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(54) Title: EX VIVO PERFUSION OF DONOR ORGANS PRIOR TO TRANSPLANTATION USING MESENCHYMAL STEM CELLS

(57) Abstract: The invention relates to methods of treating a donor organ by *ex* vivo perfusion of the donor organ with mesenchymal stem cells (MSCs) prior to transplantation to a patient.

EX VIVO PERFUSION OF DONOR ORGANS PRIOR TO TRANSPLANTATION USING MESENCHYMAL STEM CELLS

RELATED APPLICATIONS

[1] This application claims priority to U.S. Provisional Application No. 61/828,509, filed on May 29, 2013, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[2] The present invention generally relates to the use of mesenchymal stem cells to perfuse donor organs such as kidneys prior to transplantation.

BACKGROUND OF THE INVENTION

[3] Stem cell therapy offers a promising new option for the treatment of human disease. Adult stem cells have been used successfully to treat patients in various clinical trials across a number of clinical conditions. Mesenchymal stem cells (MSCs) are bone marrow, adipose, and/or cord blood derived cells that have the ability to differentiate into a variety of cell types under certain conditions, possess immunomodulatory properties and secrete chemokines, cytokines and growth factors (Schinkothe *et al.*, Stem Cells Dev. 2008; 17: 199–206), together making them ideal candidate therapies of various disorders (Porada *et al.,* Curr Stem Cell Res Ther. 2006; 1:365–9). MSCs have been used successfully to treat a number of conditions in animal models and are currently being evaluated in clinical trials to treat different diseases including acute kidney injury (AKI), myocardial infarction, graft versus host disease, Crohn's disease and others (Giordano *et al.*, J Cell Physiol. 2007; 211: 27–35). Additionally, Prochymal® (Osiris Therapeutics, Inc.), a hMSC therapy, has been approved for pediatric graft versus host disease in a number of countries.

[4] MSCs are effective in reducing kidney injury and enhancing recovery of kidney function in animal models of AKI, including an ischemia/reperfusion model as well as in cytotoxicity models such as the cisplatin model. Importantly, in these models, MSC do not or only rarely directly contribute to differentiated kidney cell types, *e.g.* tubular cells or endothelial

cells (Humphreys *et al.* Minerva Urol Nefrol. 2006; 58: 329–37). Instead, MSCs mediate benefit and promote kidney recovery through paracrine and endocrine mechanisms via the release of secreted mediators including stromal cell-derived factor-1 (SDF-1), vascular endothelial growth factor (VEGF) and other vasculotropic factors, insulin-like growth factor (IGF-1) (Imberti *et al.,* J Am Soc Nephrol. 2007; 18: 2921–8), epidermal growth factor (EGF) (Tögel *et al.* Am J Physiol Renal Physiol. 2007; 292: F1626–35) and other factors that promote organ repair.

[5] Delayed graft function (DGF) is a serious medical condition associated with deleterious consequences, including the need for acute dialysis, extended length of hospital stay, and long-term poor graft function in kidney transplant recipients. Unfortunately, the unmet need in DGF is critical.

SUMMARY OF THE INVENTION

[6] The invention provides methods for treating a donor organ prior to transplantation to a patient by perfusing the donor organ with an effective amount of mesenchymal stem cells (MSCs) prior to transplantation when the risk profiles of the recipient, the donor organ, or boht the recipient and the donor organ indicate that the donor organ is at an increased risk of developing delayed graft function following transplantion. For example, the donor organ may be a kidney, heart, or lung. Preferably, the donor organ is perfused *ex vivo* prior to transplantation. *Ex vivo* perfusion of the kidney(s) prior to transplantation into the patient prevents or reduces the development of delayed graft function, acute kidney injury, and/or chronic kidney disease in the patient following transplantation.

[7] In various embodiments, both the patient and the donor kidney had or are at an increased risk of developing delayed graft function and/or other kidney dysfunctions, diseases, or disorders following transplantation. By way of non-limiting example, the donor kidney may be obtained from a cadaver. In some embodiments, the cadaver organ is obtained from a donor that had or was at an increased risk for developing a kidney disease or disorder. For example, the donor was elderly, was acutely ill, had a medical history of chronic or acute kidney disease, and/or had low birthweight. In many instances, the cadaveric kidney may have been harvested from a individual with comorbidities and risk factors that increase the likelihood that delayed graft function will occur. Such risk factors include older age, a history of chronic kidney disease or acute kidney injury in the donor, a history of diabetes, hypertension or other conditions.

Moreover, the patient receiving the kidney may be elderly and/or be recognized to be at significant risk for the development of delayed graft function.

[8] By simultaneously focusing on both high risk donor and recipient patient populations, the perfusion methods described herein can be used to increase the likelihood that the kidney will survive transplantation into the patient and function well *in vivo,* without evidence of delayed graft function. Thus, the decision of whether to treat the organ prior to transplantation is made based on information about both the donor and the intended recipient. In accordance with the methods described herein, perfusion with MSCs is warranted when the donor and/or the recipient exhibit one or more qualities and/or risk factors that make the patient more likely to develop delayed graft function, acute kidney injury, chronic kidney disease, and/or other kidney dysfunction following transplantation.

[9] In one embodiment, perfusion is warranted when the donor organ is obtained from a cadaver from a donor that had (or was at risk for developing) a kidney disease or disorder and the recipient suffers from or is at risk of suffering from (or developing) delayed graft function. In another embodiment, perfusion is warranted when the donor organ is not obtained from a cadaver from a donor that had or was at an increased risk for developing a kidney disease or disorder and the recipient suffers from or is at high risk of developing delayed graft function. In a further embodiment, perfusion is warranted when the donor organ is obtained from a cadaver from a donor that had (or was at risk for developing) a kidney disease or disorder and the recipient does not suffer from and is not at high risk of suffering from (or developing) delayed graft function.

[10] The effective amount of MSCs used to perfuse the donor organ prior to transplantation may be about 25 million cells to about 300 million cells. Determination of this effective amount of MSCs to use in the methods of the invention is well within the routine level of skill in the art.

[11] Poor kidney function can be measured by one or more serum/blood biomarkers, one or more urine biomarkers, or both. Serum/blood biomarkers include serum creatinine (SCr), blood urea nitrogen (BUN), and/or Cystatin C, and/or Beta-trace protein (BTP) (also known as Prostaglandin D Synthase). Urine biomarkers include Podocalyxin, Nephrin, Alpha 1-microglobulin, Beta 2-microglobulin, Glutathione S-transferase, Interleukin-18, Kidney Injury Molecule-1 (KIM-1), Liver-Type Fatty Acid-Binding Protein, Netrin-1, Neutrophil Gelatinase-

Associated Lipocalcin (NGAL), and/or N-Acetyl-Beta-D-Glucosaminidase (NAG). Those skilled in the art will recognize that the major markers used for evaluating kidney function are serum creatinine (SCr) and/or blood urea nitrogen (BUN). Thus, poor kidney function and/or delayed graft function can be measured by serum creatinine (SCr) and/or blood urea nitrogen alone or in combination with one or more biomarkers selected from Cystatin C, Beta-trace protein (BTP) (also known as Prostaglandin D Synthase), Podocalyxin, Nephrin, Alpha 1-microglobulin, Beta 2-microglobulin, Glutathione S-transferase, Interleukin-18, Kidney Injury Molecule-1 (KIM-1), Liver-Type Fatty Acid-Binding Protein, Netrin-1, Neutrophil Gelatinase-Associated Lipocalcin (NGAL), and/or N-Acetyl-Beta-D-Glucosaminidase (NAG).

[12] The donor organ (*i.e.*, the kidney) can be perfused *ex vivo* using any methods known in the art. In some embodiments, the MSCs are in a biologically and physiologically compatible solution. Preferably, the solution is not enriched for pluripotent hematopoietic stem cells.

[13] The MSCs can be autologous or allogeneic cells. Additionally, the MSCs can be non-transformed stem cells. Moreover, the patient may be any living organisms such as humans, non-human animals (*e.g.*, monkeys, cows, sheep, horses, pigs, cattle, goats, dogs, cats, mice, or rats), cultured cells therefrom, and transgenic species thereof.

[14] The MSCs are expanded *in vitro* to produce an enriched population of human MSCs. Any expansion method known in the art can be used to produce the enriched population.

[15] In addition, the MSCs can be obtained from any source known in the art. For example, in one embodiment, the MSCs are isolated from a bone marrow aspirate and adhere to a culture dish while substantially all other cell types remain in suspension. In other embodiments, the MSCs are obtained from a bone marrow sample, from fat, from cord blood, from placenta, from a cryopreserved sample, from a Master Cell Bank (MCB), and/or from any other source known to those skilled in the art.

[16] By way of non-limiting example, the MSCs are expanded in a platelet lysate (PL) supplemented culture medium. Those skilled in the art will recognize that MSCs that have been cultured in PL supplemented culture media will express Prickle 1 at a higher degree than MSCs that have been cultured in fetal bovine serum (FBS) supplemented culture media. For example, the population of human MSCs expresses Prickle 1 to an eight-fold higher degree than MSCs that have been cultured in FBS supplemented culture media. (*See, e.g.*, Lange et al., Cellular Therapy and Transplantation 1:49-53 (2008), which is herein incorporated by reference in its

entirety). Those skilled in the art will recognize that a population of human MSCs that has been cultured in platelet lysate may be less immunogenic than MSCs that have been cultured in fetal calf serum supplemented culture media. Moreover the use of PL instead of FBS supplemented culture media reduces infectious risk and overall safety and regulatory concerns associated with the use of FBS.

[17] Human MSCs suitable for use in the methods of the invention preferably have 32 or fewer GT repeats in both alleles of the human heme oxygenase (HO-1) promoter region. For example, the human MSCs utilized may have two short alleles, two medium alleles, or one short and one medium allele in the HO-1 promoter region, wherein a short allele has ≤ 26 GT repeats in the HO-1 promoter region and wherein a medium allele has between 27 and 32 GT repeats in the HO-1 promoter region. MSCs containing one or more long alleles are less therapeutically effective. Therefore, ideally, the human MSCs do not have any long alleles, wherein a long allele has > 32 GT repeats in the HO-1 promoter region.

