The skilled artisan can readily determine the amount of cells and optional additives, vehicles, and/or carrier in compositions to be administered in methods of the invention. Typically, any additives (in addition to the cells) are present in an amount of 0.001 to 50 wt % in solution, such as in phosphate buffered saline. The active ingredient is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt %, preferably about 0.0001 to about 1 wt %, most preferably about 0.0001 to about 0.05 wt % or about 0.001 to about 20 wt %, preferably about 0.01 to about 10 wt %, and most preferably about 0.05 to about 5 wt %.

[0072] For any composition to be administered to an animal or human, and for any particular method of administration, it is preferred to determine therefore: toxicity, such as by determining the lethal dose (LD) and LD50 in a suitable animal model, e.g., rodent such as mouse or rat; and, the dosage of the composition(s), concentration of components therein, and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure, and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

[0073] In some embodiments Enhanced MSC are encapsulated for administration, particularly where encapsulation enhances the effectiveness of the therapy, or provides advantages in handling and/or shelf life. Encapsulation in some embodiments where it increases the efficacy of ENHANCED MSC mediated immunosuppression may, as a result, also reduce the need for immunosuppressive drug therapy.

[0074] Also, encapsulation in some embodiments provides a barrier to a subject's immune system that may further reduce a subject's immune response to the Enhanced MSC (which generally are not immunogenic or are only weakly immunogenic in allogeneic transplants), thereby reducing any graft rejection or inflammation that might occur upon administration of the cells.

[0075] In a variety of embodiments where enhanced MSC are administered in admixture with cells of another type, which are more typically immunogenic in an allogeneic or xenogeneic setting, encapsulation may reduce or eliminate adverse host immune responses to the non-enhanced MSC cells and/or GVHD that might occur in an immunocompromised host if the admixed cells are immunocompetent and recognize the host as non-self.

[0076] ¶Enhanced MSC may be encapsulated by membranes, as well as capsules, prior to implantation. It is contemplated that any of the many methods of cell encapsulation available may be employed. In some embodiments, cells are individually encapsulated. In some embodiments, many cells are encapsulated within the same membrane. In embodiments in which the cells are to be removed following implantation, a relatively large size structure encapsulating many cells, such as within a single membrane, may provide a convenient means for retrieval.

[0077] A wide variety of materials may be used in various embodiments for microencapsulation of Enhanced MSC. Such materials include, for example, polymer capsules, alginate-poly-L-lysine-alginate microcapsules, barium poly-L-lysine alginate

capsules, barium alginate capsules, polyacrylonitrile/polyvinylchloride (PAN/PVC) hollow fibers, and polyethersulfone (PES) hollow fibers.

[0078] Techniques for microencapsulation of cells that may be used for administration of Enhanced MSC are known to those of skill in the art and are described, for example, in Chang, P., et al., 1999; Matthew, H. W., et al., 1991; Yanagi, K., et al., 1989; Cai Z. H., et al., 1988; Chang, T. M., 1992 and in U.S. Pat. No. 5,639,275 (which, for example, describes a biocompatible capsule for long-term maintenance of cells that stably express biologically active molecules. Additional methods of encapsulation are in European Patent Publication No. 301,777 and U.S. Pat. Nos. 4,353,888; 4,744,933; 4,749,620; 4,814,274; 5,084,350; 5,089,272; 5,578,442; 5,639,275; and 5,676,943. All of the foregoing are incorporated herein by reference in parts pertinent to encapsulation of Enhanced MSC.

[0079] Certain embodiments incorporate Enhanced MSC into a polymer, such as a biopolymer or synthetic polymer. Examples of biopolymers include, but are not limited to, fibronectin, fibin, fibrinogen, thrombin, collagen, and proteoglycans. Other factors, such as the cytokines discussed above, can also be incorporated into the polymer. In other embodiments of the invention, Enhanced MSC may be incorporated in the interstices of a three-dimensional gel. A large polymer or gel, typically, will be surgically implanted. A polymer or gel that can be formulated in small enough particles or fibers can be administered by other common, more convenient, non-surgical routes.

[0080] Pharmaceutical compositions of the invention may be prepared in many forms that include tablets, hard or soft gelatin capsules, aqueous solutions, suspensions, and liposomes and other slow-release formulations, such as shaped polymeric gels. Oral liquid pharmaceutical compositions may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups, or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid pharmaceutical compositions may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. An oral dosage form may be formulated such that cells are released into the intestine after passing through the stomach. Such formulations are described in U.S. Pat. No. 6,306,434 and in the references contained therein.

[0081] Pharmaceutical compositions suitable for rectal administration can be prepared as unit dose suppositories. Suitable carriers include saline solution and other materials commonly used in the art.

[0082] For administration by inhalation, cells can be conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

[0083] Alternatively, for administration by inhalation or insufflation, a means may take the form of a dry powder composition, for example, a powder mix of a modulator and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator. For intra-nasal administration, cells may be administered via a liquid spray, such as via a plastic bottle atomizer.

[0084] Enhanced MSC may be administered with other pharmaceutically active agents. In some embodiments one or more of such agents are formulated together with Enhanced MSC for administration. In some embodiments the Enhanced MSC and the one or more agents are in separate formulations. In some embodiments the compositions comprising the Enhanced MSC and/or the one or more agents are formulated with regard to adjunctive use with one another.

[0085] Enhanced MSC may be administered in a formulation comprising a immunosuppressive agents, such as any combination of any number of a corticosteroid, cyclosporin A, a cyclosporin-like immunosuppressive agent, cyclophosphamide, antithymocyte globulin, azathioprine, rapamycin, FK-506, and a macrolide-like immunosuppressive agent other than FK-506 and rapamycin. In certain embodiments, such agents include a corticosteroid, cyclosporin A, azathioprine, cyclophosphamide, rapamycin, and/or FK-506. Immunosuppressive agents in accordance with the foregoing may be the only such additional agents or may be combined with other agents, such as other agents noted herein. Other immunosuppressive agents include Tacrolimus, Mycophenolate mofetil, and Sirolimus.

[0086] Such agents also include antibiotic agents, antifungal agents, and antiviral agents, to name just a few other pharmacologically active substances and compositions that may be used in accordance with embodiments of the invention.

[0087] Typical antibiotics or anti-mycotic compounds include, but are not limited to, penicillin, streptomycin, amphotericin, ampicillin, gentamicin, kanamycin,

mycophenolic acid, nalidixic acid, neomycin, nystatin, paromomycin, polymyxin, puromycin, rifampicin, spectinomycin, tetracycline, tylosin, zeocin, and cephalosporins, aminoglycosides, and echinocandins.

