

MODIFIED PLURIPOTENT CELLS

I. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/846,399, filed May 10, 2019 and U.S. Provisional Application No. 62/855,499, filed May 31, 2019, each of which are incorporated herein by reference in their entirety.

II. FIELD OF THE INVENTION

[0002] The invention relates to regenerative cell therapy. In some embodiments, the regenerative cell therapy comprises transplanting cell lines into patients in need thereof. In some embodiments, the cell lines comprise O Rh- hypoinmunogenic pluripotent cells. In some embodiments, the regenerative cell therapy reduces the propensity for the cell transplant recipient's immune system to reject allogeneic material. In some embodiments, the regenerative cell therapy is used in the treatment of injured organs and tissue. In some embodiments, the regenerative cell therapy is used for rehabilitating damaged tissues after myocardial infarction.

III. BACKGROUND OF THE INVENTION

[0003] Regenerative cell therapy is an important potential treatment for regenerating injured organs and tissue. With the low availability of organs for transplantation and the accompanying lengthy wait, the possibility of regenerating tissue by transplanting readily available cell lines into patients is understandably appealing. Regenerative cell therapy has shown promising initial results for rehabilitating damaged tissues after transplantation in animal models (e.g. after myocardial infarction). The propensity for the transplant recipient's immune system to reject allogeneic material, however, greatly reduces the potential efficacy of therapeutics and diminishes the possible positive effects surrounding such treatments.

[0004] Autologous induced pluripotent stem cells (iPSCs) theoretically constitute an unlimited cell source for patient-specific cell-based organ repair strategies. Their generation, however, poses technical and manufacturing challenges and is a lengthy process that conceptually prevents any acute treatment modalities. Allogeneic iPSC-based therapies or embryonic stem cell-based therapies are easier from a manufacturing standpoint and allow the generation of well-screened, standardized, high-quality cell products. Because pluripotent stem cells can be differentiated into any cell type of the three germ layers, the potential application of stem cell therapy is wide-ranging. Differentiation can be performed *ex vivo* or *in vivo* by transplanting progenitor cells that continue to

differentiate and mature in the organ environment of the implantation site. *Ex vivo* differentiation allows researchers or clinicians to closely monitor the procedure and ensures that the proper population of cells is generated prior to transplantation. Because of their allogeneic origin, however, such cell products could undergo rejection.

[0005] The art seeks stem cells capable of producing cells that are used to regenerate or replace diseased or deficient cells. Pluripotent stem cells (PSCs) may be used because they rapidly propagate and differentiate into many possible cell types. The family of PSCs includes several members generated via different techniques and possessing distinct immunogenic features. Patient compatibility with engineered cells or tissues derived from PSCs determines the risk of immune rejection and the requirement for immunosuppression.

[0006] Embryonic stem cells (ESCs) isolated from the inner cell mass of blastocysts can exhibit the histocompatibility antigens that are mismatches with recipients. This immunological barrier cannot be solved by human leukocyte antigen (HLA)-typed banks of ESCs because even HLA-matched PSC grafts undergo rejection because of mismatches in non-HLA molecules that function as minor antigens. This is also true for allogeneic induced pluripotent stem cells (iPSCs).

[0007] To circumvent the problem of rejection, different techniques for the generation of patient-specific pluripotent stem cells have been developed. These include the transfer of a somatic cell nucleus into an enucleated oocyte (somatic cell nucleus transfer (SCNT) stem cells), the fusion of a somatic cell with an ESC (hybrid cell), and the reprogramming of somatic cells using certain transcription factors (induced PSCs or iPSCs). SCNT stem cells and iPSCs, however, may have immune incompatibilities with the nucleus or cell donor, respectively, despite chromosomal identity. SCNT stem cells carry mitochondrial DNA (mtDNA) passed along from the oocyte. mtDNA-coded proteins can act as relevant minor antigens and trigger rejection. DNA and mtDNA mutations and genetic instability associated with reprogramming and culture-expansion of iPSCs can also create minor antigens relevant for immune rejection. This hurdle decreases the likelihood of successful, large-scale engineering of compatible patient-specific tissues using SCNT stem cells or iPSCs.

[0008] More recently, the advent of hypoimmune pluripotent (HIP) cells that evade rejection by the host allogeneic immune system has resulted in a major breakthrough for allogeneic transplantations. See WO 2018/132783. These cells are engineered to reduce HLA-I and HLA-II expression to avoid initiation of an immune response and to increase production of CD47 to suppress phagocytic innate immune surveillance. The inventions described herein build on this technology and further reduce HIP cell rejection.

IV. SUMMARY OF THE INVENTION

[0009] A previously unrecognized cause of rejection of transplanted cells has now been linked to blood group antigens. Blood products can be classified into different groups according to the presence or absence of antigens on the surface of every red blood cell in a person's body (ABO Blood Type). The A, B, AB, and A1 antigens are determined by the sequence of oligosaccharides on the glycoproteins of erythrocytes. The genes in the blood group antigen group provide instructions for making antigen proteins. Blood group antigen proteins serve a variety of functions within the cell membrane of red blood cells. These protein functions include transporting other proteins and molecules into and out of the cell, maintaining cell structure, attaching to other cells and molecules, and participating in chemical reactions.

[0010] The Rhesus Factor (Rh) blood group is the second most important blood group system, after the ABO blood group system. The Rh blood group system consists of 49 defined blood group antigens, among which five antigens, D, C, c, E, and e, are the most important. Rh(D) status of an individual is normally described with a positive or negative suffix after the ABO type. The terms "Rh factor," "Rh positive," and "Rh negative" refer to the Rh(D) antigen only. Antibodies to Rh antigens can be involved in hemolytic transfusion reactions and antibodies to the Rh(D) and Rh(c) antigens confer significant risk of hemolytic disease of the fetus and newborn. ABO antibodies develop in early life in every human. However, rhesus antibodies in Rh- humans develop only when the person is sensitized. This occurs by giving birth to a rh+ baby or by receiving an Rh+ blood transfusion.

[0011] This invention provides ABO blood type O and/or Rhesus Factor negative (O-) populations of pluripotent (PSCO-) cells suitable for transplantation and/or differentiation. The PSCO- cells include induced iPSCs (iPSCO-), embryonic ESCs (ESCO-), and cells differentiated from those cells, including O- endothelial cells, O- cardiomyocytes, O- hepatocytes, O- dopaminergic neurons, O- pancreatic islet cells, O- retinal pigment endothelium cells, and other O- cell types used for transplantation and medical therapies. These would include O- chimeric antigen receptor (CAR) cells, such as CAR-T cells, CAR-NK cells, and other engineered cell populations. In some embodiments, the cells are not hematopoietic stem cells. The invention further provides universally acceptable "off-the-shelf" ESCO-s and PSCO-s and derivatives thereof for generating or regenerating specific tissues and organs.

[0012] Another aspect of the invention provides methods of generating populations of PSCO-, iPSCO-, ESCO- and other O- cells for transplantation. The invention also provides methods of

treating diseases, disorders, and conditions that benefit from the transplantation of pluripotent or differentiated cells.

[0013] In some embodiments of the invention, the ABO blood group type O results from a reduced ABO blood group protein expression. In other aspects, the ABO blood group is endogenously type O. In some aspects of the invention, the HIPO- cell has an ABO blood group type O that results from a disruption in human Exon 7 of the ABO gene. In some embodiments, both alleles of Exon 7 of the ABO gene are disrupted. In some embodiments, the disruption in both alleles of Exon 7 of the ABO gene results from a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 reaction that disrupts both of the alleles.

[0014] In other aspects, the ABO blood group type O results from an enzymatic modification of an ABO gene product on a surface of the cell. In a preferred aspect, the enzymatic modification removes a carbohydrate from the ABO gene product. In another preferred aspect, the enzymatic modification removes a carbohydrate from an ABO A1 antigen, A2 antigen, or B antigen.

[0015] In some embodiments of the invention, the Rh blood group is endogenously type Rh-. In another aspect, the Rh- blood group results from reducing or eliminating Rh protein expression. In another aspect, the type Rh- results from disrupting the gene encoding Rh C antigen, Rh E antigen, Kell K antigen (KEL), Duffy (FY) Fya antigen, Duffy Fy3 antigen, Kidd (JK) Jkb antigen, or/and or Kidd SLC14A1. In some embodiments the disruption results from a CRISPR/Cas9 reaction that disrupts both alleles of the gene encoding Rh C antigen, Rh E antigen, Kell K antigen (KEL), Duffy (FY) Fya antigen, Duffy Fy3 antigen, Kidd (JK) Jkb antigen, or/and or Kidd SLC14A1.

[0016] In some embodiments of the invention, the O- cells (e.g., PSCO-, iPSCO-, ESCO- and cells derived therefrom) of the invention are of mammalian origin, for example, human, bovine, porcine, chicken, turkey, horse, sheep, goat, donkey, mule, duck, goose, buffalo, camel, yak, llama, alpaca, mouse, rat, dog, cat, hamster, or guinea pig origin.