[18] As used herein, a "short" allele can have ≤ 26 GT repeats in the HO-1 promoter region (*e.g.*, between about 21 and about 26 GT repeats); a "medium" allele can have between about 27 and about 32 GT repeats in the HO-1 promoter region; and a "long" allele can have >32 GT repeats in the HO-1 promoter region (*e.g.*, between about 33 and about 44 GT repeats).

[19] Those skilled in the art will recognize that the number of GT repeats in an allele of the HO-1 promoter region can be analyzed using any suitable method known in the art, including, but not limited to Fragment Length Analysis and DNA sequencing methodologies.

[20] In some embodiments, the MSCs are genetically modified, to augment the renoprotective potency of said cells prior to administration to the patient.

[21] In any of the methods described herein, the MSCs can be pre-differentiated *in vitro* prior to *ex vivo* perfusion of the transplanted organ. By way of non-limiting example, the MSCs are pre-differentiated into endothelial cells and/or into renal tubular cells.

[22] Those skilled in the art will recognize that, following transplantation of the perfused organ, a therapeutically effective amount of MSCs can also be administered to the patient. For example, therapeutically effective amount can be administered following transplantation to treat, ameliorate, and/or delay the development or progression of conditions including, but not limited to, stroke, multi-organ failure (MOF), AKI of native kidneys, AKI of native kidneys in multi-organ failure, AKI in transplanted kidneys, kidney dysfunction, multi-organ dysfunction, delayed

graft function, and/or wound repair.

DETAILED DESCRIPTION OF THE INVENTION

[23] The details of one or more embodiments of the invention have been set forth in the accompanying description below. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural references unless the context clearly dictates otherwise. All patents and publications cited in this specification are incorporated by reference in their entirety.

[24] For convenience, certain terms used in the specification, examples and claims are collected here. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

[25] As used herein, the terms "patient," "individual," "subject", "host", "recipient" or the like are used interchangeably herein to refer to either a human or a non-human animal.

[26] *Assaying MSCs for Therapeutic Effectiveness or Potency*

[27] MSCs can be evaluated for their therapeutic effectiveness or potency. The number of GT repeats in the HO-1 promoter region of MSCs may be indicative of the therapeutic efficacy of the MSCs. Analyzing the number of GT repeats in both donor alleles (whether obtained from a cryopreserved MSC sample, from fresh blood, from a Master Cell Bank and/or from other suitable genetic material), helps to determine whether the MSC population is enriched to be robust, and, thus, be therapeutically effective.

[28] Preferably, the number of GT repeats in both HO-1 alleles is not too long. Indeed, as described herein, MSCs having fewer GT repeats in both HO-1 alleles express higher HO-1 protein levels and are more likely to be therapeutically effective.

[29] A (GT)n repeat region that can decrease transcription is located between -190 and -270 of the human HO-1 promoter and is absent in the mouse HO-1 gene. (*See* Sikorski et al. at page F429). In addition, DNA length polymorphisms of this region vary between human subjects and correlate with activity of various diseases, such as emphysema, coronary artery

disease, and other disorders. Typically, individuals with shorter repeats (<25) demonstrate higher induced HO-1 protein levels and milder disease manifestations, whereas individuals with longer repeats have lower HO-1 levels and more severe disease. (*See* Sikorski et al., Am J Physiol Renal Physiol 286:F424-F441 (2004); Zarjou et al., Am J Physiol Renal Physiol 300:F254-F262 (2011); Exner et al., Free Radical Biology & Medicine 37(8):1097-104 (2004), which are herein incorporated by reference in their entireties).

[30] As used herein, the term "short allele" refers to MSC HO-1 alleles having ≤ 26 GT repeats in the human HO-1 promoter region.

[31] As used herein, the term "medium allele" refers to MSC HO-1 alleles having between 27 and 32 GT repeats in the human HO-1 promoter region.

[32] As used herein, the term "long allele" refers to MSC HO-1 alleles having >32 GT repeats in the human HO-1 promoter region.

[33] Studies in mice have demonstrated that HO-1 is essential for their therapeutic potential in cisplatin-induced AKI. (*See* Zarjou et al., Am J Physiol Renal Physiol 300:F254-F262 (2011)). Moreover, the absence of HO-1 expression in MSCs limit their protective paracrine effects including the angiogenic potential of MSCs and for growth factor and/or reparative factor secretion and expression by MSC. (*See* Zarjou et al. at p. F260).

[34] Moreover, the number of GT repeats in the HO-1 promoter region of any nucleated cell of the human body may be measured by any method known in the art. For example, Fragment Length Analysis can be used. Briefly, PCR is used to amplify fragments from both HO-1 alleles per cell using PCR primers that flank the HO-1 promoter region containing the GT repeats. The resulting PCR fragments are separated on a column and the "predicted" sizes are reported (in base pairs). Fragment Length Analysis is, thus, able to report relative size differences between different alleles. The absolute size of the PCR fragments can subsequently be determined using methods well known to those of ordinary skill in the relevant art.

[35] Fragment Length Analysis (*see* Exner et al., Free Radical Biology & Medicine 37(8):1097-104 (2004)) is used to determine the number of GT repeats. Briefly, PCR is used to amplify fragments from both HO-1 alleles per MSC using PCR primers, one of which is fluorescently labeled, that flank the HO-1 promoter region containing the GT repeats. The resulting PCR fragments are separated on a column (for example, at an external vendor), and the "predicted" sizes are reported (in base pairs).

[36] Fragment Length Analysis is a commonly used method for determining the length of FAM-labeled PCR fragments. However, fragment length analysis only predicts the *relative* size of different fragments and the relative differences between different alleles. Based upon the fragment length data, it is believed that a PCR fragment size of 302 base pairs corresponds to 23 GT repeats. However, those skilled in the art will appreciate that the apparent fragment length could differ on a different column.

[37] In accordance with the methods of the instant invention, donors or MSCs will be excluded if they have one or more long GT repeat alleles. Thus, only those donors or MSCs having two short alleles, two medium alleles, or one medium and one short allele will be accepted. Only MSCs without a long allele will be used clinically.

[38] According to certain embodiments of the invention, other MSC markers are also measured. For example, the presence of CD105 and/or CD90 is measured in some embodiments. In other embodiments, the absence of CD34 and/or CD45 is measured. The presence of CD105 and/or CD90 as well as the absence of CD34 and/or CD45 is indicative of the MSC phenotype. In other embodiments, adipogenic, osteogenic and/or chondrogenic assays are used to show that the MSCs possess the characteristic ability of trilineage differentiation.

[39] *Mesenchymal Stem Cells Cultured in Platelet Lysate (PL) Supplemented Media*

[40] MSCs may be passaged or expanded according to any methods known in the art. For example, published PCT application WO2010/017216 and US patent publication US20110293576, which are incorporated herein by reference in their entireties, describe methods for the culture and expansion of MSCs in platelet lysate (PL) supplemented media.

[41] The invention provides MSCs with unique properties that make them particularly beneficial for use in the treatment of kidney pathology. The MSCs of the invention are grown in media containing PL, as described in greater detail below. The culturing of MSCs in PL-supplemented media creates MSCs that are more protective against ischemia-reperfusion damage than MSCs grown in FBS.

[42] The MSCs of the invention, cultured in PL-supplemented media constitute a population with (i) surface expression of antigens such as CD105, CD90, CD73, CD44, and MHC I, but lacking hematopoietic markers such as CD45, CD34 and CD14; (ii) preservation of the multipotent trilineage (osteoblasts, adipocytes and chondrocytes) differentiation capability after expansion with PL, however the adipogenic differentiation was delayed and needed longer

times of induction. This decreased adipogenic/lipogenic ability is a favorable property because in mice the intra-arterial injection of MSCs for treatment of kidney injury has revealed formation of adipocytes (Kunter et al., J Am Soc Nephrol 2007 Jun;18(6):1754-64). These results are reflected in the gene expression profile of PL-generated cells revealing a down-regulation of genes involved in fatty acid metabolism, described in greater detail below.

[43] The MSCs of the invention, cultured in PL-supplemented media have been described to act immunomodulatory by impairing T-cell activation without inducing anergy. There is a dilution of this effect *in vitro* in mixed lymphocyte cultures (MLC) leading eventually to an activation of T-cells if decreasing amounts of MSCs, not cultured in PL- supplemented media, are added to the MLC reaction. This activation process is not observed when PL-generated MSCs are used in the MLC as a third party, as shown in greater detail below. It was concluded that the MSCs of the invention, cultured in PL-supplemented media are less immunogenic and that growing MSCs in FBS-supplemented media may act as a strong antigen or at least has adjuvant function in T-cell stimulation. This result again is reflected in differential gene expression showing a down-regulation of MHC II molecules verifying the decreased immunostimulation by MSC, as shown below.

[44] Moreover, the MSCs of the invention, cultured in PL-supplemented media show up-regulation of genes involved in the cell cycle (*e.g.* cyclins and cyclin dependent kinases) and the DNA replication and purine metabolism when compared to MSCs cultured in FBS-supplemented media. On the other hand, genes functionally active in cell adhesion/extracellular matrix (ECM) - receptor interaction, differentiation/development, TGF-β signaling and TSP-I induced apoptosis could be shown to be down-regulated in the MSCs of the invention, cultured in PL-supplemented media when compared to MSCs cultured in FBS-supplemented media, again supporting the results of faster growth and accelerated expansion.

[45] The MSCs of the invention, cultured in PL-supplemented media, when administered (*e.g.*, intra-arterially) lead to improvement of repair and regeneration of injured tissue by ameliorating local inflammation, decreasing apoptosis, and by delivering growth factors and other mediators needed for the repair and/or regeneration of the damaged cells. Injured cells or organs secrete SDF-1 that draws MSCs to the site of injury through the chemokine receptor 4 (CXCR4).