[0088] Further additives of this type relate to the fact that Enhanced MSC, like other stems cells, following administration to a subject may "home" to an environment favorable to their growth and function. Such "homing" often concentrates the cells at sites where they are needed, such as sites of immune disorder, dysfunction, or disease. A number of substances are known to stimulate homing. They include growth factors and trophic signaling agents, such as cytokines. They may be used to promote homing of Enhanced MSC to therapeutically targeted sites. They may be administered to a subject prior to treatment with Enhanced MSC, together with enhanced MSC, or after enhanced MSC are administered.

[0089] Certain cytokines, for instance, alter or affect the migration of enhanced MSC or their differentiated counterparts to sites in need of therapy, such as immunocompromised sites. Cytokines that may be used in this regard include, but are not limited to, stromal cell derived factor-1 (SDF-1), stem cell factor (SCF), angiopoietin-1, placenta-derived growth factor (PIGF), granulocyte-colony stimulating factor (G-CSF), cytokines that stimulate expression of endothelial adhesion molecules such as ICAMs and VCAMs, and cytokines that engender or facilitate homing.

[0090] They may be administered to a subject as a pre-treatment, along with Enhanced MSC, or after enhanced MSC have been administered, to promote homing to desired sites and to achieve improved therapeutic effect, either by improved homing or by other mechanisms. Such factors may be combined with Enhanced MSC in a formulation suitable for them to be administered together. Alternatively, such factors may be formulated and administered separately.

[0091] Order of administration, formulations, doses, frequency of dosing, and routes of administration of factors (such as the cytokines discussed above) and Enhanced MSC generally will vary with the disorder or disease being treated, its severity, the subject, other therapies that are being administered, the stage of the disorder or disease, and prognostic factors, among others. General regimens that have been established for other treatments provide a framework for determining appropriate dosing in enhanced MSC-mediated direct or adjunctive therapy. These, together with the additional information provided herein, will enable the skilled artisan to determine appropriate administration

procedures in accordance with embodiments of the invention, without undue experimentation.

[0092] Enhanced MSC can be administered to a subject by any of a variety of routes known to those skilled in the art that may be used to administer cells to a subject.

[0093] Among methods that may be used in this regard in embodiments of the invention are methods for administering enhanced MSC by a parenteral route. Parenteral routes of administration useful in various embodiments of the invention include, among others, administration by intravenous, intraarterial, intracardiac, intraspinal, intrathecal, intraosseous, intraarticular, intrasynovial, intracutaneous, intradermal, subcutaneous, and/or intramuscular injection. In some embodiments intravenous, intraarterial, intracutaneous, intradermal, subcutaneous and/or intramuscular injection are used. In some embodiments intravenous, intraarterial, intracutaneous, subcutaneous, and/or intramuscular injection are used.

[0094] In various embodiments of the invention enhanced MSC are administered by systemic injection. Systemic injection, such as intravenous injection, offers one of the simplest and least invasive routes for administering enhanced MSC. In some cases, these routes may require high enhanced MSC doses for optimal effectiveness and/or homing by the enhanced MSC to the target sites. In a variety of embodiments enhanced MSC may be administered by targeted and/or localized injections to ensure optimum effect at the target sites.

[0095] Enhanced MSC may be administered to the subject through a hypodermic needle by a syringe in some embodiments of the invention. In various embodiments, enhanced MSC are administered to the subject through a catheter. In a variety of embodiments, enhanced MSC are administered by surgical implantation. Further in this regard, in various embodiments of the invention, Enhanced MSC are administered to the subject by implantation using an arthroscopic procedure. In some embodiments Enhanced MSC are administered to the subject in or on a solid support, such as a polymer or gel. In various embodiments, Enhanced MSC are administered to the subject in an encapsulated form.

[0096] In additional embodiments of the invention, Enhanced MSC are suitably formulated for oral, rectal, epicutaneous, ocular, nasal, and/or pulmonary delivery and are administered accordingly.

[0097] Compositions can be administered in dosages and by techniques well known to those skilled in the medical and veterinary arts taking into consideration such factors as

the age, sex, weight, and condition of the particular patient, and the formulation that will be administered (e.g., solid vs. liquid). Doses for humans or other mammals can be determined without undue experimentation by the skilled artisan, from this disclosure, the documents cited herein, and the knowledge in the art.

[0098] The dose of enhanced MSC appropriate to be used in accordance with various embodiments of the invention will depend on numerous factors. It may vary considerably for different circumstances. The parameters that will determine optimal doses of enhanced MSC to be administered for primary and adjunctive therapy generally will include some or all of the following: the disease being treated and its stage; the species of the subject, their health, gender, age, weight, and metabolic rate; the subject's immunocompetence; other therapies being administered; and expected potential complications from the subject's history or genotype. The parameters may also include: whether the Enhanced MSC are syngeneic, autologous, allogeneic, or xenogeneic; their potency (specific activity); the site and/or distribution that must be targeted for the Enhanced MSC to be effective; and such characteristics of the site such as accessibility to Enhanced MSC and/or engraftment of Enhanced MSC. Additional parameters include co-administration with Enhanced MSC of other factors (such as growth factors and cytokines). The optimal dose in a given situation also will take into consideration the way in which the cells are formulated, the way they are administered, and the degree to which the cells will be localized at the target sites following administration. Finally, the determination of optimal dosing necessarily will provide an effective dose that is neither below the threshold of maximal beneficial effect nor above the threshold where the deleterious effects associated with the dose of Enhanced MSC outweighs the advantages of the increased dose.

[0099] The optimal dose of enhanced MSC for some embodiments will be in the range of doses used for autologous, mononuclear bone marrow transplantation. For fairly pure preparations of enhanced MSC, optimal doses in various embodiments will range from 10×10^4 to 10×10^8 enhanced MSC cells/kg of recipient mass per administration. In some embodiments the optimal dose per administration will be between 10×10^5 to 10×10^7 enhanced MSC cells/kg. In many embodiments the optimal dose per administration will be 5×10^5 to 5×10^6 enhanced MSC cells/kg. By way of reference, higher doses in the foregoing are analogous to the doses of nucleated cells used in autologous mononuclear bone marrow transplantation. Some of the lower

doses are analogous to the number of CD34.sup.+ cells/kg used in autologous mononuclear bone marrow transplantation.