[0017] In a specific embodiment, the invention provides hypoimmune pluripotent ABO blood type O Rhesus Factor negative (HIPO-) cells that evade rejection by the host allogeneic immune system and avoid blood antigen type rejection. In some embodiments, the HIPO- cells are engineered to reduce or eliminate HLA-I and HLA-II expression, increase expression of an endogenous protein that reduces the susceptibility of the pluripotent cell to macrophage phagocytosis, and comprise a universal blood group O Rh- ("O-") blood type. The universal blood type may be achieved by eliminating ABO blood group A and B antigens and Rh factor expression, or by starting with an O- cell

line. These novel HIPO- cells evade host immune rejection because they have an impaired antigen presentation capacity, protection from innate immune clearance, and lack blood group rejection.

[0018] In certain embodiments, HIPO- cells of the invention comprise: an endogenous Major Histocompatibility Antigen Class I (HLA-I) function that is reduced when compared to an unmodified pluripotent cell; an endogenous Major Histocompatibility Antigen Class II (HLA-II) function that is reduced when compared to an unmodified pluripotent cell; increased expression of CD47 as compared to an unmodified pluripotent cell that reduces susceptibility to NK cell killing; an ABO blood group type O (O); and a Rhesus Factor (Rh) blood type negative (-); wherein the human hypoinmunogenic pluripotent O- (HIPO-) cell is less susceptible to rejection when transplanted into a subject when compared with an otherwise similar hypoinmunogenic pluripotent (HIP) cell that is an ABO blood group or Rh factor mismatch to the subject.

[0019] In certain embodiments of the invention, the HIPO- cell has a reduced HLA-I function by virtue of a reduction in β -2 microglobulin protein expression. In another aspect, a gene encoding the β -2 microglobulin protein is knocked out. In some embodiments, both alleles of the MB2 gene are disrupted. In some embodiments, that disruption results from a CRISPR/Cas9 reaction. In some embodiments of the invention, the HIPO- cell has a reduced HLA-I function by virtue of a reduction in HLA-A protein expression. In another aspect, a gene encoding the HLA-A protein is knocked out. In another embodiment, the HLA-I function is reduced by a reduction in HLA-B protein expression. In some embodiments, a gene encoding the HLA-B protein is knocked out. In other embodiments of the invention, the HIPO- cell has a reduced HLA-I function by virtue of a reduction in HLA-C protein expression. In some embodiments, a gene encoding the HLA-C protein is knocked out. In some embodiments, the HIPO- cell does not comprise an HLA-I function.

[0020] In certain embodiments of the invention, the HIPO- cell has a reduced HLA-II function by virtue of a reduction in CIITA protein expression. In another embodiment, a gene encoding the CIITA protein is knocked out. In an aspect of the invention, the HIPO- cell has a reduced HLA-II function by virtue of a reduction in HLA-DP protein expression. In another aspect, a gene encoding the HLA-DP protein is knocked out. In another aspect, the HLA-II function is reduced by a reduction in HLA-DR protein expression. In another aspect, a gene encoding the HLA-DR protein is knocked out. In another aspect, the HLA-II function is reduced by a reduction in HLA-DQ protein expression. In another aspect, a gene encoding the HLA-DQ protein is knocked out. In another aspect, the hypoinmunogenic pluripotent cell does not comprise an HLA-II function.

[0021] In some embodiments, the HIPO- cells of the invention are engineered to have increased expression of a protein that reduces the susceptibility of the pluripotent cell to macrophage phagocytosis. In some embodiments, the increased expression results from a modification to an endogenous gene locus. In some embodiments, the HIPO- cells have a reduced susceptibility to NK cell killing resulting from an increased expression of a CD47 protein. In another aspect, the increased CD47 protein expression results from a modification to an endogenous CD47 gene locus. In another aspect, the increased CD47 protein expression results from addition of a CD47 transgene. In some embodiments, the increased CD7 protein expression results from introducing at least one copy of a human CD47 gene under the control of a promoter into the cell. In a preferred aspect, the promoter is a constitutive promoter.

[0022] In some imbodiments, the HIPO- cells of the invention further comprise a suicide gene that is activated by a trigger that causes the hypoimmunogenic pluripotent cell to die. In another aspect, the suicide gene is a herpes simplex virus thymidine kinase gene (HSV-tk) and the trigger is ganciclovir. In another aspect, the suicide gene is an *Escherichia coli* cytosine deaminase gene (EC-CD) and the trigger is 5-fluorocytosine (5-FC). In another aspect, the suicide gene encodes an inducible Caspase protein and the trigger is a chemical inducer of dimerization (CID). In another more preferred aspect, the CID is AP1903.

[0023] One aspect of the invention, provides cells derived from the PSCO-, iPSCO-, ESCO-, and or HIPO- cells described herein, wherein the cells are selected from the group consisting of a chimeric antigen receptor (CAR) cell, an endothelial cell, a dopaminergic neuron, a pancreatic islet cell, and a retinal pigment endothelium cell. In a preferred aspect, the CAR cell is a CAR-T cell.

[0024] The invention further provides a method of treating a disease, disorder, or condition that can benefit from transplantation with the cells of the invention or derivatives thereof. The method comprises administering PSCO-, iPSCO-, ESCO-, HIPO- and/or other ABO- cells as described herein. In one aspect, the PSCO-, iPSCO-, ESCO-, HIPO- and/or other ABO- cells are selected from the group consisting of chimeric antigen receptor (CAR) cells, endothelial cells, dopaminergic neurons, pancreatic islet cells, and retinal pigment endothelium cells. In some embodiments, the disease is selected from the group consisting of Type I Diabetes, cardiac diseases, neurological diseases, cancers, ocular diseases, and vascular diseases. In some embodiments of the invention, the method comprises transplanting ABO- cells of the invention, including PSCO-, iPSCO-, ESCO-, HIPO- cells and cells derived from PSCO-, iPSCO-, ESCO-, and HIPO- cells into a mammalian subject. In some

embodiments, the subject is a human, cow, pig, chicken, turkey, horse, sheep, goat, donkey, mule, duck, goose, buffalo, camel, yak, llama, alpaca, mouse, rat, dog, cat, hamster, guinea pig.

[0025] Another aspect of the invention provides a method for generating a hypoimmunogenic pluripotent ABO group O Rh factor negative (HIPO-) cells from an O- iPSCs comprising: (a) eliminating the Major Histocompatibility Antigen Class I (HLA-I) function or reducing HLA-I function as compared to an unmodified pluripotent cell; (b) eliminating the Major Histocompatibility Antigen Class II (HLA-II) function or reducing HLA-II function as compared to an unmodified pluripotent cell; (c) increasing the expression of CD47 as compared to expression of CD47 in an unmodified iPSC. In some embodiments, the starting iPSC is not O- and thus the method further comprises: (d) eliminating any ABO blood group antigen to provide ABO blood type O; and (e) eliminating any Rhesus Factor (Rh) blood group antigen to provide Rh type negative (-).

[0026] The invention also provides one or more cells that are derived or differentiated from the induced ABO blood group O- Rh factor (-) pluripotent (e.g., PSCO-, iPSCO, ESCO-) cells described herein. These cells comprise an ABO blood group type O (O) and a Rhesus Factor (Rh) blood type negative (-), rendering them less susceptible to rejection when transplanted into a subject when compared with cells that are ABO blood group or Rh factor mismatched to the subject.

[0027] The invention also provides for the screening and/or stratifying of subjects for administration of hypoimmunogenic cells, wherein the ABO blood group type of the hypoimmunogenic cells is matched with the subject's ABO blood group type prior to the administration of the cells. For example, the invention may provide for the administration of ABO blood group type A hypoimmunogenic cells to a subject who is determined to be ABO blood group type A or AB; the administration of ABO blood type B hypoimmunogenic cells to a subject who is determined to be ABO blood group type B or AB; the administration of ABO blood group type AB hypoimmunogenic cells to a subject who is determined to be ABO blood group type AB; or the administration of ABO blood group type O hypoimmunogenic cells to a subject who is determined to be ABO blood group type A, B, AB, or O.

[0028] Another aspect of the invention provides for the screening and/or stratifying of subjects for administration of hypoimmunogenic cells, wherein the ABO blood group type and the Rh blood type of the hypoimmunogenic cells are matched with the subject's ABO blood group type and Rh blood type prior to the administration of the cells. For example, the invention may provide for the administration of ABO blood group type A, Rh positive (+) hypoimmunogenic cells to a subject who is determined to be ABO blood group type A or AB and Rh positive (+). In another example, the

invention may provide for the administration of ABO blood group type B, Rh positive (+) hypoimmunogenic cells to a subject who is determined to be ABO blood group type B or AB and Rh positive (+). In another example, the invention may provide for the administration of ABO blood group type AB, Rh positive (+) hypoimmunogenic cells to a subject who is determined to be ABO blood group type AB and Rh positive (+). In another example, the invention may provide for the administration of ABO blood group type O, Rh positive (+) hypoimmunogenic cells to a subject who is determined to be ABO blood group type A, B, AB or O, and Rh positive (+). In another example, the invention may provide for the administration of ABO blood group type A, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type A or AB and Rh negative (-). In another example, the invention may provide for the administration of ABO blood group type B, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type B or AB and Rh negative (-). In another example, the invention may provide for the administration of ABO blood group type AB, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type AB and Rh negative (-). In another example, the invention may provide for the administration of ABO blood group type O, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type A, B, AB or O, and Rh negative (-).