[46] The MSCs of the invention, cultured in PL-supplemented media are particularly good

candidates for regenerative therapy in central nervous system (CNS) damage. They express the gene Prickle 1 to an eight-fold higher degree compared to MSCs cultured in FBS -supplemented media which is involved in neuroregeneration. Mouse Prickle 1 and Prickle 2 are expressed in postmitotic neurons and promote neuronal outgrowth (Okuda et al., FEBS Lett. 2007 Oct 2;581(24):4754-60). Furthermore, MAG (Myelin-associated glycoprotein) is expressed at 13-fold lower levels in the MSCs of the invention, cultured in PL-supplemented media. MAG is a cell membrane glycoprotein and may be involved in myelination during nerve regeneration. The lack of recovery after CNS injury is caused, in part, by myelin inhibitors including MAG. MAG acts as a neurite outgrowth inhibitor for most neurons tested but stimulates neurite outgrowth in immature dorsal root ganglion neurons (Vyas et al., Proc Natl Acad Sci U S A, 2002;99(12):8412-7). These differentially regulated genes would favor the use of PL cultured hMSC for regeneration of neuronal injury.

[47] Additionally, the expression of retinoic acid receptor (RAR) responsive gene TIG1, shows 12 fold higher expression in the MSCs of the invention, cultured in PL-supplemented media) (Liang et al. Nature Genetics 2007;39(2):178-188), Keratin 18 (9 fold higher expression in the MSCs of the invention, cultured in PL-supplemented media) (Bühler et al, Mol Cancer Res. 2005;3(7):365-71), CRBPl (cellular retinol binding protein 1, 5.7 fold higher expression in the MSCs of the invention cultured in PL-supplemented media) (Roberts et al., DNA Cell Biol. 2002;21(l):l l-9.) and Prickle 1 suggest a less tumorigenic phenotype of the MSCs of the invention, cultured in PL-supplemented media.

[48] Furthermore, MSCs grown in PL-supplemented medium are more protective against ischemia-reperfusion damage than MSCs grown in FBS-supplemented medium.

[49] *Methods of Producing Mesenchymal Stem Cells*

[50] In certain embodiments, the mesenchymal stem cells (MSCs) of the invention are cultured in media supplemented with PL or FBS. In one embodiment of the method of producing MSCs of the invention, the starting material for the MSCs is bone marrow isolated from healthy donors. Preferably, these donors are mammals. More preferably, these mammals are humans. In one embodiment of the method of producing MSCs of the invention, the bone marrow is cultured in tissue culture cell factories between 2 and 10 days (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days) prior to washing non-adherent cells from the cell factory. Optionally, the number of days of culture of bone marrow cells prior to washing non-adherent cells is 2 to 3 days.

Preferably the bone marrow is cultured in PL containing media. 25-125 mL (*e.g.*, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, or 125 mL) of bone marrow aspirate is cultured in 400-1500 mL (*e.g.*, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, or 1500 mL) of PL supplemented media in a multi layered cell factory or other adequate tissue culture vessels, automated closed system bioreactors, or suspension bead technology (including enough media volume for each culture vessel technology).

[51] After washing away the non-adherent cells, the adherent cells are also cultured in media that has been supplemented with PL or FBS. Thrombocytes (platelets) are a well-characterized human product already widely used clinically for patients in need. Platelets are known to produce a wide variety of factors, *e.g.* PDGF-BB, TGF-β, IGF-1, and VEGF. In one embodiment of the method of producing MSCs of the invention, an optimized preparation of PL is used. This optimized preparation of PL is made up of pooled platelet rich plasma (PRP) from at least 10 (*e.g.*, about 10 to about 100; for example, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 75, about 80, about 85, about 90, about 95, or about 100) donors with a minimal concentration of 3×10^9 thrombocytes/mL.

[52] According to preferred embodiments of the method of producing MSCs of the invention, PL was prepared either from pooled thrombocyte concentrates designed for human use or from 7-13 (*e.g.*, 7, 8, 9, 10, 11, 12, or 13) pooled buffy coats after centrifugation with 200xg for 20 min. Preferably, the PRP was aliquoted into small portions, frozen at –80°C, and thawed immediately before use. Thawing of PRP causes lysis of thrombocytes, generating PL, and release of growth factors that facilitate robust MSC growth. Multiple freeze and thaw cycles may increase the potency of the PL. PL-containing medium is prepared freshly for each lot production. In a preferred embodiment, medium contained αMEM (minimum essential medium alpha) as basic medium supplemented with 5 IU Heparin/mL and 2-10% (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10%) of freshly thawed PL, which can be used for up to 28 days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 days) without significant loss of MSC growth supporting properties. The method of producing MSCs of the invention uses a method to prepare PL that differs from others according to the thrombocyte concentration and centrifugation forces. The composition of this PL is described in greater

detail, below.

[53] In one embodiment of the method of producing MSCs, the adherent cells are cultured in PL-supplemented media at 37 °C with approximately 5% CO_2 under hypoxic conditions. Preferably, the hypoxic conditions are an atmosphere of 5% O_2. In some situations hypoxic culture conditions allow MSCs to grow more quickly. This allows for a reduction of days needed to grow the cells to 90-100% confluence. Generally, it reduces the growing time by three days. In another embodiment of the method of producing MSCs of the invention, the adherent cells are cultured in PL-supplemented media at 37 °C with approximately 5% CO_2 under normoxic conditions, *i.e.* wherein the O_2 concentration is the same as atmospheric O_2, approximately 20.9%. Preferably, the adherent cells are cultured between 9 and 12 days (*e.g.*, 9, 10, 11, or 12 days), being fed every 3-5 days (*e.g.*, 3, 4, or 5 days) with PL-supplemented media. In one embodiment of the method of producing MSCs of the invention, the adherent cells are grown to between 80 and 100% confluence. Preferably, once this level of confluence is reached, the cell monolayers are detached from the culture vessel enzymatically by using recombinant porcine trypsin. The detached cells in suspension are plated for subsequent culture. The process of successive detaching and plating of cells is called passage.

[54] In certain embodiments, the population of cells that is isolated from the culture vessel is between 50-99% MSCs. In other embodiments, isolated MSCs are enriched in MSCs so that 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% of the cell population are MSCs. In other embodiments, the MSCs are greater than 95% of the isolated cell population.

[55] Preferably, the MSCs used in any of the methods, compositions, and kits described herein are free of infectious agents. In some embodiments, the MSCs have undergone fewer than 30 population doublings and are cultured to 80 to 100% confluence. Moreover, using the various methods described herein, MSC cell viability should be greater or equal to 70% (*e.g.*, 70%, 75%, 80%, 85%, 90%, 95% or greater viability).

[56] In another embodiment of the method of producing MSCs of the invention, the cells are frozen after they are released from the tissue culture vessel. Freezing is performed in a step-wise manner in a physiologically acceptable carrier, 2 to 10% serum albumin (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, and/or 10%) and 2-10% DMSO (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, and/or 10%). Thawing is also performed in a step-wise manner. Preferably, when thawed, the frozen MSCs of the invention

are diluted about 2-8 fold (*e.g.*, 2, 3, 4, 5, 6, 7, or 8-fold) to reduce DMSO concentration. In some embodiments, frozen MSCs of the invention are thawed quickly at 37 °C and administered intravenously without any dilution or washings. Optionally, the cells are administered following any protocol that is adequate for the transplantation of hematopoietic stem cells (HSCs). Preferably, the serum albumin is human serum albumin (HSA).

[57] In another embodiment of the method of producing MSCs of the invention, the cells are frozen in aliquots of 10^4-10^{12} cells in 10 to 20 mL (*e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mL) of physiologically acceptable carrier and HSA. The cells are frozen in aliquots of 10^6-10^8 cells in 10 to 20 mL (*e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mL) of physiologically acceptable carrier and HSA. In another embodiment of the method of producing MSCs of the invention, the cells administered following transplantation of a perfused kidney in a dose of 10^6-10^8 cells per kg of subject body weight, in 50-150 mL (*e.g.*, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mL) of physiologically acceptable carrier and HSA. If the cells are administered IV, the dose of cells may be included in up to 1 L of physiologically acceptable carrier and HSA.

[58] Those skilled in the art will recognize that any cryopreservation protocol or process known in the art can be used to freeze the MSCs of the invention.

[59] When an effective amount of MSCs for *ex vivo* perfusion and/or a therapeutic dose of MSCs is being assembled, the appropriate number of cryovials is thawed in order to prepare the appropriate number of cells for the effective amount and/or the therapeutic dose based on the patient's body weight. Any thawing protocol or process known in the art can be used to thaw the MSCs of the invention prior to perfusion and/or administration. When determining a therapeutic dose, the number of cryovials is chosen based on the weight of the patient. The vials are thawed in a water bath and placed in a sterile infusion bag with 2 -10% HSA (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, and/or 10%). Once in the bag, the MSCs do not aggregate and viability remains greater than 70% even when the MSCs are stored at room temperature for at least 8 hours. This provides ample time to perfuse the donor organ and/or administer the MSCs of the invention to a patient. Optionally, the physiologically acceptable carrier is Plasma-lyte A. Preferably the HSA is present at a concentration of 5-10% (*e.g.*, 5, 6, 7, 8, 9, and/or 10%) w/v. Suspending the 10^6-10^8 cells MSCs of the invention in greater than 50 mL of physiological carrier is critical to their biological activity. If the cells are suspended in lower volumes, the cells are prone to

aggregation. Administration of aggregated MSCs to animals has resulted in cardiac infarction. Thus, it is crucial that non-aggregated MSCs be administered according to the methods of the invention. The presence of HSA is also critical because it prevents aggregation of the MSCs and also prevents the cells from sticking to plastic containers the cells pass through (*e.g.*, when administered to subjects).