[00100] It is to be appreciated that a single dose may be delivered all at once, fractionally, or continuously over a period of time. The entire dose also may be delivered to a single location or spread fractionally over several locations.

[00101] In various embodiments, Enhanced MSC may be administered in an initial dose, and thereafter maintained by further administration of Enhanced MSC. Enhanced MSC may be administered by one method initially, and thereafter administered by the same method or one or more different methods. The subject's ENHANCED MSC levels can be maintained by the ongoing administration of the cells. Various embodiments administer the Enhanced MSC either initially or to maintain their level in the subject or both by intravenous injection. In a variety of embodiments, other forms of administration, are used, dependent upon the patient's condition and other factors, discussed elsewhere herein.

[00102] It is noted that human subjects are treated generally longer than experimental animals; but, treatment generally has a length proportional to the length of the disease process and the effectiveness of the treatment. Those skilled in the art will take this into account in using the results of other procedures carried out in humans and/or in animals, such as rats, mice, non-human primates, and the like, to determine appropriate doses for humans. Such determinations, based on these considerations and taking into account guidance provided by the present disclosure and the prior art will enable the skilled artisan to do so without undue experimentation.

[00103] Suitable regimens for initial administration and further doses or for sequential administrations may all be the same or may be variable. Appropriate regimens can be ascertained by the skilled artisan, from this disclosure, the documents cited herein, and the knowledge in the art.

[00104] The dose, frequency, and duration of treatment will depend on many factors, including the nature of the disease, the subject, and other therapies that may be administered. Accordingly, a wide variety of regimens may be used to administer Enhanced MSC.

[00105] In some embodiments Enhanced MSC are administered to a subject in one dose. In others Enhanced MSC are administered to a subject in a series of two or more doses in succession. In some other embodiments wherein Enhanced MSC are administered in a single dose, in two doses, and/or more than two doses, the doses may be

the same or different, and they are administered with equal or with unequal intervals between them.

[00106] Enhanced MSC may be administered in many frequencies over a wide range of times. In some embodiments, enhanced MSC are administered over a period of less than one day. In other embodiment they are administered over two, three, four, five, or six days. In some embodiments Enhanced MSC are administered one or more times per week, over a period of weeks. In other embodiments they are administered over a period of weeks for one to several months. In various embodiments they may be administered over a period of months. In others they may be administered over a period of one or more years. Generally lengths of treatment will be proportional to the length of the disease process, the effectiveness of the therapies being applied, and the condition and response of the subject being treated.

[00107] The immunomodulatory properties of enhanced MSC may be used in treating a wide variety of disorders, dysfunctions and diseases, such as those that, intrinsically, as a secondary effect or as a side effect of treatment, present with deleterious immune system processes and effects. Several illustrations are discussed below.

[00108] Many embodiments in this regard involve administering Enhanced MSC to a subject having a weakened (or compromised) immune system, either as the sole therapy or as adjunctive therapy with another treatment. In a variety of embodiments in this regard Enhanced MSC are administered to a subject adjunctively to radiation therapy or chemotherapy or a combination of radiation and chemotherapies that either have been, are being, or will be administered to the subject. In many such embodiments, the radiation therapy, chemotherapy, or a combination of radiation and chemotherapies are part of a transplant therapy. And in a variety of embodiments Enhanced MSC are administered to treat a deleterious immune response, such as HVG or GVHD.

[00109] In a variety of embodiments in this regard, the subject is the recipient of a non-syngeneic, typically allogeneic, blood cell or bone marrow cell transplant, the immune system of the subject has been weakened or ablated by radiation therapy, chemotherapy, or a combination of radiation and chemotherapy, immunosuppressive drugs are being administered to the subject, the subject is at risk to develop or has developed graft versus host disease, and Enhanced MSC are administered to the subject adjunctively to any one or more of the transplant, the radiation therapy and/or the chemotherapy, and the immunosuppressive drugs to treat, such as ameliorate, arrest, or eliminate, graft versus host disease in the subject.

[00110] In various embodiments, Enhanced MSC are administered to a subject suffering from a neoplasm, adjunctive to a treatment thereof. For example, in some embodiments of the invention in this regard, the subject is at risk for or is suffering from a neoplasm of blood or bone marrow cells and has undergone or will undergo a blood or bone marrow transplant. Using the methods described herein for enhanced MSC isolation, characterization, and expansion, together with the disclosures herein on immune-suppressing properties of Enhanced MSC, enhanced MSC are administered to treat, such as to prevent, suppress, or diminish, the deleterious immune reactions, such as HVG and GVHD, that may complicate the transplantation therapy.

[00111] In a variety of embodiments involving transplant therapies, enhanced MSC can be used alone for an immunosuppressive purpose, or together with other agents. Enhanced MSC can be administered before, during, or after one or more transplants. If administered during transplant, enhanced MSC can be administered separately or together with transplant material. If separately administered, the Enhanced MSC can be administered sequentially or simultaneously with the other transplant materials. Furthermore, Enhanced MSC may be administered well in advance of the transplant and/or well after, alternatively to or in addition to administration at or about the same time as administration of the transplant.

[00112] Other agents that can be used in conjunction with enhanced MSC, in transplantation therapies in particular, include immunomodulatory agents, such as those described elsewhere herein, particularly immunosuppressive agents, more particularly those described elsewhere herein, especially in this regard, one or more of a corticosteroid, cyclosporin A, a cyclosporin-like immunosuppressive compound, azathioprine, cyclophosphamide, methotrexate, and an immunosuppressive monoclonal antibody agent.

[00113] Among neoplastic disorders of bone marrow that are treated with Enhanced MSC in embodiments of the invention in this regard are myeloproliferative disorders ("MPDs"); myelodysplastic syndromes (or states) ("MDSs"), leukemias, and lymphoproliferative disorders including multiple myeloma and lymphomas.

[00114] MPDs are distinguished by aberrant and autonomous proliferation of cells in blood marrow. The disorder may involve only one type of cell or several. Typically, MPDs involve three cell lineages and are erythrocytic, granulocytic, and thrombocytic. Involvement of the three lineages varies from one MPD to another and between occurrences of the individual types. Typically, they are differently affected and one cell

lineage is affected predominately in a given neoplasm. MPDs are not clearly malignant; but, they are classified as neoplasms and are characterized by aberrant, self-replication of hematopoietic precursor cells in blood marrow. MPDs have the potential, nonetheless, to develop into acute leukemias.