[0029] Another aspect of the invention provides for the screening and/or stratifying of subjects for administration of hypoimmunogenic cells, wherein the ABO blood group type of the hypoimmunogenic cells are matched with the subject's ABO blood group type, but the Rh blood type of the hypoimmunogenic cells and the Rh blood type of the subject are not matched. In this aspect, the cells are only administered if they are Rh blood type negative (-) and they are administered to a subject who is Rh blood type positive (+). For example, the invention may provide for the administration of ABO blood group type A, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type A or AB and Rh positive (+). In another example, the invention may provide for the administration of ABO blood group type B, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type B or AB and Rh positive (+). In another example, the invention may provide for the administration of ABO blood group type AB, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type AB and Rh positive (+). In another example, the invention may provide for the

administration of ABO blood group type O, Rh negative (-) hypoimmunogenic cells to a subject who is determined to be ABO blood group type A, B or AB, and Rh positive (+).

V. BRIEF DESCRIPTION OF THE DRAWINGS

[0030] **Fig. 1A** shows that adaptive and innate immune cells were not activated in immune assays using blood from Macaque Rhesus monkeys in which hypoimmunogenic B2M^{-/-} CIITA^{-/-} rhesus CD47 tg endothelial cells did not survive. Hypoimmunogenic B2M^{-/-} CIITA^{-/-} rhesus CD47 tg endothelial cells were not rejected by T cells, Cytotoxic T cells, or NK cells. **Fig. 1B** shows that hypoimmunogenic B2M^{-/-} CIITA^{-/-} rhesus CD47 tg endothelial cells did not trigger antibody production and were not rejected by macrophages. Because the cells were cleared from the monkeys, these results suggested another mechanism for cell death.

[0031] **Fig. 2A** shows that adaptive immune cells (T cells, Cytotoxic T cells, and B cells) were activated in immune assays using blood from Macaque Rhesus monkeys that rejected unmodified human iPSC-derived endothelial cells. **Fig. 2B** shows that innate immune cells (NK cells and macrophages) were activated in immune assays using blood from Macaque Rhesus monkeys that rejected HLA-I deficient / HLA-II deficient human iPSC-derived endothelial cells. **Fig. 2C** shows that Rhesus Monkey Blood Type B Serum Causes Complement-Dependent Cytotoxicity (CDC) of wild-type (wt) induced endothelial cells (iECs) of blood type A. **Fig. 2D** shows that iECs of blood type O were unaffected. **Fig. 2E** shows that embryonic stem cell-derived ECs undergo the same ABO blood type-dependent CDC as iPSC-derived iECs.

[0032] **Fig. 3:** Targeted cell killing by ABO blood type-incompatible serum was confirmed by incubating human B2M^{-/-} CIITA^{-/-} rhesus CD47 tg hypoimmunogenic endothelial cells with rhesus macaque serum. When human hypoimmunogenic endothelial cells (blood type A) are incubated with rhesus macaque serum (blood type B), cells are killed immediately. Depletion of either IgM or IgG antibodies demonstrated that the ABO-antibodies were from the IgM type.

[0033] **Fig. 4** shows that the human B2M^{-/-} CIITA^{-/-} rhesus CD47 tg cells were not rejected by other pre-formed antibodies when transplanted across the xenogeneic barrier. Human B2M^{-/-} CIITA^{-/-} rhesus CD47 tg iPSC-derived endothelial cells (blood type A) were killed when incubated with ABO-mismatched rhesus macaque serum (blood type B). When serum from rhesus macaque with blood type AB was used, however, the human cells survived.

[0034] **Fig. 5A** shows that human hypoimmunogenic iPSC-derived cardiomyocytes (blood type A) survive when incubated with allogeneic human serum blood type A and AB. Serum containing pre-

formed antibodies against A (blood type O and B), however, killed the cells immediately. **Fig. 5B** shows that mature cardiomyocytes (blood type A) survive when incubated with ABO matched allogeneic human serum (blood type A and AB), but they are killed when incubated with serum containing pre-formed antibodies against A (blood type O and B).

[0035] **Fig. 6** shows that blood type A human hepatocytes survive when incubated with ABO matched serum (blood type A and AB) but are killed when incubated with ABO mismatched serum (blood type B and O). Similarly, blood type AB human hepatocytes survive when incubated with ABO matched serum (blood type AB) but are killed when incubated with ABO mismatched serum (blood type A, B, and O).

[0036] **Fig. 7A** shows that endothelial cells (blood type A Rh+ or Rh-) are able to survive when incubated with an ABO matched serum (blood type A) which is either Rh+ or Rh- when the serum has not been previously sensitized to the Rh factor. **Fig. 7B** shows that endothelial cells (blood type A or B Rh+) are killed when incubated with an ABO-compatible serum (blood type AB) that is Rh- when the serum contains anti-Rh antibodies due to a previous sensitization to the Rh factor. Endothelial cells (blood type A Rh-) are not killed when incubated with the same blood type AB serum that contains anti-Rh antibodies because the EC cells don't express the Rh antigen. Hypoimmunogenic endothelial cells (B2M-/- CIITA -/- CD47 tg) (blood type A Rh+) are also killed when incubated with an ABO-compatible serum (blood type AB) that is Rh- and that has been previously sensitized to the Rh factor. **Fig. 7C** shows that endothelial cells that are blood type O Rh+ are killed when using O Rh- serum that has been previously sensitized to the Rh factor. **Fig. 7D** shows that when H9-derived ECs of blood type A Rh+ were incubated with ABO-compatible serum containing Rh antibodies, they underwent CDC killing. **FIG. 7E** shows that HEK293 cells incubated with serum containing Rh antibodies did not undergo killing.

VI. DETAILED DESCRIPTION OF THE INVENTION

[0037] The invention provides Rhesus Factor negative pluripotent cells of any ABO blood type, such as blood type O, that avoid or minimize host immune responses due to one or more genetic or enzymatic manipulations as outlined herein. The cells lack major blood group and immune antigens that trigger immune responses and may also be engineered to avoid rejection, phagocytosis, or killing. This allows the derivation of "off-the-shelf" cell products for generating specific tissues and organs. The use of human allogeneic PSCO-, iPSCO-, ESCO-, or HIPO- cells and their derivatives in human patients provides significant benefits, including the ability to avoid cell transplant rejection without requiring long-term adjunct immunosuppressive therapy and drug use generally seen in

allogeneic transplantations. It also provides significant cost savings as cell therapies can be used without requiring individual treatments for each patient. Recently, it was shown that cell products generated from autologous cell sources may become subject to immune rejection with few or even one single antigeneic mutation. Thus, autologous cell products are not inherently non-immunogenic. Also, individual patient cell engineering and quality control is very labor and cost intensive and thus, autologous cells are rarely available for acute treatment options. Allogeneic cell products will be able to be used for a bigger patient population only if the immune hurdle can be overcome. The PSCO-, iPSCO, ESCO-, and HIPO- cells of the invention and cells derived from those cells will provide a universal cell source for the generation of universally-acceptable derivatives.

[0038] In addition to an O- blood type, the present invention, in part, exploits the fetomaternal tolerance that exists in pregnant women. Although half of a fetus' human leukocyte antigens (HLA) are paternally inherited and the fetus expresses major HLA mismatched antigens, the maternal immune system does not recognize the fetus as an allogeneic entity and does not initiate an immune response, *e.g.* as is seen in a "host versus graft" type of immune reaction. Fetomaternal tolerance is mainly mediated by syncytiotrophoblast cells in the fetal-maternal interface. Syncytiotrophoblast cells show little or no proteins of the major histocompatibility complexes I and II (MHC-I and MHC-II), as well as increased expression of CD47, known as the "don't eat me" protein that suppresses phagocytic innate immune surveillance and elimination of HLA-devoid cells. Surprisingly, the same tolerogenic mechanisms that prevent rejection of the fetus during pregnancy also allow the HIPO-cells of the invention to escape rejection and facilitate long-term survival and engraftment of these cells after allogeneic transplantation.

[0039] Fetomaternal tolerance can be introduced with as little as three genetic modifications (as compared to unmodified iPSCs, *e.g.* hiPSCs): two reductions in activity ("knock outs" as further described herein) and one increase in activity (a "knock in" as described herein). Generally, others have attempted to suppress immunogenicity of iPSCs but have been only partially successful; see Rong *et al.*, *Cell Stem Cell* 14:121-130 (2014) and Gornalusse *et al.*, *Nature Biotech* doi:10.1038/nbt.3860), WO2018/132783 and U.S. Prov. App. Nos. 62/698,941, 62/698,965, 62/698,973, 62/698,978, 62/698,981, and 62/698,984, each of which are incorporated by reference herein in their entirety.

[0040] Autologous induced pluripotent stem cells (iPSCs) constitute an unlimited cell source for patient-specific, autologous cell-based organ repair strategies. As noted above, however, generation and subsequent differentiation of autologous iPSCs into tissue cells pose technical and

manufacturing challenges and are lengthy processes that preclude their use in acute treatment modalities. These shortcomings can only be overcome with availability of prefabricated ready-to-use cell or tissue products of allogeneic origin.

[0041] The starter cell line for prefabricated ready-to-use cell or tissue products of allogeneic origin, must be “universal”. The PSCO-, iPSCO-, ESCO-, or HIPO- cells of the invention provide for the first time, readily accessible non-immunogenic pluripotent cells for maintenance, differentiation into desired cell and tissue types, and ultimately transplantation of their derivatives into patients in need thereof.