[60] In certain embodiments, the culture system is used in conjunction with a medium for expansion of MSCs which does not contain any animal proteins, *e.g.* PL. FBS has been connected with adverse effects after *in vivo* application of FBS-expanded cells, *e.g.* formation of anti-FBS antibodies, anaphylactic or Arthus-like immune reactions or arrhythmias after cellular cardioplasty. FBS may introduce unwanted animal xenogeneic antigens, viral, prion and zoonose contaminations into cell preparations making new alternatives desirable.

[61] *Manufacturing Summary*

[62] In one embodiment, a bone marrow aspirate is suspended in culture media and then plated in multilayer cell factory. Mesenchymal progenitors naturally attach to the surface of the cell factory and then expand after several passages to become a relatively homogeneous population of MSC. After 1 to 3 days the cells remaining in suspension are washed out of the cell factory and discarded.

[63] When the MSCs have expanded to cover the culture surface, they are enzymatically detached and harvested. The harvested cells are seeded in more cell factories and the expansion process is repeated. Feeding and harvesting of the cells takes place in a completely closed system using sterile welders.

[64] After 2-6 rounds of expansion (12-20 days), the cells are harvested and cryopreserved in vapor phase liquid N_2 at <-130 °C. Representative units are tested for sterility, mycoplasma, endotoxin, identity by flow cytometry and trilineage differentiation, as well as an array of viral tests.

[65] Preferably, bone marrow aspirates are donated by healthy adult volunteers. Potential donors undergo rigorous testing including health questionnaire, physical examination, and testing for various infectious diseases.

[66] Cryopreserved units (1-2) are thawed, cultured and expanded in a manner similar to the bone marrow aspirate cultures. The cells are expanded for two additional rounds at large scale to obtain the final product. The final harvested product is concentrated and washed using a

scalable downstream process (*e.g.*, Tangential Flow Filtration (TFF) and/or closed system centrifugation).

[67] The MSC population is then packaged into cryogenic vials, frozen to -80 °C in a stepwise manner using a controlled rate freezer, and stored at <-130 °C in vapor phase liquid N_2. Moreover, the population is also tested for sterility, mycoplasma, endotoxin, and identity.

[68] Unlike dead end filtration, TFF or closed system centrifugation is an efficient process for retaining and concentrating larger particulates (cells) while removing non-particulates (culture media). In TFF or closed system centrifugation, the system efficiently separates cells from culture media without the clogging that occurs in dead end filtration.

[69] Determination of suitable protocols for cryopreservation and/or thawing of the MSCs prior to use are within the routine skill in the art.

[70] Thus, this manufacturing system represents the next generation in cutting edge processes for MSC production. Specifically, it is scalable, performed in a closed culturing system, and free of animal origin products. Moreover, it employs a closed system centrifugation or TFF downstream processing system, which preserves cell viability. Likewise, it also uses a closed vialing system.

[71] *Methods of Using Mesenchymal Stem Cells*

[72] The MSCs can be used to perfuse donor organs prior to transplantation and/or to treat, ameliorate, and/or delay the development or progression of conditions including, but not limited to, stroke, multi-organ failure (MOF), AKI of native kidneys, AKI of native kidneys in multi-organ failure, AKI in transplanted kidneys, kidney dysfunction, multi-organ dysfunction, delayed graft function, and/or wound repair that refer to conditions known to one of skill in the art. Descriptions of these conditions may be found in medical texts, such as Brenner & Rector's The Kidney, WB Saunders Co., Philadelphia, last edition, 2012, which is incorporated herein in its entirety by reference. In one preferred embodiment, the methods described herein are used to prevent or reduce delayed graft function (*i.e.*, lack of new kidney function) in patients with kidney failure prior to receiving a transplant.

[73] When used to perfuse donor organs, an effective amount (*e.g.*, about 25 million cells to about 300 million cells) of the MSCs can be administered (*i.e.*, perfused) to the donor organ prior to transplantation. The donor organ can be perfused with MSCs for any sufficient length of time (*e.g.*, 5 min, 15 min, 30 min, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6, hours, 7 hours, 8

hours, 9 hours, 10 hours, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, 5 days, 6 days, 1 week, 2 weeks, or more)

[74] Multi-organ Failure (MOF) is a condition in which kidneys, lungs, liver and/or heart are impaired simultaneously or successively, associated with mortality rates as high as 100% despite the modern medical support. MOF patients frequently require intubation and respirator support because their lungs may develop Adult Respiratory Distress Syndrome (ARDS), resulting in inadequate oxygen uptake and CO_2 elimination. MOF patients may also depend on hemodynamic support, vasopressor drugs to maintain adequate blood pressures. MOF patients with liver failure may exhibit bleeding along with accumulation of toxins that often impair mental function. Patients may need blood transfusions and clotting factors to prevent or stop bleeding. It is considered that MOF patients may be given MSC therapy to address AKI and MOF.

[75] Early graft dysfunction (EGD), delayed graft function (DGF), or transplant associated-acute kidney injury (TA-AKI) is Acute Kidney Injury (AKI) that affects the transplanted kidney in the first few days after implantation. The more severe TA-AKI, the more likely it is that patients will suffer from the same complications as those who have AKI in their native kidneys, as above. The severity of TA-AKI is also a determinant of enhanced graft loss due to rejection(s) in the subsequent years. These are two strong indications for the prompt treatment of TA-AKI with the MSCs of the present invention.

[76] Chronic renal failure (CRF) or Chronic Kidney Disease (CKD) is the progressive loss of nephrons and consequent loss of renal function due to a variety of causes, including diabetic nephropathy and hypertensive nephropathy, resulting in End Stage Renal Disease (ESRD), at which time patient survival depends on dialysis support or kidney transplantation. The need for MSC therapy of the present invention will be determined on the basis of physical and laboratory abnormalities described above.

[77] In some embodiments, the MSCs may be administered to patients in need thereof (for example in transplantation of a donor kidney that has been perfused *ex vivo* with an effective amount of MSCs). In other embodiments of methods of use of MSCs of the invention, the MSCs of the invention are administered as a first line therapy following transplantation of a perfused kidney.

[78] In certain embodiments, the MSCs are administered to a subject once following

transplantation of a perfused kidney. This one dose is sufficient treatment in some embodiments. In other embodiments the MSCs are administered 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times following transplantation of a perfused kidney in order to attain or sustain a therapeutic effect. For example, in some instances, the cells are administered chronically and/or on an on-going basis following transplantation of a perfused kidney.

[79] Monitoring patients for a therapeutic effect of the MSCs delivered to a patient in need thereof following transplantation of a perfused kidney and assessing further treatment will be accomplished by techniques known to one of skill in the art. For example, renal function will be monitored by determination of SCr and BUN levels, serum electrolytes, measurement of renal blood flow (ultrasonic method), creatinine and insulin clearances, urine output, and other methods. A positive response to therapy for AKI includes return of excretory kidney function, normalization of urine output, blood chemistries and electrolytes, repair of the organ and survival. For MOF, positive responses also include improvement in blood pressure, blood oxygenation, and improvement in function of one or all organs.

[80] In other embodiments the MSCs are used to effectively repopulate dead or dysfunctional kidney cells following transplantation of a perfused kidney in subjects that are suffering from chronic kidney pathology including CKD. The effect may be the results of the paracrine and/or endocrine effects of the MSCs that induce endogenous progenitor cells in the kidney. Additionally (or alternatively), this effect may be because of the "plasticity" of the MSC populations. The term "plasticity" refers to the phenotypically broad differentiation potential of cells that originate from a defined stem cell population. MSC plasticity can include differentiation of stem cells derived from one organ into cell types of another organ. "Transdifferentiation" refers to the ability of a fully differentiated cell, derived from one germinal cell layer, to differentiate into a cell type that is derived from another germinal cell layer.

[81] It was previously assumed that stem cells gradually lose their pluripotency and thus their differentiation potential during organogenesis. It was thought that the differentiation potential of somatic cells was restricted to cell types of the organ from which respective stem cells originate. This differentiation process was thought to be unidirectional and irreversible. However, recent studies have shown that somatic stem cells maintain some of their differentiation potential. (*See* Hombach-Klonich eta l., J Mol Med (Berl).86(12):1301-1314

(2008)). For example, stem cells may be able to transdifferentiate into muscle, neurons, liver, myocardial cells, and kidney cell populations. It is possible that as yet undefined signals that originate from injured and not from intact tissue act as transdifferentiation signals.

[82] In certain embodiments, a therapeutically effective dose of MSCs is delivered to the patient following transplantation of a perfused kidney. An effective dose for treatment following transplantation of a perfused kidney will be determined by the body weight of the patient receiving treatment, and may be further modified, for example, based on the severity or phase of the stroke, kidney or other organ dysfunction, for example the severity of AKI, the phase of AKI in which therapy is initiated, and the simultaneous presence or absence of MOF. In some embodiments of the methods of use of the MSCs of the invention, from about $1x10^5$ to about $1x10^{10}$ MSCs per kilogram of recipient body weight are administered in a therapeutic dose following transplantation of a perfused kidney. Preferably from about $1x10^5$ to about $1x10^8$ MSCs per kilogram of recipient body weight is administered in a therapeutic dose following transplantation of a perfused kidney. More preferably from about $7x10^5$ to about $5x10^8$ MSCs per kilogram of recipient body weight is administered in a therapeutic dose following transplantation of a perfused kidney. More preferably from about $1x10^6$ to about $1x10^8$ MSCs per kilogram of recipient body weight is administered in a therapeutic dose. More preferably from about $7x10^5$ to about $7x10^6$ MSCs per kilogram of recipient body weight is administered in a therapeutic dose following transplantation of a perfused kidney. More preferably about $2x10^6$ to about $5x10^6$ MSCs per kilogram of recipient body weight is administered in a therapeutic dose following transplantation of a perfused kidney. The number of MSCs used will depend on the weight and condition of the recipient, the number of or frequency of administrations, the route of administration, and other variables known to those of skill in the art. For example, a therapeutic dose following transplantation of a perfused kidney may be one or more administrations of the therapy.