[00115] Enhanced MSC can modulate immune responses. In particular in this regard, it has been found that Enhanced MSC can suppress immune responses, including but not limited to immune responses involved in, for example, HVG response and GVHD, to name just two. In an even more detailed particular in this regard, it has been found that Enhanced MSC can suppress proliferation of T-cells, even in the presence of potent T-cell stimulators, such as Concanavalin A and allogeneic or xenogeneic stimulator cells.

[00116] Moreover, it has been found that even relatively small amounts of enhanced MSC can suppress these responses. Indeed, only 3% Enhanced MSC in mixed lymphocyte reactions is sufficient to reduce T-cell response by 50% in vitro.

[00117] In one embodiment of the invention, reduced numbers of enhanced MSC in a patient is used as a diagnostic for predisposition to degenerative disorders.

[00118] Accordingly, embodiments of the invention provide compositions and methods and the like for treating, such as for ameliorating, and/or curing or eliminating, neoplasms, such as neoplasms of hematopoietic cells, particularly those of bone marrow.

[00119] Embodiments of the invention relate to using enhanced MSC immunomodulation to treat an immune dysfunction, disorder, or disease, either solely, or as an adjunctive therapy. Embodiments in this regard relate to congenital immune deficiencies and autoimmune dysfunctions, disorders, and diseases. Various embodiments relate, in this regard, to using Enhanced MSC to treat, solely or adjunctively, Crohn's disease, Guillain-Barre syndrome, lupus erythematosus (also called "SLE" and systemic lupus erythematosus), multiple sclerosis, myasthenia gravis, optic neuritis, psoriasis, rheumatoid arthritis, Graves' disease, Hashimoto's disease, Ord's thyroiditis, diabetes mellitus (type 1), Reiter's syndrome, autoimmune hepatitis, primary biliary cirrhosis, antiphospholipid antibody syndrome ("APS"), opsoclonus-myoclonus syndrome ("OMS"), temporal arteritis, acute disseminated encephalomyelitis ("ADEM" and "ADE"), Goodpasture's, syndrome, Wegener's granulomatosis, celiac disease, pemphigus, polyarthritis, autism, autism spectrum disorder, post traumatic stress disorder, and warm autoimmune hemolytic anemia.

[00120] Particular embodiments among these relate to Crohn's disease, lupus erythematosus (also called "SLE" and systemic lupus erythematosus), multiple sclerosis,

myasthenia gravis, psoriasis, rheumatoid arthritis, Graves' disease, Hashimoto's disease, diabetes mellitus (type 1), Reiter's syndrome, primary biliary cirrhosis, celiac disease, polyarthritis, and warm autoimmune hemolytic anemia.

[00121] In addition, enhanced MSC are used in a variety of embodiments in this regard, solely and, typically, adjunctively, to treat a variety of diseases thought to have an autoimmune component, including but not limited to embodiments that may be used to treat endometriosis, interstitial cystitis, neuromyotonia, scleroderma, progressive systemic scleroderma, vitiligo, vulvodynia, Chagas' disease, sarcoidosis, chronic fatigue syndrome, and dysautonomia.

[00122] In one embodiment higher expression of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein and lower expression of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1 is utilized to select MSC generated from MSC progenitors and/or pluripotent cells

[00123] Inherited immune system disorders include Severe Combined Immunodeficiency (SCID) including but not limited to SCID with Adenosine Deaminase Deficiency (ADA-SCID), SCID which is X-linked, SCID with absence of T & B Cells, SCID with absence of T Cells, Normal B Cells, Omenn Syndrome, Neutropenias including but not limited to Kostmann Syndrome, Myelokathexis; Ataxia-Telangiectasia, Bare Lymphocyte Syndrome, Common Variable Immunodeficiency, DiGeorge Syndrome, Leukocyte Adhesion Deficiency; and phagocyte Disorders (phagocytes are immune system cells that can engulf and kill foreign organisms) including but not limited to Chediak-Higashi Syndrome, Chronic Granulomatous Disease, Neutrophil Actin

Deficiency, Reticular Dysgenesis. Enhanced MSC may be administered adjunctively to a treatment for any of the foregoing diseases.

[00124] In one embodiment tissue culture supernatant is derived from cultures of enhanced MSC and utilized for therapeutic applications. Use of tissue culture supernatant is described in the following patents and incorporated by reference 8,703,710; 9,192,632; 6,642,048; 7,790,455; 9,192,632; and the following patent applications; 20160022738; 20160000699; 20150024483; 20130251670; 20120294949; 20120276215; 20120195969; 20110293583; 20110171182; 20110129447; 20100159588; 20080241112.

[00125] Example 1

[00126] Extraordinary clinical results in patients receiving MSCs were noticed. An analysis of the cells used in treatment yielded data showing that the extraordinary results were among individuals who had received a limited number of lots of MSCs. A group of six of those lots (Enhanced MSCs) was compiled and compared to a group of six lots of cells that had been used clinically without subjects experiencing extraordinary clinical results (Normal MSCs) and two groups were compared in an experiment that was designed to determine if there were differences in the proteins of the cell lysates from each group. The cells were lysed and placed in m-Per with HALT protease inhibitor and normalized for protein concentration.

[00127] To perform the experiment, all cells' suspensions were transferred to 1.5ml cryovials and then centrifuged at 2500g for 10minutes. Supernatant was removed, M-PER with Halt protease additive (500ul of 1X M-PER + 5.5ul of 100X HALT) was added. Samples were then placed in a rocker for 15 minutes at room temperature (22°C), and then centrifuged at 14000g for 6 minutes. Supernatants placed in amber cryovials and labeled. Protein concentrations were determined using the Bradford Method. The lysates were sent to Somalogics for the SOMAscan™ assay. A description of the assay from the company follows:

[00128] “The SOMAscan™ assay is comprised of 1129 proprietary SOMAmer® molecules that function as affinity reagents to detect 1,129 proteins within a small amount of sample. The assay is a multi-step, semiautomated process that converts protein signal to SOMAmer signal that is quantified on a DNA microarray. The median %CV over all 1129 SOMAmer reagents is 5%, the median lower limit of quantification is 100 fM, and the median quantification range is 4.2 logs per SOMAmer (8 logs across all SOMAmer reagents). SOMAmer reagents are DNA-based affinity reagents with modified nucleotides that confer high specificity and affinity. Each SOMAmer contains four

functional moieties: a unique protein recognition sequence, a biotin for capture, a photocleavable linker, and a fluorescent molecule for detection. For the SOMAscan assay, SOMAmer reagents are grouped into large mixes specific for sample type and dilution. Each mixture of SOMAmer reagents is pre-bound to streptavidin beads prior to incubation with the respective sample dilutions. Proteins bind to cognate SOMAmer molecules during equilibration. Afterwards the streptavidin beads are washed to remove non-specifically associated proteins. Next, an NHS-biotin reagent is added to label the proteins bound to their cognate SOMAmer molecule. After the labeling reaction, washing occurs to remove the NHS-biotin reagent. Afterwards the beads are exposed to a UV light source to cleave the photocleavable linker which releases the SOMAmer molecules from the beads. The photocleavage eluate, which contains all of the SOMAmer reagents, some bound to a biotin-labeled protein and some free, is separated from the beads and then incubated with a second streptavidin-coated bead. This second streptavidin capture binds the biotin-labeled proteins and associated SOMAmer molecules. The unbound

[00129] SOMAmer reagents are removed during subsequent washing steps. In the final elution step, SOMAmer molecules are released from their protein using denaturing conditions. The eluate is hybridized to custom Agilent DNA microarrays and the fluorophore from the SOMAmer molecule is quantified in relative fluorescent units (RFU). The RFU is proportional to the amount of target protein in the initial sample.”

[00130] Once the data were received from Somalogic, an analysis of the average RFU for each protein were compared and a student t-test was performed to determine the significance of differences for each analyte.

[00131] Results of highly significant difference in marker concentration between Enhanced and Normal mesenchymal stem cells are presented in table below:

Molecule	RFU		
	Enhanced MSCs	Normal MSCs	P Value t-test
Mitogen-activated protein kinase 8	2878	11245	0.00000024
Connective tissue growth factor	10362	18786	0.00006127
Apolipoprotein E (isoform E4)	5187	9725	0.00011003
Interleukin-12 receptor subunit beta-2	6910	9005	0.00038706

Molecule	RFU		P Value t-test
	Enhanced MSCs	Normal MSCs	
AMP Kinase			
(alpha2beta2gamma1)	14085	25314	0.00064698
Tyrosine-protein kinase Fer	198	930	0.00066166
Sorting nexin-4	8442	24016	0.00094100
Moesin	2574	2984	0.00097830
Complement factor I	6244	19736	0.00098805
Tyrosine-protein kinase CSK	27942	69711	0.00106210
Calcium/calmodulin-dependent protein kinase type II subunit beta	15457	21760	0.00169880
Protein kinase C beta type (splice variant beta-II)	2054	3391	0.00206408
Neurogenic locus notch homolog protein 1	1343	1915	0.00208523
Tenascin	8991	10957	0.00261107
TATA-box-binding protein	6461	10911	0.00326294
3-phosphoinositide-dependent protein kinase 1	10666	21709	0.00381177
Calcium/calmodulin-dependent protein kinase type II subunit alpha	5297	7687	0.00500954
Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex	20252	25204	0.00539456
Interferon alpha-2	886	1152	0.00572893
Inosine-5'-monophosphate dehydrogenase 1	36435	56299	0.00596655
Cystatin-C	3127	6027	0.00605327
Histone acetyltransferase type B catalytic subunit	29221	50103	0.00641762

Molecule	RFU		
	Enhanced MSCs	Normal MSCs	P Value t-test
Sphingosine kinase 2	2239	2751	0.00671356
Disintegrin and metalloproteinase domain-containing protein 12	4130	4551	0.00737758
C-type mannose receptor 2	8904	17241	0.00933531
Neural cell adhesion molecule L1	240	264	0.00973630
Thymidylate synthase	1165	1062	0.00030100
Cytochrome c	121105	33510	0.00101035
Carbohydrate sulfotransferase 15	923	628	0.00108565
C-C motif chemokine 16	574	539	0.00208533
Tropomyosin alpha-1 chain	11020	10230	0.00353268
Trypsin-3	2388	2168	0.00411373
C-C motif chemokine 17	5491	5161	0.00412279
Troponin I, cardiac muscle	1769	1686	0.00723458
Parathyroid hormone-related protein	4280	4001	0.00728418

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Claims

1. A method of selecting for mesenchymal stem cells possessing enhanced efficacy, said method comprising the steps of: a) obtaining mesenchymal stem cells; b) identifying expression of one or more markers selected from a group comprising of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein, Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1; c) selecting for cells that express lower levels of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1; d) identifying one more markers selected from a group comprising of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein, Mitogen-activated protein kinase 8, Connective

tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1; and e) selecting for cells that express higher levels of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein.

2. The method of claim 1, wherein said mesenchymal stem cells are naturally occurring mesenchymal stem cells.
3. The method of claim 1, wherein said mesenchymal stem cells are generated in vitro.
4. The method of claim 2, wherein said naturally occurring mesenchymal stem cells are tissue derived.
5. The method of claim 2, wherein said naturally occurring mesenchymal stem cells are derived from a bodily fluid.
6. The method of claim 4, wherein said tissue derived mesenchymal stem cells are selected from a group comprising of: a) bone marrow; b) perivascular tissue; c) adipose tissue; d) placental tissue; e) amniotic membrane; f) omentum; g) tooth; h) umbilical cord tissue; i) fallopian tube tissue; j) hepatic tissue; k) renal tissue; l) cardiac tissue; m) tonsillar tissue; n) testicular tissue; o) ovarian tissue; p) neuronal tissue; q) auricular tissue; r) colonic tissue; s) submucosal tissue; t) hair follicle tissue; u) pancreatic tissue; v) skeletal muscle tissue; and w) subepithelial umbilical cord tissue.