Definitions

[0042] The term “pluripotent cells” refers to cells that can self-renew and proliferate while remaining in an undifferentiated state and that can, under the proper conditions, be induced to differentiate into specialized cell types. The term “pluripotent cells,” as used herein, encompass embryonic stem cells and other types of stem cells, including fetal, amnionic, or somatic stem cells. Exemplary human stem cell lines include the H9 human embryonic stem cell line. Additional exemplary stem cell lines include those made available through the National Institutes of Health Human Embryonic Stem Cell Registry and the Howard Hughes Medical Institute HUES collection (as described in Cowan, C. A. *et al.*, *New England J. Med.* 350:13. (2004), incorporated by reference herein in its entirety).

[0043] “Pluripotent stem cells” as used herein have the potential to differentiate into any of the three germ layers: endoderm (*e.g.* the stomach lining, gastrointestinal tract, lungs, etc), mesoderm (*e.g.* muscle, bone, blood, urogenital tissue, etc) or ectoderm (*e.g.* epidermal tissues and nervous system tissues). The term “pluripotent stem cells,” as used herein, also encompasses “induced pluripotent stem cells”, or “iPSCs”, a type of pluripotent stem cell derived from a non-pluripotent cell. Examples of parent cells include somatic cells that have been reprogrammed to induce a pluripotent, undifferentiated phenotype by various means. Such “iPS” or “iPSC” cells can be created by inducing the expression of certain regulatory genes or by the exogenous application of certain proteins. Methods for the induction of iPS cells are known in the art and are further described below. (See, *e.g.*, Zhou *et al.*, *Stem Cells* 27 (11): 2667-74 (2009); Huangfu *et al.*, *Nature Biotechnol.* 26 (7): 795 (2008); Woltjen *et al.*, *Nature* 458 (7239): 766-770 (2009); and Zhou *et al.*, *Cell Stem Cell* 8:381-384 (2009); each of which is incorporated by reference herein in their entirety.) The generation of induced pluripotent stem cells (iPSCs) is outlined below. As used herein, “hiPSCs” are human induced pluripotent stem cells, and “miPSCs” are murine induced pluripotent stem cells.

[0044] Several characteristics of pluripotent stem cells distinguish them from other cells. For example, the ability to give rise to progeny that can undergo differentiation, under the appropriate conditions, into cell types that collectively demonstrate characteristics associated with cell lineages from all of the three germinal layers (endoderm, mesoderm, and ectoderm) is a pluripotent stem cell characteristic. Expression or non-expression of certain combinations of molecular markers are also pluripotent stem cell characteristics. For example, human pluripotent stem cells express at least several, and in some embodiments, all of the markers from the following non-limiting list: SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, ALP, Sox2, E-cadherin, UTF-1, Oct4, Rex1, and Nanog. Cell morphologies associated with pluripotent stem cells are also pluripotent stem cell characteristics. Cells do not need to pass through pluripotency to be reprogrammed into endodermal progenitor cells and/or hepatocytes.

[0045] As used herein, "multipotent" or "multipotent cell" refers to a cell type that can give rise to a limited number of other particular cell types. For example, induced multipotent cells are capable of forming endodermal cells. Additionally, multipotent blood stem cells can differentiate itself into several types of blood cells, including lymphocytes, monocytes, neutrophils, etc.

[0046] As used herein, the term "oligopotent" refers to the ability of an adult stem cell to differentiate into only a few different cell types. For example, lymphoid or myeloid stem cells are capable of forming cells of either the lymphoid or myeloid lineages, respectively.

[0047] As used herein, the term "unipotent" means the ability of a cell to form a single cell type. For example, spermatogonial stem cells are only capable of forming sperm cells.

[0048] As used herein, the term "totipotent" means the ability of a cell to form an entire organism. For example, in mammals, only the zygote and the first cleavage stage blastomeres are totipotent.

[0049] As used herein, "non-pluripotent cells" refer to mammalian cells that are not pluripotent cells. Examples of such cells include differentiated cells as well as progenitor cells. Examples of differentiated cells include, but are not limited to, cells from a tissue selected from bone marrow, skin, skeletal muscle, fat tissue and peripheral blood. Exemplary cell types include, but are not limited to, fibroblasts, hepatocytes, myoblasts, neurons, osteoblasts, osteoclasts, and T-cells. The starting cells employed for generating the induced multipotent cells, the endodermal progenitor cells, and the hepatocytes can be non-pluripotent cells.

[0050] Differentiated cells include, but are not limited to, multipotent cells, oligopotent cells, unipotent cells, progenitor cells, and terminally differentiated cells. In particular embodiments, a less potent cell is considered “differentiated” in reference to a more potent cell.

[0051] A “somatic cell” is a cell forming the body of an organism. Somatic cells include cells making up organs, skin, blood, bones and connective tissue in an organism, but not germ cells.

[0052] Cells can be from, for example, human or non-human mammals. Exemplary non-human mammals include, but are not limited to, mice, rats, cats, dogs, rabbits, guinea pigs, hamsters, sheep, pigs, horses, bovines, and non-human primates. In some embodiments, a cell is from an adult human or non-human mammal. In some embodiments, a cell is from a neonatal human, an adult human, or non-human mammal.

[0053] As used herein, the terms “subject” or “patient” refers to any animal, such as a domesticated animal, a zoo animal, or a human. The “subject” or “patient” can be a mammal like a dog, cat, bird, livestock, or a human. Specific examples of “subjects” and “patients” include, but are not limited to, individuals (particularly human) with a disease or disorder related to the liver, heart, lung, kidney, pancreas, brain, neural tissue, blood, bone, bone marrow, and the like.

[0054] Mammalian cells can be from humans or non-human mammals. Exemplary non-human mammals include, but are not limited to, mice, rats, cats, dogs, rabbits, guinea pigs, hamsters, sheep, pigs, horses, bovines, and non-human primates (e.g., chimpanzees, macaques, and apes).

[0055] By “hypoimmunogenic pluripotent” cell or “HIP” cell herein is meant a pluripotent cell that retains its pluripotent characteristics and yet gives rise to a reduced immunological rejection response when transferred into an allogeneic host. In preferred embodiments, HIP cells do not give rise to an immune response. Thus, “hypoimmunogenic” refers to a significantly reduced or eliminated immune response when compared to the immune response of an unmodified or wild type (*i.e.* “wt”) pluripotent cell, *i.e.*, a cell prior to immunoengineering as outlined herein. In many cases, the HIP cells are immunologically silent and yet retain pluripotent capabilities. Assays for HIP characteristics are outlined below.

[0056] By “hypoimmunogenic pluripotent cell O-” “hypoimmunogenic pluripotent ORh-” cell or “HIPO-” cell herein is meant a HIP cell that is also ABO blood group O and Rhesus Factor Rh-. HIPO-cells may have been generated from O- cells, enzymatically modified to be O-, or genetically engineered to be O-.

[0057] By “HLA” or “human leukocyte antigen” complex herein is meant a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins that make up the HLA complex are responsible for the regulation of the immune response to antigens. In humans, there are two MHCs, class I and class II, “HLA-I” and “HLA-II”. HLA-I includes three proteins, HLA-A, HLA-B and HLA-C, which present peptides from the inside of the cell, and antigens presented by the HLA-I complex attract killer T-cells (also known as CD8+ T-cells or cytotoxic T cells). The HLA-I proteins are associated with β -2 microglobulin (β 2M). HLA-II includes five proteins, HLA-DP, HLA-DM, HLA-DOB, HLA-DQ and HLA-DR, which present antigens from outside the cell to T lymphocytes. This stimulates CD4+ cells (also known as T-helper cells). It should be understood that the use of either “MHC” or “HLA” is not meant to be limiting. Thus, as it relates to mammalian cells, these terms may be used interchangeably herein.

[0058] By “gene knock out” herein is meant a process that renders a particular gene inactive in the host cell in which it resides, resulting either in no protein of interest being produced or an inactive form. As will be appreciated by those in the art and further described below, this can be accomplished in a number of different ways, including removing nucleic acid sequences from a gene, or interrupting the sequence with other sequences, altering the reading frame, or altering the regulatory components of the nucleic acid. For example, all or part of a coding region of the gene of interest can be removed or replaced with “nonsense” sequences, all or part of a regulatory sequence such as a promoter can be removed or replaced, translation initiation sequences can be removed or replaced, etc.

[0059] By “gene knock in” herein is meant a process that adds a genetic function to a host cell. This causes increased levels of the encoded protein. As will be appreciated by those in the art, this can be accomplished in several ways, including adding one or more additional copies of the gene to the host cell or altering a regulatory component of the endogenous gene increasing expression of the protein is made. This may be accomplished by modifying the promoter, adding a different promoter, adding an enhancer, or modifying other gene expression sequences.

[0060] “ β -2 microglobulin” or “ β 2M” or “B2M” protein refers to the human β 2M protein that has the amino acid and nucleic acid sequences shown below; the human gene has accession number NC_000015.10:44711487-44718159.

[0061] “CD47 protein” protein refers to the human CD47 protein that has the amino acid and nucleic acid sequences shown below; the human gene has accession number NC_000016.10:10866208-10941562.