[83] The therapeutic dose of MSCs following transplantation of a perfused kidney is administered in a suitable solution for injection (*i.e.*, infusion or bolus). Solutions are those that are biologically and physiologically compatible with the cells and with the recipient, such as buffered saline solution, Plasma-lyte or other suitable excipients or formulations, known to one of skill in the art.

[84] In certain embodiments of the MSCs of the invention are administered to a subject

following transplantation of a perfused kidney at a rate between approximately 0.5 and 1.5 mL (*e.g.*, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, or 1.5 mL) of MSCs in physiologically compatible solution per second. Preferably, the MSCs of the invention are administered to a subject following transplantation of a perfused kidney at a rate between approximately 0.83 and 1.0 mL per second (*e.g.*, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, or 1.0 mL). More preferably, the MSCs are suspended in approximately 100 mL of physiologically compatible solution and are completely injected into a subject between approximately one and three minutes. More preferably the 100 mL of MSCs in physiologically compatible solution is completely infused in approximately one to three minutes. Determination of injection and/or infusion rate for a given mode of administration following transplantation of a perfused kidney is within the routine level of skill in the art.

[85] In other embodiments, the MSCs are used in trauma or surgical patients scheduled to undergo high-risk surgery such as the repair of an aortic aneurysm. In the case of poor outcome, including infected and non-healing wounds, development of MOF post-surgery, the patient's own MSCs, prepared according to the methods of the invention, that are cryopreserved may be thawed out and administered as detailed above. Following transplantation of a perfused kidney, patients with severe AKI affecting a transplanted kidney may either be treated with MSCs, prepared according to the methods of the invention, from an unrelated donor or the donor of the transplanted kidney (allogeneic) or with cells from the recipient (autologous). Allogeneic or autologous MSCs, prepared according to the methods of the invention, are an immediate treatment option in patients with TA-AKI and for the same reasons as in patients with AKI of their native kidneys.

[86] In certain embodiments following transplantation of a perfused kidney, the MSCs of the invention are administered to the patient by infusion intravenously or intra-arterially (for example, for renal indications, via femoral artery into the supra-renal aorta). Preferably, the MSCs of the invention are administered via the supra-renal aorta. In certain embodiments, the MSCs of the invention are administered through a catheter that is inserted into the femoral artery at the groin. Preferably, the catheter has the same diameter as a 12-18 gauge needle. More preferably, the catheter has the same diameter as a 15 gauge needle. The diameter is relatively small to minimize damage to the skin and blood vessels of the subject during MSC administration. Preferably, the MSCs of the invention are administered at a pressure that is

approximately 50% greater than the pressure in the subject's aorta. More preferably, the MSCs of the invention are administered at a pressure of between about 120 and 160 psi (*e.g.*, about 120, 130, 140, 150, or 160 psi). Generally, at least 95% of the MSCs of the invention survive injection and/or infusion into the subject. Moreover, the MSCs are generally suspended in a physiologically acceptable carrier containing about 5-10% (*e.g.*, 5, 6, 7, 8, 9, or 10%) HSA. The HSA, along with the concentration of the cells prevents the MSCs from sticking to the catheter or the syringe, which also insures a high (*i.e.* greater than 95%) rate of survival of the MSCs when they are administered to a subject. The catheter is advanced into the supra-renal aorta to a point approximately 20 cm above the renal arteries. Preferably, blood is aspirated to verify the intravascular placement and to flush the catheter. More preferably, the position of the catheter is confirmed through a radiographic or ultrasound based method. Preferably the methods are transesophageal echocardiography (TEE), an X-ray, or fluoroscopy. The MSCs of the invention are then transferred to a syringe that is connected to the femoral catheter. The MSCs, suspended in the physiologically compatible solution are then infused over approximately one to three minutes into the patient. Preferably, after injection and/or infusion of the MSCs of the invention, the femoral catheter is flushed with normal saline. Optionally, the pulse of the subject found in the feet is monitored, before, during and after administration of the MSCs of the invention. The pulse can be monitored to ensure that the MSCs do not clump during administration.

[87] In certain embodiments, a therapeutically effective dose of MSCs is delivered intravenously (IV) to the patient following transplantation of a perfused kidney. The therapeutic dose of MSCs in a suitable solution for injection is administered via IV injection, infusion, or bolus or other suitable methods into a peripheral, femoral, jugular, or other vein known to one of ordinary skill in the art.

[88] *Dose Rationale*

[89] A dose of 2 x 10^6 human MSCs (hMSC)/kg of bodyweight of a preparation of human MSC designed for clinical use has been selected for further investigation of the preparation in clinical studies of AKI. Data from a Phase 1 study, other clinical investigations of hMSC, as well as nonclinical investigations support selection of this dose.

[90] The Phase 1 study evaluated three dose levels of PL-produced hMSC, designated AC607, including 7 x 10^5, 2 x 10^6 and 7 x 10^6 hMSC/kg. All doses of AC607 were safe and well tolerated in this study, with no treatment related adverse events or serious adverse events

observed in any dose cohort. In other clinical studies, hMSC have been administered to subjects across a range of doses with no reported safety issues. Doses of hMSC in these other studies have typically ranged from 150 to 300 million MSC per subject (approximately 2 to 4 x 10^6 MSC/kg for a 70-kg subject), consistent with the selected dose. (*See* Ankrum et al., Trends Mol Med. 16(5):203-09 (2010)). Moreover, published data suggest that hMSC doses of at least 1 x 10^6 MSC/kg are pharmacologically active in non-AKI clinical indications. (*See* Hare et al., J. Am Coll Cardiol 2227-86 (2009)).

[91] In a rat I/R model of AKI, hMSC at an intra-arterial dose of 1 x 10^6 hMSC/kg significantly reduced serum creatinine (SCr) when administered to animals after the onset of AKI, as evidenced by a 7-fold increase in SCr. (*See* Cao et al., Biotechnol Lett 32:725-32 (2010)). Consistent with data for hMSC, a nonclinical study demonstrated that intra-arterial administration of rat MSC (rMSC) significantly lowered SCr in the rat I/R model of AKI at doses of 2 x 10^6 rMSC/kg or 5 x 10^6 rMSC/kg, but not at 0.5 x 10^6 rMSC/kg. (*See* Tögel et al., Stem Cells Dev 18:475-85 (2009)). Further, another nonclinical investigation demonstrated that a single intra-arterial injection of rMSC at doses up to 15 x 10^6 rMSC/kg was well tolerated in rats with AKI.

[92] Collectively, these clinical and nonclinical data support selection of 2 x 10^6 MSC/kg of AC607 as a safe and pharmacologically active dose for future clinical studies of AKI.

[93] *Clinical Data*

[94] In the Phase 1 study, a single intra-arterial injection of AC607 at 7 x 10^5 hMSC/kg, 2 x 10^6 hMSC/kg, or 7 x 10^6 hMSC/kg was safe and well tolerated in 16 subjects undergoing elective cardiac surgery who were at risk for developing postoperative AKI.

[95] In summary, a single, intra-arterial dose of up to 7 x 10^6 hMSC/kg of AC607 was safe and well tolerated when administered to subjects after cardiac surgery.

[96] Currently, there are over 150 clinical studies of hMSC (not limited to AKI trials) currently listed on ClinicalTrials.gov. In these clinical investigations, hMSC doses most commonly range from 2 x 10^6 MSC/kg to 4 x 10^6 MSC/kg. (*See* Ankrum et al., Trends Mol Med 16(5):203-209 (2010)). Moreover, hMSC have been safely administered to subjects at doses of up to 8 x 10^6 MSC/kg with no reported treatment related adverse events. (*See* Kebriaei et la., Biol Blood Marrow Transplant. 15:804-11 (2009)).

[97] In a double-blind, placebo-controlled study of 60 patients with acute myocardial

infarction, subjects were randomized 2:1 to receive either hMSC or placebo. (*See* Hare et al., J Am Coll Cardiol 54:2227-86 (2009)). hMSC were administered at doses of 0.5 x 10^6 MSC/kg, 1.6 x 10^6 MSC/kg, or 5 x 10^6 MSC/kg. The rate of arrhythmias was 4-fold less in subjects that received hMSC compared to the placebo group (8.8% versus 36.8%, $P = 0.025$). hMSC-treated subjects experienced fewer premature ventricular contractions (PVC) compared to those treated with placebo ($P = 0.017$), and the percentage of patients that experienced more than 10 PVC per hour was significantly reduced in hMSC-treated compared to placebo-treated subjects (10.0% versus 24%, $P = 0.001$). Interestingly, the rate of PVC exhibited a dose-response effect with reductions in PVC detected in the 1.6 x 10^6 MSC/kg and 5 x 10^6 MSC/kg groups but not in the 0.5 x 10^6 MSC/kg group, compared to the placebo group.

[98] A randomized, multicenter, double-blind, placebo-controlled study is currently underway to test AC607 for the treatment of acute kidney injury in cardiac surgery subjects. *See*, ClinicalTrials.gov Identifier: NCT01602328, incorporated herein by reference. This phase 2 clinical study evaluates the safety and efficacy of AC607 for the treatment of kidney injury in cardiac surgery subjects (ACT-AKI). The clinical study will test about 200 subjects that are at least 21 years in age. Subjects entering the study will have undergone cardiac surgery, *e.g.*, coronary artery bypass grafting, valve surgery, and/or other surgery utilizing cardiopulmonary bypass. Those who experience kidney injury within 48 hours of their surgery (*e.g.*, subjects exhibiting laboratory evidence of kidney injury within 48 hours of surgery) will be enrolled in the study. For example, a subject enrolled in the study will have AKI, as measured by a 0.5 mg/dL or greater increase in SCr from baseline within 48 hours of surgery.

[99] Once enrolled, subjects receive a single administration of AC607 or placebo (vehicle only). Subjects are randomly assigned (1:1 ratio) to AC607 or placebo, with approximately 100 subjects per group. In study, AC607 is provided as a single administration at a target dose of 2 x 10^6 human MSC/kg body weight.