7. The method of claim 4, wherein said tissue derived mesenchymal stem cells are isolated from tissues containing cells selected from a group of cells comprising of: endothelial cells, epithelial cells, dermal cells, endodermal cells, mesodermal cells, fibroblasts, osteocytes, chondrocytes, natural killer cells, dendritic cells, hepatic cells, pancreatic cells, stromal cells, salivary gland mucous cells, salivary gland serous cells, von Ebner's gland cells, mammary gland cells, lacrimal gland cells, ceruminous gland cells, eccrine sweat gland dark cells, eccrine sweat gland clear cells, apocrine sweat gland cells, gland of Moll cells, sebaceous gland cells, Bowman's gland cells, Brunner's gland cells, seminal vesicle cells, prostate gland cells, bulbourethral gland cells, Bartholin's gland cells, gland of Littre cells, uterus endometrium cells, isolated goblet cells, stomach lining mucous cells, gastric gland zymogenic cells, gastric gland oxyntic cells, pancreatic acinar cells, Paneth cells, type II pneumocytes, Clara cells, somatotropes, lactotropes, thyrotropes, gonadotropes, corticotropes, intermediate pituitary cells, magnocellular neurosecretory cells, gut cells, respiratory tract cells, thyroid epithelial cells, parafollicular cells, parathyroid gland cells, parathyroid chief cell, oxyphil cell, adrenal gland cells, chromaffin cells, Leydig cells, theca interna cells, corpus luteum cells, granulosa lutein cells, theca lutein cells, juxtaglomerular cell, macula densa cells, peripolar cells, mesangial cell, blood vessel and lymphatic vascular endothelial fenestrated cells, blood vessel and lymphatic vascular endothelial continuous cells, blood vessel and lymphatic vascular endothelial splenic cells, synovial cells, serosal cell (lining peritoneal, pleural, and pericardial cavities), squamous cells, columnar cells, dark cells, vestibular membrane cell (lining endolymphatic space of ear), stria vascularis basal cells, stria vascularis marginal cell (lining endolymphatic space of ear), cells of Claudius, cells of Boettcher, choroid plexus cells, pia-arachnoid squamous cells, pigmented ciliary epithelium cells, nonpigmented ciliary epithelium cells, corneal endothelial cells, peg cells, respiratory tract ciliated cells, oviduct ciliated cell, uterine endometrial ciliated cells, rete testis ciliated cells, ductulus efferens ciliated cells, ciliated ependymal cells, epidermal keratinocytes, epidermal basal cells, keratinocyte of fingernails and toenails, nail bed basal cells, medullary hair shaft cells, cortical hair shaft cells, cuticular hair shaft cells, cuticular hair root sheath cells, hair root sheath cells of Huxley's layer, hair root sheath cells of Henle's layer, external hair root sheath cells, hair matrix cells, surface epithelial cells of stratified squamous epithelium, basal cell of epithelia, urinary epithelium cells, auditory inner hair cells of organ of Corti, auditory outer hair cells of organ of Corti, basal cells of olfactory epithelium, cold-sensitive primary sensory

neurons, heat-sensitive primary sensory neurons, Merkel cells of epidermis, olfactory receptor neurons, pain-sensitive primary sensory neurons, photoreceptor rod cells, photoreceptor blue-sensitive cone cells, photoreceptor green-sensitive cone cells, photoreceptor red-sensitive cone cells, proprioceptive primary sensory neurons, touch-sensitive primary sensory neurons, type I carotid body cells, type II carotid body cell (blood pH sensor), type I hair cell of vestibular apparatus of ear (acceleration and gravity), type II hair cells of vestibular apparatus of ear, type I taste bud cells cholinergic neural cells, adrenergic neural cells, peptidergic neural cells, inner pillar cells of organ of Corti, outer pillar cells of organ of Corti, inner phalangeal cells of organ of Corti, outer phalangeal cells of organ of Corti, border cells of organ of Corti, Hensen cells of organ of Corti, vestibular apparatus supporting cells, taste bud supporting cells, olfactory epithelium supporting cells, Schwann cells, satellite cells, enteric glial cells, astrocytes, neurons, oligodendrocytes, spindle neurons, anterior lens epithelial cells, crystallin-containing lens fiber cells, hepatocytes, adipocytes, white fat cells, brown fat cells, liver lipocytes, kidney glomerulus parietal cells, kidney glomerulus podocytes, kidney proximal tubule brush border cells, loop of Henle thin segment cells, kidney distal tubule cells, kidney collecting duct cells, type I pneumocytes, pancreatic duct cells, nonstriated duct cells, duct cells, intestinal brush border cells, exocrine gland striated duct cells, gall bladder epithelial cells, ductulus efferens nonciliated cells, epididymal principal cells, epididymal basal cells, ameloblast epithelial cells, planum semilunatum epithelial cells, organ of Corti interdental epithelial cells, loose connective tissue fibroblasts, corneal keratocytes, tendon fibroblasts, bone marrow reticular tissue fibroblasts, nonepithelial fibroblasts, pericytes, nucleus pulposus cells, cementoblast/cementocytes, odontoblasts, odontocytes, hyaline cartilage chondrocytes, fibrocartilage chondrocytes, elastic cartilage chondrocytes, osteoblasts, osteocytes, osteoclasts, osteoprogenitor cells, hyalocytes, stellate cells (ear), hepatic stellate cells (Ito cells), pancreatic stelle cells, red skeletal muscle cells, white skeletal muscle cells, intermediate skeletal muscle cells, nuclear bag cells of muscle spindle, nuclear chain cells of muscle spindle, satellite cells, ordinary heart muscle cells, nodal heart muscle cells, Purkinje fiber cells, smooth muscle cells, myoepithelial cells of iris, myoepithelial cell of exocrine glands, melanocytes, retinal pigmented epithelial cells, oogonia/oocytes, spermatids, spermatocytes, spermatogonium cells, spermatozoa, ovarian follicle cells, Sertoli cells, thymus epithelial cell, and/or interstitial kidney cells.

8. The method of claim 1, wherein said mesenchymal stem cells are plastic adherent.
9. The method of claim 1, wherein said mesenchymal stem cells express a marker selected from a group comprising of: a) CD73; b) CD90; and c) CD105.
10. The method of claim 1, wherein said mesenchymal stem cells lack expression of a marker selected from a group comprising of: a) CD14; b) CD45; and c) CD34.
11. The method of claim 6, wherein said mesenchymal stem cells from umbilical cord tissue express markers selected from a group comprising of; a) oxidized low density lipoprotein receptor 1, b) chemokine receptor ligand 3; and c) granulocyte chemotactic protein.
12. The method of claim 6, wherein said mesenchymal stem cells from umbilical cord tissue do not express markers selected from a group comprising of: a) CD117; b) CD31; c) CD34; and CD45;
13. The method of claim 6, wherein said mesenchymal stem cells from umbilical cord tissue express, relative to a human fibroblast, increased levels of interleukin 8 and reticulon 1
14. The method of claim 6, wherein said mesenchymal stem cells from umbilical cord tissue have the potential to differentiate into cells of at least a skeletal muscle, vascular smooth muscle, pericyte or vascular endothelium phenotype.
15. The method of claim 6, wherein said mesenchymal stem cells from umbilical cord tissue express markers selected from a group comprising of: a) CD10; b) CD13; c) CD44; d) CD73; and e) CD90.
16. The method of claim 6, wherein said umbilical cord tissue mesenchymal stem cell is an isolated umbilical cord tissue cell isolated from umbilical cord tissue substantially free of blood that is capable of self-renewal and expansion in culture,
17. The method of claim 16, wherein said umbilical cord tissue mesenchymal stem cells has the potential to differentiate into cells of other phenotypes.