[0062] "CIITA protein" protein refers to the human CIITA protein that has the amino acid and nucleic acid sequences shown below; the human gene has accession number NC_000003.12:108043094-108094200.

[0063] By "wild type" in the context of a cell herein is meant a cell found in nature. However, in the context of a pluripotent stem cell, as used herein, it also means an iPSC that may contain nucleic acid changes resulting in pluripotency but did not undergo the gene editing procedures of the invention to achieve hypoinmunogenicity.

[0064] By "syngeneic" herein is meant the genetic similarity or identity of a host organism and a cellular transplant where there is immunological compatibility; *e.g.* no immune response is generated.

[0065] By "allogeneic" herein is meant the genetic dissimilarity of a host organism and a cellular transplant where an immune response is generated.

[0066] By "B2M-/-" herein is meant a diploid cell that has had the B2M gene inactivated in both chromosomes. As described herein, this can be done in a variety of ways.

[0067] By "CIITA -/-" herein is meant a diploid cell that has had the CIITA gene inactivated in both chromosomes. As described herein, this can be done in a variety of ways.

[0068] By "CD47 tg" (standing for "transgene") or "CD47+" herein is meant that the host cell expresses CD47 in some cases by having at least one additional copy of the CD47 gene.

[0069] An "Oct polypeptide" refers to any of the naturally-occurring members of the Octamer family of transcription factors, or variants thereof. They maintain a similar transcription factor activity within at least 50%, 80%, or 90% when compared to the closest related naturally occurring family member. An Oct polypeptide may also comprise at least the DNA-binding domain of the naturally occurring family member and may further comprise a transcriptional activation domain. Exemplary Oct polypeptides include Oct-1, Oct-2, Oct-3/4, Oct-6, Oct-7, Oct-8, Oct-9, and Oct-11. Oct3/4 (referred to herein as "Oct4") and contain a POU domain, a 150 amino acid sequence conserved among Pit-1, Oct-1, Oct-2, and uric-86. (See, Ryan, A. K. & Rosenfeld, M. G., *Genes Dev.* 11:1207-1225 (1997), incorporated herein by reference in its entirety.) In some embodiments, variants have at least 85%, 90%, or 95% amino acid sequence identity across their whole sequence compared to a naturally occurring Oct polypeptide family member such as those listed above or in Genbank accession number NP-002692.2 (human Oct4) or NP-038661.1 (mouse Oct4). Oct

polypeptides (e.g., Oct3/4 or Oct 4) can be from human, mouse, rat, bovine, porcine, or other animals. In some embodiments, the same species of protein will be used with the species of cells being manipulated. The Oct polypeptide(s) can be a pluripotency factor that can help induce multipotency in non-pluripotent cells.

[0070] A "Klf polypeptide" refers to any of the naturally-occurring members of the family of Krüppel-like factors (Klfs), zinc-finger proteins that contain amino acid sequences similar to those of the *Drosophila* embryonic pattern regulator Krüppel, or variants of the naturally-occurring members that maintain transcription factor activity similar (within at least 50%, 80%, or 90% activity) compared to the closest related naturally occurring family member, or polypeptides comprising at least the DNA-binding domain of the naturally occurring family member, and can further comprise a transcriptional activation domain. (See, Dang, D. T., Pevsner, J. & Yang, V. W., *Cell Biol.* 32:1103-1121 (2000), incorporated by reference herein in its entirety.) Exemplary Klf family members including, Klf1, Klf2, Klf3, Klf-4, Klf5, Klf6, Klf7, Klf8, Klf9, Klf10, Klf11, Klf12, Klf13, Klf14, Klf15, Klf16, and Klf17. Klf2 and Klf-4 were found to be factors capable of generating iPS cells in mice, and related genes Klf1 and Klf5 did as well, although with reduced efficiency. (See, Nakagawa, et al., *Nature Biotechnology* 26:101-106 (2007), incorporated by reference herein in its entirety.) In some embodiments, variants have at least 85%, 90%, or 95% amino acid sequence identity across their whole sequence compared to a naturally occurring Klf polypeptide family member such as to those listed above or such as listed in Genbank accession number CAX16088 (mouse Klf4) or CAX14962 (human Klf4). Klf polypeptides (e.g., Klf1, Klf4, and Klf5) can be from human, mouse, rat, bovine, porcine, or other animals. Generally, the same species of protein will be used with the species of cells being manipulated. The Klf polypeptide(s) can be a pluripotency factor. The expression of the Klf4 gene or polypeptide can help induce multipotency in a starting cell or a population of starting cells.

[0071] A "Myc polypeptide" refers to any of the naturally-occurring members of the Myc family. (See, e.g., Adhikary, S. & Eilers, M., *Nat. Rev. Mol. Cell Biol.* 6:635-645 (2005), incorporated by reference herein in its entirety.) It also includes variants that maintain similar transcription factor activity when compared to the closest related naturally occurring family member (i.e. within at least 50%, 80%, or 90% activity). It further includes polypeptides comprising at least the DNA-binding domain of a naturally occurring family member, and can further comprise a transcriptional activation domain. Exemplary Myc polypeptides include, e.g., c-Myc, N-Myc and L-Myc. In some embodiments, variants have at least 85%, 90%, or 95% amino acid sequence identity across their whole sequence compared to a naturally occurring Myc polypeptide family member, such as to those listed above or

such as listed in Genbank accession number CAA25015 (human Myc). Myc polypeptides (*e.g.*, c-Myc) can be from human, mouse, rat, bovine, porcine, or other animals. Generally, the same species of protein will be used with the species of cells being manipulated. The Myc polypeptide(s) can be a pluripotency factor.

[0072] A "Sox polypeptide" refers to any of the naturally-occurring members of the SRY-related HMG-box (Sox) transcription factors, characterized by the presence of the high-mobility group (HMG) domain, or variants thereof that maintain similar transcription factor activity when compared to the closest related naturally occurring family member (*i.e.* within at least 50%, 80%, or 90% activity). It also includes polypeptides comprising at least the DNA-binding domain of the naturally occurring family member and can further comprise a transcriptional activation domain. (*See, e.g.*, Dang, D. T. et al., *Int. J. Biochem. Cell Biol.* 32:1103-1121 (2000), incorporated by reference herein in its entirety.) Exemplary Sox polypeptides include, *e.g.*, Sox1, Sox-2, Sox3, Sox4, Sox5, Sox6, Sox7, Sox8, Sox9, Sox10, Sox11, Sox12, Sox13, Sox14, Sox15, Sox17, Sox18, Sox-21, and Sox30. Sox1 has been shown to yield iPS cells with a similar efficiency as Sox2, and genes Sox3, Sox15, and Sox18 have also been shown to generate iPS cells, although with somewhat less efficiency than Sox2. (*See, Nakagawa, et al., Nature Biotechnology* 26:101-106 (2007), incorporated by reference herein in its entirety.) In some embodiments, variants have at least 85%, 90%, or 95% amino acid sequence identity across their whole sequence compared to a naturally occurring Sox polypeptide family member such as to those listed above or such as listed in Genbank accession number CAA83435 (human Sox2). Sox polypeptides (*e.g.*, Sox1, Sox2, Sox3, Sox15, or Sox18) can be from human, mouse, rat, bovine, porcine, or other animals. Generally, the same species of protein will be used with the species of cells being manipulated. The Sox polypeptide(s) can be a pluripotency factor. As discussed herein, SOX2 proteins find particular use in the generation of iPSCs.

[0073] By "differentiated ABO- cell" *e.g.*, differentiated PSCO- or HIPO cells herein is meant pluripotent cells that are blood group O, Rh factor – and may have been engineered to possess hypoinmunogenicity (*e.g.* by the knock out of B2M and CIITA and the knock in of CD47) and then are differentiated into a cell type for ultimate transplantation into subjects. Thus, for example HIPO-cells can be differentiated into hepatocytes ("dHIPO- hepatocytes"), into beta-like pancreatic cells or islet organoids ("dHIPO- beta cells"), into endothelial cells ("dHIPO- endothelial cells"), etc.

[0074] The term percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum

correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0075] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., *infra*).

[0076] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).

[0077] "Inhibitors," "activators," and "modulators" affect a function or expression of a biologically-relevant molecule. The term "modulator" includes both inhibitors and activators. They may be identified using *in vitro* and *in vivo* assays for expression or activity of a target molecule.

[0078] "Inhibitors" are agents that, e.g., inhibit expression or bind to target molecules or proteins. They may partially or totally block stimulation or have protease inhibitor activity. They may reduce, decrease, prevent, or delay activation, including inactivation, desensitization, or down regulation of the activity of the described target protein. Modulators may be antagonists of the target molecule or protein.

[0079] "Activators" are agents that, *e.g.*, induce or activate the function or expression of a target molecule or protein. They may bind to, stimulate, increase, open, activate, or facilitate the target molecule activity. Activators may be agonists of the target molecule or protein.