[100] The MSCs (*e.g.*, AC607) of the present invention can be administered to a subject in need thereof (*e.g.*, following transplantation of a perfused kidney). "Subjects in need thereof" can include subjects who experience kidney injury and/or a decline in kidney function. For example, in this study, a subject who experiences kidney injury and/or a decline in kidney function has an increase in serum creatinine level from baseline of at least 0.5 mg/dL. Alternatively or additionally, a subject who experiences kidney injury and/or a decline in kidney

function has a SCr level greater than the normal SCr level (*e.g.*, 1 mg/dL).

[101] Therapeutic efficacy may be determined by any outcomes known in the art, including, but not limited to, time to kidney recovery, need for dialysis, death, and/or any other methods of assessment described herein. A time to kidney recovery that is reduced in subjects that have been administered hMSCs compared to subjects that have been administered placebo or no treatment indicates therapeutic efficacy of the hMSCs.

[102] For example, the baseline SCr level is determined from a subject. Then, the SCr level is monitored following transplantation of a perfused kidney and after dosing with hMSCs (*e.g.*, within 30, 25, 20, 15, 10, 5, 2, or 1 days after dosing with hMSCs). The first occurrence of a post-dosing SCr level that is less than or equal to the SCr baseline level is the time to kidney recovery.

[103] In another example, the post-dosing SCr level is compared to a normal SCr level (*e.g.*, about 1.0 mg/dL). A post-dosing SCr level that is the same as or less than a normal SCr level indicates recovery from kidney injury and therapeutic effectiveness of the hMSCs.

[104] In addition to SCr level, any other measurements of renal function described herein can also be used to evaluate therapeutic efficacy and time to kidney recovery. For example, a baseline BUN level can be measured from a subject within 30 days (*e.g.*, within 30, 25, 20, 15, 10, 5, 2, or 1 days) prior to surgery. Then, the BUN level is monitored following transplantation of a perfused kidney and after dosing with hMSCs. The first occurrence of a post-dosing BUN level that is less than or equal to the BUN baseline level is the time to kidney recovery. In another example, the post-dosing BUN level is compared to a normal BUN level (*e.g.*, about 20 mg/dL). A post-dosing BUN level that is the same as or less than a normal BUN level indicates recovery from kidney injury and therapeutic effectiveness of the hMSCs.

[105] The invention will be further illustrated in the following non-limiting examples.

EXAMPLES

[106] Example 1. DNA Isolation from Human Blood Samples

[107] The objective of this Example is to ensure that a sufficient quantity of DNA is isolated from human blood samples using the Qiagen DNeasy Blood and Tissue Kit for subsequent determination of the GT repeat lengths in both HO-1 promoter alleles. This protocol

is designed for use in the isolation of total DNA from human blood samples. DNA samples are sent to an outside vendor for fragment length analysis to determine the GT repeat lengths in the HO-1 promoter region.

[108] *Required Materials*

1. Anti-coagulated human blood in and EDTA-vacutainer (from a refrigerated or a thawed, frozen sample)
2. Qiagen DNeasy Blood & Tissue Kit (Cat. #69504)
   - -Proteinase K
   - -Buffer AL
   - -Buffer AW 1
   - -Buffer AW2
   - -Buffer AE
   - -Spin Columns
   - -Collection Tubes
3. Ethanol (96-100%)
4. Water bath set to 56°C
5. 1.5 mL microcentrifuge tubes
6. Phosphate-buffered saline (PS), Lonza catalog #17-513F (or equivalent)
7. Assorted serological pipettes

[109] 25 mL ethanol was added to Buffer AW and 30 mL ethanol was added to Buffer AW2 prior to procedure. All centrifugations were performed at room temperature. Four separate DNeasy columns were used for each donor's blood sample, and the 4 DNA samples purified from the same donor were combined at the end of the purification procedure.

[110] *Procedure*

1. For each blood sample, 4 microcentrifuge tubes were with the blood sample identification.
2. 20 µl proteinase K were added to each of the 4 microcentrifuge tubes. The blood sample vacutainer tube was thoroughly mixed by vortexing and 100 µl anti-coagulated blood was transferred to each microcentrifuge tube, then 100 µl PBS was added to each microcentrifuge tube.

3. Vacutainer tube was capped and wrapped with parafilm. The remaining blood was stored in the freezer.
4. 200µL Buffer AL was added to each microcentrifuge tube and mixed thoroughly by vortexing. Tubes were incubated at 56°C for 10 minutes.
5. 200 µL ethanol (96-100%) were added to each tube and mixed thoroughly by vortexing.
6. The mixture was pipette from each tube into a separate DNeasy Mini spin column placed in a 2mL collection tube. Tubes were centrifuged for 1 min at ≥ 6000 x g. Flow-through and collection tube were discarded.
7. Each spin column was placed in a fresh 2 mL collection tube. 500 µl Buffer AW1 was added to each spin column. Tubes were centrifuged for 1 min at ≥ 6000 x g. Flow-through and collection tube were discarded.
8. Each spin column was placed in a fresh 2 mL collection tube. 500 µl Buffer AW2 was added to each spin column. Tubes were centrifuged for 3 min at ≥ 20,000 x g (14,000 rpm). Flow-through and collection tube were discarded.
9. Each spin column was transferred to a fresh 1.5 mL micro-centrifuge tube. DNA was eluted by adding 200 µl Buffer AE to the center of each spin column membrane. Tubes were incubated for 1 minute at room temperature (15-25 °C) and were centrifuged for 1 minute at ≥ 6000 x g.
10. The 4 DNA samples purified from the same donor were combined into a single 1.5 L microcentrifuge tube.
11. The purified DNA was quantitated by measuring the optical density (OD) 260.
    a. 20 µl of the combined DNA sample was diluted with 80 µl of water in a fresh 1.5 mL tube.
    b. the diluted DNA was pipette into a well of a 96-well UV compatible plate.
    c. the OD at 260 and 280 nanometers was measured.
    d. the formula of $OD_{260/280}$ of 1 = 50 µg/mL DNA was used
        i. For example, an $OD_{260/280}$ of 0.015 = 0.75 µg/mL DNA
    e. the DNA concentration was confirmed using the nanodrop method, if available.
12. DNA sample tube was stored at -20°C.

13. Date of DNA isolation was recorded.
14. A sufficient quantity of DNA was submitted for fragment analysis. The GT repeat length was determined by comparing the resulting fragment size to the published HO-1 promoter sequence and fragment sizes of synthetic DNA fragments with known GT repeat lengths.

[111] Example 2. DNA Isolation from Cryopreserved MSC

[112] The objective of this Example is to ensure that a sufficient quantity of DNA is isolated from cryopreserved MSC samples using the Qiagen DNeasy Blood and Tissue Kit for subsequent determination of the GT repeat lengths in both alleles of the HO-1 promoter. This protocol is designed for use in the isolation of total DNA from frozen MSC samples. DNA samples are sent to an outside vendor for fragment length analysis to determine the GT repeat lengths in the HO-1 promoter region.

[113] *Required Materials*

1. Cryopreserved MSC
2. Qiagen DNeasy Blood & Tissue Kit (Cat. #69504)
   - -Proteinase K
   - -Buffer AL
   - -Buffer AW 1
   - -Buffer AW2
   - -Buffer AE
   - -Spin Columns
   - -Collection Tubes
3. Ethanol (96-100%)
4. Water bath set to 56°C
5. 1.5 mL microcentrifuge tubes
6. Phosphate-buffered saline (PS), Lonza catalog #17-513F (or equivalent)
7. Assorted serological pipettes

[114] 25 mL ethanol was added to Buffer AW and 30 mL ethanol was added to Buffer AW2 prior to procedure. All centrifugations were performed at room temperature.

[115] *Procedure*

1. A frozen MSC sample (approximately 1 x 10^5 to 5 x 10^6 MSC) was thawed in a 37°C water bath and the cells were transferred to a 1.5 mL microcentrifuge tube. Cells were spun for 1 minute at 6000 x g (8000 rpm). Supernatant was aspirated and 200 µl PBS was added, mixed, and then 20 µL Proteinase K was added.
2. 200µL Buffer AL was added and mixed thoroughly by vortexing. Tubes were incubated at 56°C for 10 minutes.
3. 200 µL ethanol (96-100%) was and mixed thoroughly by vortexing.
4. The mixture was pipetted into a DNeasy Mini spin column placed in a 2mL collection tube and centrifuged for 1 min at ≥ 6000 x g. Flow-through and collection tube were discarded.
5. The spin column was placed in a fresh 2 mL collection tube. 500 µl Buffer AW1 was added and tube was centrifuged for 1 min at ≥ 6000 x g. Flow-through and collection tube were discarded.
6. Spin column was placed in a fresh 2 mL collection tube. 500 µl Buffer AW2 was added and tube was centrifuged for 3 min at ≥ 20,000 x g (14,000 rpm). Flow-through and collection tube were discarded.
7. Spin column was transferred to a fresh 1.5 mL micro-centrifuge tube. DNA was eluted by adding 200 µl Buffer AE to the center of the spin column membrane and tube was incubated for 1 minute at room temperature (15-25 °C) and centrifuged for 1 minute at ≥ 6000 x g.
8. DNA was quantitated by measuring the optical density (OD) 260.
    a. 20 µl of the DNA sample was diluted with 80 µl of water in a fresh 1.5 mL tube.
    b. the diluted DNA was pipette into a well of a 96-well UV compatible plate.
    c. the OD at 260 and 280 nanometers was measured.
    d. the formula of $OD_{260/280}$ of 1 = 50 µg/mL DNA was used
        i. For example, an $OD_{260/280}$ of 0.015 = 0.75 µg/mL DNA
    e. the DNA concentration was confirmed using the nanodrop method, if available.

9. DNA was stored at -20°C.
10. A sufficient quantity of DNA was submitted for fragment analysis. The GT repeat length was determined by comparing the resulting fragment size to the published HO-1 promoter sequence and fragment sizes synthetic DNA fragments with known GT repeat lengths.