18. The method of claim 17, wherein said other phenotypes comprise: a) osteocytic; b) adipogenic; and c) chondrogenic differentiation.
19. The method of claim 6, wherein said cord tissue derived mesenchymal stem cells can undergo at least 20 doublings in culture.
20. The method of claim 6, wherein said cord tissue derived mesenchymal stem cell maintains a normal karyotype upon passaging
21. The method of claim 6, wherein said cord tissue derived mesenchymal stem cell expresses a marker selected from a group of markers comprised of: a) CD10 b) CD13; c) CD44; d) CD73; e) CD90; f) PDGFr-alpha; g) PD-L2; and h) HLA-A,B,C
22. The method of claim 6, wherein said cord tissue mesenchymal stem cells does not express one or more markers selected from a group comprising of; a) CD31; b) CD34; c) CD45; d) CD80; e) CD86; f) CD117; g) CD141; h) CD178; i) B7-H2; j) HLA-G and k) HLA-DR,DP,DQ.
23. The method of claim 6, wherein said umbilical cord tissue-derived cell secretes factors selected from a group comprising of: a) MCP-1; b) MIP1beta; c) IL-6; d) IL-8; e) GCP-2; f) HGF; g) KGF; h) FGF; i) HB-EGF; j) BDNF; k) TPO; l) RANTES; and m) TIMP1
24. The method of claim 6, wherein said umbilical cord tissue derived cells express markers selected from a group comprising of: a) TRA1-60; b) TRA1-81; c) SSEA3; d) SSEA4; and e) NANOG.
25. The method of claim 6, wherein said umbilical cord tissue-derived cells are positive for alkaline phosphatase staining.
26. The method of claim 6, wherein said umbilical cord tissue-derived cells are capable of differentiating into one or more lineages selected from a group comprising of; a) ectoderm; b) mesoderm, and; c) endoderm.
27. The method of claim 6, wherein said bone marrow derived mesenchymal stem cells possess markers selected from a group comprising of: a) CD73; b) CD90; and c) CD105.

28. The method of claim 6, wherein said bone marrow derived mesenchymal stem cells possess markers selected from a group comprising of: a) LFA-3; b) ICAM-1; c) PECAM-1; d) P-selectin; e) L-selectin; f) CD49b/CD29; g) CD49c/CD29; h) CD49d/CD29; i) CD29; j) CD18; k) CD61; l) 6-19; m) thrombomodulin; n) telomerase; o) CD10; p) CD13; and q) integrin beta.
29. The method of claim 6, wherein said bone marrow derived mesenchymal stem cell is a mesenchymal stem cell progenitor cell.
30. The method of claim 29, wherein said mesenchymal progenitor cells are a population of bone marrow mesenchymal stem cells enriched for cells containing STRO-1
31. The method of claim 30, wherein said mesenchymal progenitor cells express both STRO-1 and VCAM-1.
32. A method of claim 30, wherein said STRO-1 expressing cells are negative for at least one marker selected from the group consisting of: a) CBFA-1; b) collagen type II; c) PPAR. γ 2; d) osteopontin; e) osteocalcin; f) parathyroid hormone receptor; g) leptin; h) H-ALBP; i) aggrecan; j) Ki67, and k) glycophorin A.
33. The method of claim 6, wherein said bone marrow mesenchymal stem cells lack expression of CD14, CD34, and CD45.
34. The method of claim 32, wherein said STRO-1 expressing cells are positive for a marker selected from a group comprising of: a) VACM-1; b) TKY-1; c) CD146 and; d) STRO-2
35. The method of claim 6, wherein said bone marrow mesenchymal stem cell express markers selected from a group comprising of: a) CD13; b) CD34; c) CD56 and; d) CD117
36. The method of claim 35, wherein said bone marrow mesenchymal stem cells do not express CD10.
37. The method of claim 35, wherein said bone marrow mesenchymal stem cells do not express CD2, CD5, CD14, CD19, CD33, CD45, and DR11.

38. The method of claim 35, wherein said bone marrow mesenchymal stem cells express CD13, CD34, CD56, CD90, CD117 and nestin, and which do not express CD2, CD3, CD10, CD14, CD16, CD31, CD33, CD45 and CD64.

39. The method of claim 6, wherein said skeletal muscle stem cells express markers selected from a group comprising of: a) CD13; b) CD34; c) CD56 and; d) CD117

40. The method of claim 40, wherein said skeletal muscle mesenchymal stem cells do not express CD10.

41. The method of claim 40, wherein said skeletal muscle mesenchymal stem cells do not express CD2, CD5, CD14, CD19, CD33, CD45, and DR11.

42. The method of claim 40, wherein said bone marrow mesenchymal stem cells express CD13, CD34, CD56, CD90, CD117 and nestin, and which do not express CD2, CD3, CD10, CD14, CD16, CD31, CD33, CD45 and CD64.

43. The method of claim 6, wherein said subepithelial umbilical cord derived mesenchymal stem cells possess markers selected from a group comprising of; a) CD29; b) CD73; c) CD90; d) CD166; e) SSEA4; f) CD9; g) CD44; h) CD146; and i) CD105

44. The method of claim 43, wherein said subepithelial umbilical cord derived mesenchymal stem cells do not express markers selected from a group comprising of; a) CD45; b) CD34; c) CD14; d) CD79; e) CD106; f) CD86; g) CD80; h) CD19; i) CD117; j) Stro-1 and k) HLA-DR.

45. The method of claim 43, wherein said subepithelial umbilical cord derived mesenchymal stem cells express CD29, CD73, CD90, CD166, SSEA4, CD9, CD44, CD146, and CD105.

46. The method of claim 43, wherein said subepithelial umbilical cord derived mesenchymal stem cells do not express CD45, CD34, CD14, CD79, CD106, CD86, CD80, CD19, CD117, Stro-1, and HLA-DR.