[0080] "Homologs" are bioactive molecules that are similar to a reference molecule at the nucleotide sequence, peptide sequence, functional, or structural level. Homologs may include sequence derivatives that share a certain percent identity with the reference sequence. Thus, in one embodiment, homologous or derivative sequences share at least a 70 percent sequence identity. In a specific embodiment, homologous or derivative sequences share at least an 80 or 85 percent sequence identity. In a specific embodiment, homologous or derivative sequences share at least a 90 percent sequence identity. In a specific embodiment, homologous or derivative sequences share at least a 95 percent sequence identity. In a more specific embodiment, homologous or derivative sequences share at least an 50, 55, 60, 65, 70, 75, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence identity. Homologous or derivative nucleic acid sequences may also be defined by their ability to remain bound to a reference nucleic acid sequence under high stringency hybridization conditions. Homologs having a structural or functional similarity to a reference molecule may be chemical derivatives of the reference molecule. Methods of detecting, generating, and screening for structural and functional homologs as well as derivatives are known in the art.

[0081] "Hybridization" generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al*, Current Protocols in Molecular Biology, Wiley Interscience Publishers (1995), incorporated by reference herein in its entirety.

[0082] "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures.

[0083] "Stringent conditions" or "high stringency conditions", as defined herein, can be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during

hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 Mm sodium phosphate buffer at Ph 6.5 with 750 Mm sodium chloride, 75 Mm sodium citrate at 42°C; or (3) overnight hybridization in a solution that employs 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 Mm sodium phosphate (Ph 6.8), 0.1 % sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µl/m1), 0.1% SDS, and 10% dextran sulfate at 42°C, with a 10 minute wash at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) followed by a 10 minute high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

[0084] It is intended that every maximum numerical limitation given throughout this specification includes every lower numerical limitation, as if such lower numerical limitations were expressly written herein. Every minimum numerical limitation given throughout this specification will include every higher numerical limitation, as if such higher numerical limitations were expressly written herein. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0085] As used herein the term “modification” refers to an alteration that physically differentiates the modified molecule from the parent molecule. In one embodiment, an amino acid change in a CD47, HSVtk, EC-CD, or iCasp9 variant polypeptide prepared according to the methods described herein differentiates it from the corresponding parent that has not been modified according to the methods described herein, such as wild-type proteins, a naturally occurring mutant proteins or another engineered protein that does not include the modifications of such variant polypeptide. In another embodiment, a variant polypeptide includes one or more modifications that differentiates the function of the variant polypeptide from the unmodified polypeptide. For example, an amino acid change in a variant polypeptide affects its receptor binding profile. In other embodiments, a variant polypeptide comprises substitution, deletion, or insertion modifications, or combinations thereof. In another embodiment, a variant polypeptide includes one or more modifications that increases its affinity for a receptor compared to the affinity of the unmodified polypeptide.

[0086] In one embodiment, a variant polypeptide includes one or more substitutions, insertions, or deletions relative to a corresponding native or parent sequence. In certain embodiments, a variant polypeptide includes 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, 28, 29, 30, 31 to 40, 41 to 50, or 51 or more modifications.

[0087] The term “about” is used herein to mean approximately, in the region of, roughly, or around. When the term “about” is used in conjunction with a numerical value/range, it modifies that value/range by extending the boundaries above and below the numerical value(s) set forth. In general, the term “about” is used herein to modify a numerical value(s) above and below the stated value(s) by a variance of 20%.

[0088] “Modulated,” as used herein with respect to protein expression, means that protein expression level is increased or decreased relative to the corresponding wild type level for that protein, e.g., the expression of a protein in a modified pluripotent cell is modulated if it is increased or decreased relative to the protein expression level of the unmodified (wild type) pluripotent cell.

[0089] By “episomal vector” herein is meant a genetic vector that can exist and replicate autonomously in the cytoplasm of a cell; e.g. it is not integrated into the genomic DNA of the host cell. A number of episomal vectors are known in the art and described below.

[0090] As used herein, the terms “evade rejection,” “escape rejection,” “avoid rejection,” and similar terms are used interchangeably to refer to genetically or otherwise modified cells according to the invention that are less susceptible to rejection when transplanted into a subject when compared with corresponding cells that are not genetically modified according to the invention. In some embodiments, the genetically modified cells according to the invention are less susceptible to rejection when transplanted into a subject when compared with corresponding cells that are ABO blood group or Rh factor mismatched to the subject.

VII. Cells of the Invention

[0091] The invention provides compositions and methodologies for generating blood type O- pluripotent (PSCO-) cells. In some aspects of the invention, the cells will be O- induced pluripotent stem (iPSCO-) cells, O- embryonic stem (ESCO-) cells, hypoinmunogenic pluripotent O- (HIPO-) cells, or cells derived or differentiated therefrom. In other aspects, the unmodified cell type is O-. In other aspects, the cells are modified enzymatically or genetically to the O- Rh- blood type.

A. Methodologies for Genetic Alterations

[0092] The invention includes methods of modifying nucleic acid sequences within cells or in cell-free conditions to generate both pluripotent cells and HIP cells. Exemplary technologies include homologous recombination, knock-in, ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases), CRISPR/Cas9, and other site-specific nuclease technologies. These techniques enable double-strand DNA breaks at desired locus sites. These controlled double-strand

breaks promote homologous recombination at the specific locus sites. This process focuses on targeting specific sequences of nucleic acid molecules, such as chromosomes, with endonucleases that recognize and bind to the sequences and induce a double-stranded break in the nucleic acid molecule. The double-strand break is repaired either by an error-prone non-homologous end-joining (NHEJ) or by homologous recombination (HR).

[0093] As will be appreciated by those in the art, a number of different techniques can be used to engineer the pluripotent cells of the invention, as well as the engineering of the PSCs or iPSCs to become blood type O, Rh negative and optionally hypo-immunogenic as outlined herein.

[0094] In general, these techniques can be used individually or in combination. For example, in the generation of the HIP cells, CRISPR may be used to reduce the expression of active B2M and/or CIITA protein in the engineered cells, with viral techniques (e.g. lentivirus) to knock in the CD47 functionality. Also, as will be appreciated by those in the art, although one embodiment sequentially utilizes a CRISPR step to knock out B2M, followed by a CRISPR step to knock out CIITA with a final step of a lentivirus to knock in the CD47 functionality, these genes can be manipulated in different orders using different technologies.

[0095] As is discussed more fully below, transient expression of reprogramming genes is generally done to generate/induce pluripotent stem cells.

a. CRISPR Technologies

[0096] In one embodiment, the cells are manipulated using CRISPR/Cas technologies as is known in the art. CRISPR can be used to generate the starting PSCO-, iPSCO-, or ESCO- or to generate the HIPO- cells from the PSCO-s, iPSCO-s or ESCO-s. There are a large number of techniques based on CRISPR, see for example Doudna and Charpentier, Science doi:10.1126/science.1258096, hereby incorporated by reference. CRISPR techniques and kits are sold commercially.

b. TALEN Technologies

[0097] In some embodiments, the HIP cells of the invention are made using Transcription Activator-Like Effector Nucleases (TALEN) methodologies. TALEN are restriction enzymes combined with a nuclease that can be engineered to bind to and cut practically any desired DNA sequence. TALEN kits are sold commercially.

c. Zinc Finger Technologies

[0098] In one embodiment, the cells are manipulated using Zn finger nuclease technologies. Zn finger nucleases are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes. By taking advantage of endogenous DNA repair machinery, these reagents can be used to precisely alter the genomes of higher organisms, similar to CRISPR and TALENs.

d. Viral Based Technologies

[0099] There are a wide variety of viral techniques that can be used to generate the HIP cells of the invention (as well as for the original generation of the iPSCs), including, but not limited to, the use of retroviral vectors, lentiviral vectors, adenovirus vectors and Sendai viral vectors. Episomal vectors used in the generation of iPSCs are described below.

e. Down regulation of genes using interfering RNA

[00100] In other embodiments, genes that encode proteins used in HLA molecules are downregulated by RNAi technologies. RNA interference (RNAi) is a process where RNA molecules inhibit gene expression often by causing specific mRNA molecules to degrade. Two types of RNA molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference. They bind to the target mRNA molecules and either increase or decrease their activity. RNAi helps cells defend against parasitic nucleic acids such as those from viruses and transposons. RNAi also influences development.

[00101] sdRNA molecules are a class of asymmetric siRNAs comprising a guide (antisense) strand of 19-21 bases. They contain a 5' phosphate, 2'Ome or 2'F modified pyrimidines, and six phosphotioates at the 3' positions. They also contain a sense strand containing 3' conjugated sterol moieties, 2 phosphotioates at the 3' position, and 2'Ome modified pyrimidines. Both strands contain 2' Ome purines with continuous stretches of unmodified purines not exceeding a length of 3. sdRNA is disclosed in U.S. Patent No. 8,796,443, incorporated herein by reference in its entirety.

[00102] For all of these technologies, well known recombinant techniques are used, to generate recombinant nucleic acids as outlined herein. In certain embodiments, the recombinant nucleic acids (either encoding a desired polypeptide, *e.g.* CD47, or disruption sequences) may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate for the host cell and subject to be treated. Numerous types of appropriate expression vectors and suitable regulatory sequences are

known in the art for a variety of host cells. Typically, the one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are also contemplated. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome. In a specific embodiment, the expression vector includes a selectable marker gene to allow the selection of transformed host cells. Certain embodiments include an expression vector comprising a nucleotide sequence encoding a variant polypeptide operably linked to at least one regulatory sequence. Regulatory sequences for use herein include promoters, enhancers, and other expression control elements. In certain embodiments, an expression vector is designed for the choice of the host cell to be transformed, the particular variant polypeptide desired to be expressed, the vector's copy number, the ability to control that copy number, or the expression of any other protein encoded by the vector, such as antibiotic markers.