[116] Example 3. Human HO-1 Gene Promoter GT Repeat Analysis

[117] The objective of this example is to determine the number of GT repeats in the human HO-1 gene promoter using fragment length analysis. Total DNA purified from human blood (*see* Example 1, *supra*) or MSC samples (*see* Example 2, *supra*) were submitted to an outside vendor (University of Utah Genetics Core Facility) for fragment length analysis. Polymerase chain reaction (PCR) using a specific, forward oligonucleotides primer labeled with 6-fluorescein amidite (6-FMA) and a specific, unlabeled reverse primer flanking the GT-repeats within the HO-1 promoter were used to synthesize 6-FAM labeled DNA fragments. Fragment length analysis of the 6-FAM labeled PCR products were conducted by the outside vendor to determine the number of GT repeats in the HO-1 promoter region.

[118] *Required Materials*

1. Total DNA purified from blood or cells using DNeasy kit
   -50-100 ng per sample is needed.
2. Control DNA from Master Cell Bank (MCB) 808 or MCB 810 (50-100 ng per sample).
3. Reverse-phase HPLC purified 6-FAM labeled forward primer, synthesized and labeled by integrated technologies (IDT)
   -forward primer sequence 5'-6-FAM-TGACATTTTAGGGAGCTGGAGACA (SEQ ID NO:1)
   -the forward primer will be diluted to a 10 μM solution and used as 1 μL per 20 μL PCR reaction.
4. Reversed-phase HPLC purified unlabeled reverse primer
   -reverse primer sequence 5'-ACAAAGTCTGGCCATAGGAC (SEQ ID NO:2)
   -the reverse primer will be diluted to a 10 μM solution and used as 1 μL per 20 μL PCR reaction.

5. Microcentrifuge tubes (1.5 mL)

[119] DNA purified from human blood or MSC samples using Qiagen's DNeasy blood and tissue kit # 69504 were used. For positive controls, DNA from MCB 808 or other samples, such as synthetic DNA with known fragment lengths using the same PCR primers were submitted.

[120] *Procedure*

1. 50-100 ng of total DNA from each sample to be genotyped (or positive control DNA) were aliquoted into separate 1.5 mL microcentrifuge tubes.
2. 50 μL of the 50 μM forward and reverse primer stock solutions were aliquoted into separate 1.5 mL microcentrifuge tubes. The primers were diluted to a 10 μM working solution and were used at 1 μL PCR reactions at the external vendor.
3. The DNA samples and primer stock solutions were submitted to the external vendor.
4. Any remaining volume of the primers remained at the vendor for future PCR and fragment length analysis.

[121] *Data Analysis*

1. Fragment length data received from external vendor.
2. Confirmed that the positive control (*e.g.*, MCB 808 and 810) fragments were the expected length (in base pairs), as predicted from the published HO-1 promoter sequence.
3. Fragment sizes (in base pairs) were determined for submitted DNA samples from the plots received from the vendor.
4. Sizes of fragments and numbers of GT repeats for each sample were recorded.

[122] **Example 4. Preparation of PL**

[123] A MSC expansion medium containing PL was developed as an alternative to FBS. PL isolated from platelet rich plasma (PRP) were analyzed with either Human 27-plex (from BIO-RAD) or ELISA to show that inflammatory and anti-inflammatory cytokines as well as a variety of mitogenic factors are contained in PL, as shown below in Table 1. The human-plex method presented the concentration in [pg/mL] from undiluted PL while in the ELISA the PL was diluted to a thrombocyte concentration of 1 x 10^9/mL and used as 5% in medium (the values therefore have to be multiplied by at least 20). < : below the detection limit. Values with a black

background are anti-inflammatory cytokines and cells with a gray background are inflammatory cytokines.

Table 1. Determination of factor-concentrations in PL.

Human 27-plex (BIO-RAD) [pg/mL]

IL-1β	IL-2	IL-4	IL-5	IL-6	IL-7	IL-8	IL-9	IL-10
<	32±10	16±10	<	43.8	23±13	269±119	263±103	81±47

IL-12	IL-13	IL-15	IL-17	G-CSF	GM-CSF	IFN-γ	TNF-α	MCP-1
19.2	11±7	<	<	73±35	49.2	2073 ± 708	153±93	84±43

MIB-1β	IL-1Rα	Eotaxin	bFGF	IP-10	MIP-1α	PDGF bb	RANTES	VEGF
<	772±158	253±21	86±47	<	<	43830 ± 6767	7089 ± 1732	1023 ± 109

ELISA (n=6, 5% PL) [pg/mL]

TGF	PDGF BB	IGF-1	EGF	bFGF	HGF	VEGF
4537 ±1409	915 ± 379	634 ± 124	89 ± 24	16 ± 5	13 ± 22	50 ± 20

[124] For effective expansion of MSC, an optimized preparation of PL is needed. The protocol includes pooling PRPs from at least 10 donors (to equalize for differences in cytokine concentrations) with a minimal concentration of 3 x 10^9 thrombocytes/mL.

[125] PL was prepared either from pooled platelet concentrates designed for human use or from 7-13 pooled buffy coats after centrifugation at 200xg for 20 min. PRP was aliquoted into small portions, frozen at –80°C, thus producing PL which is thawed immediately before use. PL-containing medium was prepared fresh for each cell feeding. Medium contained αMEM as basic medium supplemented with 5 IU Heparin/mL medium (Ratiopharm) and 5% of freshly thawed PL.

[126] **Example 5. Production of MSC in PL-Supplemented Media**

[127] Bone marrow was collected from non-mobilized healthy donors. White blood cells (WBC) concentrations and CFU-F (colony forming units-fibroblasts) from bone marrow isolated

from different donors varied.

[128] Donors were tested for infectious agents prior to donation. Testing included human immunodeficiency virus, type 1 and 2 (HIV I/II), human T cell lymphotrophic virus, type I and II (HTLV I/II), hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum* (syphilis) and cytomegalovirus (CMV).

[129] 25mL-125mL (*e.g.*, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 105, 110, 115, 120, 125 mL) whole bone marrow was plated in αMEM media containing 2-10% (*e.g.*, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%) PL in a multi layered cell factory for 2-10 days (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days)to allow the MSCs to adhere. Residual non-adherent cells were washed from the cell factory. αMEM media containing 2-10% (*e.g.*, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%) PL was added to the factory. Cells were allowed to grow until 70%-100% colony confluence (*e.g.*, 70, 75, 80, 85, 90, 95, or 100%) and/or 5-15% (*e.g.*, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15%) overall surface confluence (approximately 3-33 days) with medium exchange every 4-5 days. Cells were washed with phosphate buffered saline (PBS), then detached with recombinant trypsin and re-plated into a cell factory. Cells remained in the cell factory for 6-8 days for expansion with media exchange on day 5 until they reach 80-100% surface confluence (*e.g.*, 80, 85, 90, 95, or 100%) before they are harvested.

[130] The cells were harvested by treating with trypsin (*e.g.*, recombinant) and then neutralized with a stopwash solution containing 0.5-5% HSA (*e.g.*, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5%) and were then aliquotted at 1 mL (about 10 million cells) per vial, then cryopreserved in 2-10% (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10%) DMSO, 2-10% (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10%) HSA in PlasmaLyte A using controlled-rate freezing. The cell-containing vials were stored at -130 °C or lower in vapor phase liquid nitrogen. Cell product was tested for infectious agents using methods routine in the art. Testing included human immunodeficiency virus, type 1 and 2 (HIV I/II), human T cell lymphotrophic virus, type I and II (HTLV I/II), hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum* (syphilis) and cytomegalovirus (CMV).

[131] The cell-containing vials were expanded for 2 or 3 additional rounds in cell factories using a closed system. Cells were detached with trypsin (*e.g.*, recombinate) as described above and final harvested cell product is concentrated and washed using a closed system TFF or closed system centrifugation before the cells were formulated in PlasmaLyteA, 2-10% DMSO (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10%), and 2-10% HSA (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10%). The final product

was cryopreserved using a controlled-rate freezer and stored at -130 °C or lower in vapor phase liquid nitrogen.

[132] When the cells were required for infusion, they were thawed and suspended in PlasmaLyte A containing 2-10% (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10%) HSA.

[133] The final cell product consisted of approximately 10^6-10^8 cells per kg of weight of the subject (depending on the dose schedule) suspended in a sufficient volume of PlasmaLyte A with 2-10% (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10%) HSA. No growth factors, antibodies, stimulants, or any other substances were added to the product at any time during manufacturing. The final concentration was adjusted to provide the required dose such that the volume of product that is returned to the patient remained constant.

[134] Example 6. Comparison of MSCs Grown in PL- and FBS-Supplemented Media

[135] The expansion of MSCs from bone marrow (BM) has been shown to be more effective with PL- compared to FBS-supplemented media. The size, as well as the number (Table 2), of CFU-F were considerably higher using PL as supplement in the medium (*see* WO2010/017216 or US20110293576, incorporated herein by reference).

Table 2. CFU-F from MSCs with FBS- or PL-supplemented media. Values are shown for 10^7 plated cells.

	αMEM+FBS	**αMEM+PL**
mean± SE	415 ± 97	1181 ± 244

[136] MSCs were isolated by plating 5 x 10^5 mononuclear cells/well in 3 mL. The more effective isolation of MSCs with PL-supplemented media is followed by a more rapid expansion of these cells over the whole cultivation period until senescence.

[137] Also, MSCs cultured in PL-supplemented media are less adipogenic in character when compared to MSCs cultured in FBS-supplemented media.

[138] MSC have been described to act in an immunomodulatory fashion by impairing T-cell activation without inducing anergy. A dilution of this effect has been shown *in vitro* in mixed lymphocyte cultures (MLC) leading eventually to an activation of T-cells if decreasing amounts of MSC are added to the MLC reaction. This activation process is not observed when PL-generated MSC are used in the MLC as the third party. MSCs are less immunogenic after PL-

expansion whereas FBS seems to act as a strong antigen or at least has adjuvant function in T-cell stimulation. This result is also reflected in differential gene expression showing a down-regulation of MHC II compounds.