47. The method of claim 43, wherein said subepithelial umbilical cord derived mesenchymal stem cells are positive for SOX2.

48. The method of claim 43, wherein said subepithelial umbilical cord derived mesenchymal stem cells are positive for OCT4.
49. The method of claim 43, wherein said subepithelial umbilical cord derived mesenchymal stem cells are positive for OCT4 and SOX2.
50. The method of claim 1, wherein said efficacy reflects enhanced angiogenic activity.
51. The method of claim 1, wherein said efficacy reflects enhanced regenerative activity.
52. The method of claim 1, wherein said efficacy reflects enhanced ability to stimulate endogenous regenerative activity.
53. The method of claim 1, wherein said efficacy reflects enhanced ability to induce immune modulation.
54. The method of claim 1, wherein said efficacy reflects enhanced ability to induce clinical response in a disease condition.
55. The method of claim 54, wherein said disease condition is selected from a group comprising of: a) neurological disease; b) inflammatory conditions; c) psychiatric disorders; d) inborn errors of metabolisms; e) vascular disease; f) cardiac disease; g) renal disease; h) hepatic disease; i) pulmonary disease; j) ocular conditions; k) gastrointestinal disorders; l) orthopedic disorders; m) dermal disorders; n) neoplasia; o) predisposition to neoplasia; p) hematopoietic disorders; q) reproductive disorders; r) gynecological disorders; s) urological disorders; t) immunological disorders; u) olfactory disorders; and v) auricular disorders.
56. The method of claim 1, wherein said mesenchymal stem cells selected for enhanced efficacy are utilized as a source of conditioned media.
57. The method of claim 56, wherein conditioned media is used therapeutically in the treatment of a disorder.
58. The method of claim 57, wherein said disorder is selected from a group comprising of a) neurological disease; b) inflammatory conditions; c) psychiatric disorders; d) inborn errors of metabolisms; e) vascular disease; f) cardiac disease; g) renal disease; h) hepatic

disease; i) pulmonary disease; j) ocular conditions; k) gastrointestinal disorders; l) orthopedic disorders; m) dermal disorders; n) neoplasia; o) predisposition to neoplasia; p) hematopoietic disorders; q) reproductive disorders; r) gynecological disorders; s) urological disorders; t) immunological disorders; u) olfactory disorders; and v) auricular disorders.

59. The method of claim 1, wherein mesenchymal stem cells are selected for enhanced efficacy by selecting for cells expressing higher levels of Thymidylate synthase, Cytochrome c, Carbohydrate sulfotransferase 15 C-C motif chemokine 16, Tropomyosin alpha-1 chain, Trypsin-3, C-C motif chemokine 17, Troponin I, cardiac muscle, and Parathyroid hormone-related protein.

60. The method of claim 1, wherein mesenchymal stem cells are selected for enhanced efficacy by selecting for cells expressing lower levels of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1..

61. The method of claim 1, wherein selection of cells for high or low expression of markers is performed by flow cytometry sorting.

62. The method of claim 61, wherein said flow cytometry sorting is performed by utilizing a fluorescent means that selectively induces a signal upon binding to markers of said cells.

63. The method of claim 62, wherein said flow cytometry sorting is performed by utilizing a fluorescent means that selectively induces a signal upon alteration induced by markers in said mesenchymal stem cells.

64. The method of claim 63, wherein said alteration induced by said marker is an enzymatic interaction.

65. The method of augmenting mesenchymal stem cell efficacy by inhibiting the expression and or function of Mitogen-activated protein kinase 8, Connective tissue growth factor, Apolipoprotein E (isoform E4), Interleukin-12 receptor subunit beta-2, AMP Kinase (alpha2beta2gamma1), Tyrosine-protein kinase Fer, Sorting nexin-4, Moesin, Complement factor I, Tyrosine-protein kinase CSK, Calcium/calmodulin-dependent protein kinase type II subunit beta, Protein kinase C beta type (splice variant beta-II), Neurogenic locus notch homolog protein 1, Tenascin, TATA-box-binding protein 3-phosphoinositide-dependent protein kinase 1, Calcium/calmodulin-dependent protein kinase type II subunit alpha, Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1 complex, Interferon alpha-2, Inosine-5'-monophosphate dehydrogenase, Cystatin-C, Histone acetyltransferase type B catalytic subunit, Sphingosine kinase, Disintegrin and metalloproteinase domain-containing protein 12, C-type mannose receptor 2, and Neural cell adhesion molecule L1. by means selected from: a) dominant negative protein expression; b) small molecule inhibition; c) antisense; d) RNAi induction; and, e) gene editing.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/22513

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 63/00, A01N 65/00 (2017.01)

CPC - A61K 35/28, C12N 2501/999, C12N 2501/40, A61K 35/545, A61K 31/365

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2014/128634 A1 (Pluristem Ltd.) 28 August 2014 (28.08.2014) para [002]; [005]-[006]; [0170]; Table 1, pg 49; Table 4, pg 56; Table 7, pg 69; Table 8, pg 71; Table 9, pg 75; Table 10, pg 76; Table 13, pg 85; Table 15, pg 91, 97; Table 21	1-64
A	WO 2015/121859 A1 (Brainstorm Cell Therapeutics Ltd.) 20 August 2015 (20.08.2015) pg 2, ln 21-30; pg 24, ln 21-28; pg 44, ln 5-9; pg 44, Table 3, no. 19; pg 45, Table 3, no. 42	1-64
A	US 2009/0232773 A1 (Kato et al.) 17 September 2009 (17.09.2009) para [0015]-[0018]; [0131]; Table 1d	1-64
A	US 2014/0286913 A1 (Bar-Or et al.) 25 September 2014 (25.09.2014) para [0014]; [0056]	65
A	US 2011/0263675 A1 (Federov et al.) 27 October 2011 (27.10.2011) para [0006]-[0007]; [0019]; Table 2, 4, 6	65
A	US 2014/0017787 A1 (Betancourt) 16 January 2014 (16.01.2014) para [0010]; [0046]; Table 2	65
A	US 2013/0156819 A1 (Aicher et al.) 20 June 2013 (20.06.2013) whole document	1-65
A	US 2015/0197725 A1 (Lim et al.) 16 July 2015 (16.07.2015) whole document	1-65
A	US 2013/0028871 A1 (Edinger et al.) 31 January 2013 (31.01.2013) whole document	1-65



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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"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

5 May 2017

Date of mailing of the international search report

06 JUN 2017

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