[00103] Examples of suitable mammalian promoters include, for example, promoters from the following genes: ubiquitin/S27a promoter of the hamster (WO 97/15664), Simian vacuolating virus 40 (SV40) early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, the long terminal repeat region of Rous Sarcoma Virus (RSV), mouse mammary tumor virus promoter (MMTV), Moloney murine leukemia virus Long Terminal repeat region, and the early promoter of human Cytomegalovirus (CMV). Examples of other heterologous mammalian promoters are the actin, immunoglobulin or heat shock promoter(s).

[00104] In additional embodiments, promoters for use in mammalian host cells can be obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 Jul. 1989), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40). In further embodiments, heterologous mammalian promoters are used. Examples include the actin promoter, an immunoglobulin promoter, and heat-shock promoters. The early and late promoters of SV40 are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication. Fiers et al., Nature 273: 113-120 (1978). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway, P. J. et al., Gene 18: 355-360 (1982). The foregoing references are incorporated by reference in their entirety.

B. Generation of Pluripotent Cells

[00105] The invention provides methods of producing non-immunogenic pluripotent cells from pluripotent cells. Thus, the first step is to provide the pluripotent stem cells.

[00106] The generation of mouse and human pluripotent stem cells (generally referred to as iPSCs; miPSCs for murine cells or hiPSCs for human cells) is generally known in the art. As will be appreciated by those in the art, there are a variety of different methods for the generation of iPSCs. The original induction was done from mouse embryonic or adult fibroblasts using the viral introduction of four transcription factors, Oct3/4, Sox2, c-Myc and Klf4; *see* Takahashi and Yamanaka *Cell* 126:663-676 (2006), hereby incorporated by reference in its entirety and specifically for the techniques outlined therein. Since then, a number of methods have been developed; *see* Seki *et al.*, *World J. Stem Cells* 7(1):116-125 (2015) for a review, and Lakshmipathy and Vermuri, editors, *Methods in Molecular Biology: Pluripotent Stem Cells, Methods and Protocols*, Springer 2013, both of which are hereby expressly incorporated by reference in their entirety, and in particular for the methods for generating hiPSCs (see for example Chapter 3 of the latter reference).

[00107] Generally, iPSCs are generated by the transient expression of one or more “reprogramming factors” in the host cell, usually introduced using episomal vectors. Under these conditions, small amounts of the cells are induced to become iPSCs (in general, the efficiency of this step is low, as no selection markers are used). Once the cells are “reprogrammed”, and become pluripotent, they lose the episomal vector(s) and produce the factors using the endogenous genes. This loss of the episomal vector(s) results in cells that are called “zero footprint” cells. This is desirable as the fewer genetic modifications (particularly in the genome of the host cell), the better. Thus, it is preferred that the resulting hiPSCs have no permanent genetic modifications.

[00108] As is also appreciated by those of skill in the art, the number of reprogramming factors that can be used or are used can vary. Commonly, when fewer reprogramming factors are used, the efficiency of the transformation of the cells to a pluripotent state goes down, as well as the “pluripotency”, *e.g.* fewer reprogramming factors may result in cells that are not fully pluripotent but may only be able to differentiate into fewer cell types.

[00109] In some embodiments, a single reprogramming factor, OCT4, is used. In other embodiments, two reprogramming factors, OCT4 and KLF4, are used. In other embodiments, three reprogramming factors, OCT4, KLF4 and SOX2, are used. In other embodiments, four reprogramming factors, OCT4, KLF4, SOX2 and c-Myc, are used. In other embodiments, 5, 6 or 7 reprogramming

factors can be used selected from SOKMNL; SOX2, OCT4 (POU5F1), KLF4, MYC, NANOG, LIN28, and SV40L T antigen.

[00110] In general, these reprogramming factor genes are provided on episomal vectors such as are known in the art and commercially available. For example, ThermoFisher/Invitrogen sell a sendai virus reprogramming kit for zero footprint generation of hiPSCs, see catalog number A34546. ThermoFisher also sells EBNA-based systems as well, see catalog number A14703.

[00111] In addition, there are a number of commercially available hiPSC lines; *see, e.g.*, the Gibco® Episomal hiPSC line, K18945, which is a zero footprint, viral-integration-free human iPSC cell line (*see also* Burridge et al, 2011, *supra*).

[00112] In general, as is known in the art, iPSCs are made from non-pluripotent cells such as CD34+ cord blood cells, fibroblasts, *etc.*, by transiently expressing the reprogramming factors as described herein.

[00113] For example, successful iPSCs were also generated using only Oct3/4, Sox2 and Klf4, while omitting the C-Myc, although with reduced reprogramming efficiency.

[00114] In general, iPSCs are characterized by the expression of certain factors that include KLF4, Nanog, OCT4, SOX2, ESRRB, TBX3, c-Myc and TCL1. New or increased expression of these factors for purposes of the invention may be via induction or modulation of an endogenous locus or from expression from a transgene.

[00115] For example, murine iPSCs can be generated using the methods of Diecke *et al*, *Sci Rep*. 2015, Jan. 28;5:8081 (doi:10.1038/srep08081), hereby incorporated by reference in its entirety and specifically for the methods and reagents for the generation of the miPSCs. *See also, e.g.*, Burridge *et al.*, *PLoS One*, 2011 6(4):18293, hereby incorporated by reference in its entirety and specifically for the methods outlined therein.

[00116] In some cases, the pluripotency of the cells is measured or confirmed as outlined herein, for example by assaying for generally accepted markers to indicate pluripotency such as, *e.g.*, Nanog, Oct 4, Sox2, Esrrb, Tbx3, and Tc11. Alternatively, the pluripotency of the cells can be measured or confirmed by conducting differentiation reactions as outlined in the Examples.

C. Generation of Pluripotent O- Cells

[00117] In some aspects of the invention, the iPSC cells generated as discussed above will already be blood type O, Rh factor negative cells because the process will have started with pluripotent cells having an O- blood type.

[00118] In other embodiments of the invention, the iPSCs can be made blood type O by enzymatic conversion of A and B antigens. In preferred aspects, the B antigen is converted to O using an enzyme. In more preferred aspects, the enzyme is an α -galactosidase. This enzyme eliminates the terminal galactose residue of the B antigen. Other aspects of the invention involve the enzymatic conversion of A antigen to O. In preferred aspects, the A antigen is converted to O using an α -N-acetylgalactosaminidase. Enzymatic conversion is discussed, *e.g.*, in Olsson et al., *Transfusion Clinique et Biologique* 11:33–39 (2004); U.S. Pat. Nos. 4,427,777, 5,606,042, 5,633,130, 5,731,426, 6,184,017, 4, 609,627, and 5,606,042; and Int'l Pub. No. WO9923210, each of which are incorporated by reference herein in their entirety.

[00119] Other embodiments of the invention involve genetically modifying iPSCs to blood type O by engineering the cells to knock out Exon 7 of the ABO gene (NCBI Gene ID: 80908). Any knockout methodology known in the art or described herein, such as CRISPR, TALENs, Zn fingers, or homologous recombination, may be employed.

[00120] Other embodiments of the invention involve rendering the blood type O pluripotent cell Rh factor negative by knocking out the C and/or E antigens of the Rh blood group system (RH), K in the Kell system (KEL), Fya and Fy3 in the Duffy system (FY), Jkb in the Kidd system (JK), or U and/or S in the MNS blood group system. Alternatively, the cells of the invention may be made Rh negative by or silencing the SLC14A1 (JK) gene (NCBI Gene ID: 6563). Any knockout methodology known in the art or described herein, such as CRISPR, TALENs, Zn Fingers, or homologous recombination, may be employed.

D. Generation of Hypoimmunogenic Pluripotent (HIP) Cells

[00121] Generating HIP cells from pluripotent cells or HIPO- cells from PSCO-, iPS- cells is done with as few as three genetic changes, resulting in minimal disruption of cellular activity but conferring immunosilencing to the cells.

[00122] The first two genetic changes involve a reduction or elimination in the protein activity of MHC I and II (HLA I and II when the cells are human). This can be done by altering genes encoding their component. In one embodiment, the coding region or regulatory sequences of the gene are disrupted using CRISPR. In another embodiment, gene translation is reduced using

interfering RNA technologies. The third change involves increasing expression of a gene that regulates susceptibility to macrophage phagocytosis, such as CD47. This can be effected by using a “knock in” of a gene using viral or transgene technologies.

[00123] In some cases, where CRISPR is being used for the genetic modifications, hiPSC or hiPSCO- cells that contain a Cas9 construct that enable high efficiency editing of the cell line can be used; *see, e.g.*, the Human Episomal Cas9 iPSC cell line, A33124, from Life Technologies.

1. HLA-I Reduction

[00124] The HIPO- cells of the invention include a reduction in MHC I function (HLA I when the cells are derived from human cells).