[139] Additional data from differential gene expression analysis of PL-generated compared to FBS-generated MSC showed an up-regulation of genes involved in the cell cycle (*e.g.* cyclins and cyclin dependent kinases) and the DNA replication and purine metabolism. On the other hand, genes functionally active in cell adhesion/extracellular matrix (ECM)-receptor interaction, differentiation/development, TGF-β signaling and thrombospondin induced apoptosis could be shown to be downregulated in PL-generated MSC, further supporting the results of faster growth and accelerated expansion.

[140] Furthermore, evidence demonstrates that MSCs grown in PL-supplemented medium are more protective against ischemia-reperfusion damage than MSCs grown in FBS-supplemented medium. Human kidney proximal tubular cells (HK-2) were forced to start apoptotic events by incubation with antimycin A, 2-deoxyclucose and calcium ionophore A23187 (Lee et al., J Am Soc Nephrol 13, 2753-2761 (2002); Xie et al., J Am Soc Nephrol 17, 3336-3346 (2006)). This treatment chemically mimics an ischemic event. Reperfusion was simulated by refeeding the HK-2 cells with rescue media consisting of conditioned medium incubated for 24h on confluent layers of MSCs grown with either αMEM + 10% FBS or αMEM + 5% PL.

[141] Supernatants from MSCs grown in PL-containing medium are more effective in reducing HK-2 cell death after chemically simulated ischemia/reperfusion than supernatants from MSCs grown in FBS-supplemented medium.

[142] A parallel FACS assay detecting annexin V that binds to apoptotic cells showed similar results. The proportion of viable cells (= annexin V negative) was higher in the HK-2 cells rescued with MSC-conditioned PL medium (85.7%, as compared to 78.0% in MSC-conditioned FBS medium. Thus, it appears that PL-MSCs contain a higher rate of factors that prevent kidney tubular cells from dying after ischemic events and/or less factors that promote cell death compared to FBS-MSC conditioned medium. Thus, PL appears to be the supplement of choice to expand MSCs for the clinical treatment of ischemic injury.

[143] Example 7. Safety of rMSC administration at high doses

[144] In a 30-day study, AKI was induced by I/R in 9 female Sprague-Dawley rats. Rats with

AKI received doses of rMSC of 5 x 10^6, 10 x 10^6, or 15 x 10^6 rMSC per kg body weight by intra-arterial (IA) infusion. The highest dose was 15 million rMSC/kg IA. Kidney function, as measured by SCr and BUN, was determined on days 1 and 7 after infusion. Animals were euthanized 30 days after rMSC infusion, and renal histopathology was assessed. No deaths occurred in this study. SCr and BUN values were within the expected ranges after I/R-induced AKI, and there was no evidence of deleterious consequences of rMSC administration on renal function. Kidney histopathology of samples collected 30 days after rMSC administration was normal in all animals. This study supports the safety of rMSC administration via intra-arterial infusion in the setting of AKI at high doses.

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OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

We claim:

1. A method for treating a donor organ prior to transplantation to a recipient, the method comprising perfusing the donor organ with an effective amount of mesenchymal stem cells (MSCs) prior to transplantation when the risk profiles of the recipient, the donor organ, or both the recipient and the donor organ indicate that the donor organ is at an increased risk of developing delayed graft function following transplantation.

2. The method of claim 1, wherein the donor organ is a kidney, lung, or heart.

3. The method of claim 1, wherein the donor organ is obtained from a cadaver.

4. The method of claim 3, wherein the cadaver organ is obtained from a donor that had or was at an increased risk for developing a kidney disease or disorder.

5. The method of claim 4, wherein the donor was elderly, was acutely ill, had a medical history of chronic or acute kidney disease, had low birthweight or any combination thereof.

6. The method of claim 1, wherein the recipient is elderly, is recognized to be at significant risk for the development of delayed graft function, or is both elderly and recognized to be at significant risk for the development of delayed graft function.

7. The method of claim 1, wherein the donor organ is obtained from a cadaver from a donor that had a kidney disease or disorder and wherein the recipient suffers from or is at high risk of suffering from delayed graft function.

8. The method of claim 1, wherein the donor organ is not obtained from a cadaver from a donor that had or was at an increased risk for developing a kidney disease or disorder and wherein the recipient suffers from or is at high risk of developing delayed graft function.

9. The method of claim 1, wherein the donor organ is obtained from a cadaver from a donor that had or was at an increased risk for developing a kidney disease or disorder and wherein the

recipient does not suffer from and is not at high risk of suffering from delayed graft function.

10. The method of claim 7 or claim 8, wherein poor kidney function is measured by one or more serum/blood biomarkers, one or more urine biomarkers, or both.

11. The method of claim 10, wherein the one or more serum/blood biomarkers are serum creatinine (SCr), blood urea nitrogen (BUN), or both serum creatinine (SCr) and blood urea nitrogen (BUN).

12. The method of claim 11, wherein the one or more serum/blood biomarkers are further selected from the group consisting of Cystatin C and Beta-trace protein (BTP).

13. The method of claim 11, wherein the one or more urine biomarkers are selected from the group consisting of Podocalyxin, Nephrin, Alpha 1-microglobulin, Beta 2-microglobulin, Glutathione S-transferase, Interleukin-18, Kidney Injury Molecule-1 (KIM-1), Liver-Type Fatty Acid-Binding Protein, Netrin-1, Neutrophil Gelatinase-Associated Lipocalcin (NGAL), and N-Acetyl-Beta-D-Glucosaminidase (NAG).

14. The method of claim 10, wherein poor kidney function is measured by one or more biomarkers selected from the group consisting of serum creatinine (SCr), blood urea nitrogen (BUN), Cystatin C, Beta-trace protein (BTP), Podocalyxin, Nephrin, Alpha 1-microglobulin, Beta 2-microglobulin, Glutathione S-transferase, Interleukin-18, Kidney Injury Molecule-1 (KIM-1), Liver-Type Fatty Acid-Binding Protein, Netrin-1, Neutrophil Gelatinase-Associated Lipocalcin (NGAL), and N-Acetyl-Beta-D-Glucosaminidase (NAG).

15. The method of claim 1, wherein the donor organ is perfused *ex vivo*.

16. The method of claim 15, wherein the *ex vivo* perfusion of the donor organ prior to transplantation into the patient prevents or reduces the development of delayed graft function, acute kidney injury, or chronic kidney diseases in the recipient following transplantation.

17. The method of claim 1, wherein the effective amount of MSCs is about 25 million cells to about 300 million cells.

18. The method of claim 1, wherein the effective amount of MSCs are in a biologically and physiologically compatible solution.

19. The method of claim 1 wherein the MSCs are autologous cells.

20. The method of claim 1 wherein the MSCs are allogeneic cells.

21. The method of claim 1 wherein the hMSCs are non-transformed stem cells.

22. The method of claim 1, wherein the MSCs are isolated from a bone marrow aspirate and adhere to a culture dish while substantially all other cell types remain in suspension.

23. The method of claim 1, wherein the MSCs are obtained from a bone marrow sample, from fat, from cord blood, from placenta, or any combination thereof.

24. The method of claim 1, wherein the MSCs are obtained from a cryopreserved sample.

25. The method of claim 1, wherein the MSCs are obtained from a Master Cell Bank (MCB).

26. The method of claim 1, wherein the MSCs are expanded *in vitro* to produce an enriched population of MSCs.

27. The method of claim 26, wherein the MSCs are expanded in a platelet lysate (PL)-supplemented culture medium.

28. The method of claim 1, wherein the MSCs have 32 or fewer GT repeats in both alleles of the human heme oxygenase (HO-1) promoter region.

29. The method of claim 1, wherein the MSCs have two short alleles, two medium alleles, or one short and one medium allele in the HO-1 promoter region wherein a short allele has $\leq$ 26 GT repeats in the HO-1 promoter region and wherein a medium allele has between 27 and 32 GT repeats in the HO-1 promoter region.

30. The method of claim 1, wherein the MSCs do not have any long alleles, wherein a long allele has > 32 GT repeats in the HO-1 promoter region.

31. The method of claim 1, wherein, following transplantation of the perfused organ, a therapeutically effective amount of MSCs is subsequently administered to the patient.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/039678

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0775 A01N1/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JAE W. LEE ET AL: "Therapeutic Effects of Human Mesenchymal Stem Cells in Ex Vivo Human Lungs Injured with Live Bacteria", AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, vol. 187, no. 7, 1 April 2013 (2013-04-01) , pages 751-760, XP055151331, ISSN: 1073-449X, DOI: 10.1164/rccm.201206-09900C the whole document ----- -/--	1-3, 15-18, 20-27

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 2014	19/11/2014

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/039678

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DIRK VAN RAEMDONCK ET AL: "Machine perfusion in organ transplantation", CURRENT OPINION IN ORGAN TRANSPLANTATION, vol. 18, no. 1, 1 February 2013 (2013-02-01), pages 24-33, XP055151293, ISSN: 1087-2418, DOI: 10.1097/MOT.0b013e32835c494f the whole document	1-31
A	VINCENZO CANTALUPPI ET AL: "Rationale of Mesenchymal Stem Cell Therapy in Kidney Injury", AMERICAN JOURNAL OF KIDNEY DISEASES, vol. 61, no. 2, 1 February 2013 (2013-02-01), pages 300-309, XP055151653, ISSN: 0272-6386, DOI: 10.1053/j.ajkd.2012.05.027 the whole document	1-31
A	WO 2013/070765 A1 (ALLOCURE INC [US]; BRENNER ROBERT M [US]; MOHAMED ALI S [US]; MINNING) 16 May 2013 (2013-05-16) the whole document	1-31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/039678

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013070765 A1	16-05-2013	EP 2776578 A1	17-09-2014
		US 2013129688 A1	23-05-2013
		WO 2013070765 A1	16-05-2013