[00125] As will be appreciated by those in the art, the reduction in function can be accomplished in a number of ways, including removing nucleic acid sequences from a gene, interrupting the sequence with other sequences, or altering the regulatory components of the nucleic acid. For example, all or part of a coding region of the gene of interest can be removed or replaced with “nonsense” sequences, frameshift mutations can be made, all or part of a regulatory sequence such as a promoter can be removed or replaced, translation initiation sequences can be removed or replaced, etc.

[00126] As will be appreciated by those in the art, the successful reduction of the MHC I function (HLA I when the cells are derived from human cells) in the pluripotent cells can be measured using techniques known in the art and as described below; for example, FACS techniques using labeled antibodies that bind the HLA complex; for example, using commercially available HLA-A,B, or C antibodies that bind to the the alpha chain of the human major histocompatibility HLA Class I antigens.

a. B2M Alteration

[00127] In one embodiment, the reduction in HLA-I activity is accomplished by reducing β -2 microglobulin expression. This may be achieved by disrupting the expression of the β -2 microglobulin gene in the pluripotent stem cell, the human sequence of which is disclosed herein. This alteration is generally referred to herein as a gene “knock out”, and in the HIP cells of the invention it is done on both alleles in the host cell. Generally, the techniques to do both disruptions are the same.

[00128] A particularly useful embodiment uses CRISPR technology to disrupt the gene. In some cases, CRISPR technology is used to introduce small deletions or insertions into the coding region of the gene such that no functional protein is produced. Often, the result of frameshift mutations result in the generation of stop codons and truncated, non-functional proteins are made.

[00129] Accordingly, a useful technique is to use CRISPR sequences designed to target the coding sequence of the B2M gene in mouse or the B2M gene in human. After gene editing, the transfected iPSC cultures are dissociated to single cells. Single cells are expanded to full-size colonies and tested for CRISPR edits by screening for the presence of aberrant sequences from the CRISPR cleavage site. Clones with deletions in both alleles are picked. Such clones do not express B2M as demonstrated by PCR and do not express HLA-I as demonstrated by FACS analysis (see examples 1 and 6, for example).

[00130] Assays to test whether the B2M gene has been inactivated are known and described herein. In one embodiment, the assay is a Western blot of cell lysates probed with antibodies to the B2M protein. In another embodiment, reverse transcriptase polymerase chain reactions (rt-PCR) confirms the presence of the inactivating alteration.

[00131] In addition, the cells can be tested to confirm that the HLA I complex is not expressed on the cell surface. This may be assayed by FACS analysis using antibodies to one or more HLA cell surface components as discussed above.

2. HLA-II Reduction

[00132] In addition to a reduction in HLA I, the HIPO- cells of the invention also lack MHC II function (HLA II when the cells are derived from human cells).

[00133] As will be appreciated by those in the art, the reduction in function can be accomplished in a number of ways, including removing nucleic acid sequences from a gene, adding nucleic acid sequences to a gene, disrupting the reading frame, interrupting the sequence with other sequences, or altering the regulatory components of the nucleic acid. In one embodiment, all or part of a coding region of the gene of interest can be removed or replaced with "nonsense" sequences. In another embodiment, regulatory sequences such as a promoter can be removed or replaced, translation initiation sequences can be removed or replaced, etc.

[00134] The successful reduction of the MHC II function (HLA II when the cells are derived from human cells) in the pluripotent cells or their derivatives can be measured using techniques

known in the art such as Western blotting using antibodies to the protein, FACS techniques, rt-PCR techniques, etc.

a. CIITA Alteration

[00135] In one embodiment, the reduction in HLA-II activity is done by disrupting the expression of the CIITA gene in the pluripotent stem cell, the human sequence of which is shown herein. This alteration is generally referred to herein as a gene “knock out”, and in the HIPO- cells of the invention it is done on both alleles in the host cell.

[00136] Assays to test whether the CIITA gene has been inactivated are known and described herein. In one embodiment, the assay is a Western blot of cell lysates probed with antibodies to the CIITA protein. In another embodiment, reverse transcriptase polymerase chain reactions (rt-PCR) confirms the presence of the inactivating alteration.

[00137] In addition, the cells can be tested to confirm that the HLA II complex is not expressed on the cell surface. Again, this assay is done as is known in the art and generally is done using either Western Blots or FACS analysis based on commercial antibodies that bind to human HLA Class II HLA-DR, DP and most DQ antigens as outlined below.

[00138] A particularly useful embodiment uses CRISPR technology to disrupt the CIITA gene. CRISPRs are designed to target the coding sequence of the CIITA gene, an essential transcription factor for all MHC II molecules. After gene editing, the transfected iPSC cultures are dissociated into single cells. They are expanded to full-size colonies and tested for successful CRISPR editing by screening for the presence of an aberrant sequence from the CRISPR cleavage site. Clones with deletions do not express CIITA as determined by PCR and do not express MHC II/ HLA-II as determined by FACS analysis.

3. Phagocytosis Reduction

[00139] In addition to the reduction of HLA I and II (or MHC I and II), generally using B2M and CIITA knock-outs, the HIPO- cells of the invention have a reduced susceptibility to macrophage phagocytosis and NK cell killing. The resulting HIPO- cells “escape” the immune macrophage and innate pathways due to one or more CD47 transgenes.

a. CD47 Increase

[00140] In some embodiments, reduced macrophage phagocytosis and NK cell killing susceptibility results from increased CD47 on the HIPO- cell surface. This is done in several ways as

will be appreciated by those in the art using “knock in” or transgenic technologies. In some cases, increased CD47 expression results from one or more CD47 transgenes.

[00141] Accordingly, in some embodiments, one or more copies of a CD47 gene is added to the HIPO- cells under control of an inducible or constitutive promoter, with the latter being preferred. In some embodiments, a lentiviral construct is employed as described herein or known in the art. CD47 genes may integrate into the genome of the host cell under the control of a suitable promoter as is known in the art.

[00142] The HIPO- cell lines can be generated from B2M^{-/-} CIITA^{-/-} iPSCs. Cells containing lentivirus vectors expressing CD47 may be selected using a Blasticidin marker. The CD47 gene sequence is synthesized and the DNA is cloned into the plasmid Lentivirus pLenti6/V5 with a blasticidin resistance (Thermo Fisher Scientific, Waltham, MA)

[00143] In some embodiments, the expression of the CD47 gene can be increased by altering the regulatory sequences of the endogenous CD47 gene, for example, by exchanging the endogenous promoter for a constitutive promoter or for a different inducible promoter. This can generally be done using known techniques such as CRISPR.

[00144] Once altered, the presence of sufficient CD47 expression can be assayed using known techniques such as those described in the Examples, such as Western blots, ELISA assays or FACS assays using anti-CD47 antibodies. In general, “sufficiency” in this context means an increase in the expression of CD47 on the HIPO- cell surface that silences NK cell killing. The natural expression levels on cells are too low to protect them from NK cell lysis once their MHC I is removed.

4. Other modifications

[00145] In some embodiments, the hypoimmunogenic cells are generated by editing the CCR5 and/or CXCR4 genes to reduce or eliminate CCR5 and/or CXCR4 protein surface expression, by using genome editing tools such as for example CRISPR or TALEN, as described in WO2016/073955, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein. In some embodiments, the hypoimmunogenic cells are generated by editing the CCR5 and/or CXCR4 genes in addition to the B2M gene to reduce or eliminate MHC class 1 surface expression as described in WO2016/073955, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein.

[00146] In some embodiments, the hypoimmunogenic cells are generated by editing the NLRC5, CIITA, and/or B2M genes to reduce or eliminate surface expression and/or activity of the

NLR5, CIITA, and/or B2M proteins, by use of genome editing tools such as for example CRISPR or TALEN, as described in WO2016/183041, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein. In some embodiments, the hypoinmunogenic pluripotent cells are generated by modulating expression of one or more tolerogenic factor genes such as HLA-A, HLA-B, HLA-C, HLA-E, HLA-G, PD-L1, CTLA-4-Ig, CD47, CI-inhibitor, and IL-35 as described in WO2016/183041, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein. In some embodiments, the hypoinmunogenic cells are generating by modulating expression of one or more genes such as RFX-5, RFXAP, RFXANK, NFY-A, NFY-B, NFY-C, and/or IRF-1 as described in WO2016/183041, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein. In additional embodiments, further modifications to the cells include modulating expression of one or more genes such as OX40, GITR, 4-1BB, CD28, B7-1, B7-2, ICOS, CD27, HVEM, SLAM, CD226, PD1, CTL4, LAG3, TIGIT, TIM3, CD160, BTLA, CD244, CD30, TLT, VISTA, B7-H3, PD-L2, LFA-1, CD2, CD58, ICAM-3, TCRA, TCRB, FOXP3, HELIOS, ST2, PCSK9, CCR5, and/or APOC3 as described in WO2016/183041, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein.

[00147] In some embodiments, the hypoinmunogenic cells are generated by the introduction of one or more transgenes such as PDL-1, HLA-G, CD47, CD200, FASLG, CLC21, MFGE8, and/or SERPIN B9, or a gene encoding a biologic that acts as an agonist of PDL-1, HLA-G, CD47, CD200, FASLG, CLC21, MFGE8, and/or SERPIN 9, as described in WO2018/227286, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein. In some embodiments, the hypoinmunogenic cell is generated by further introduction of one or more transgenes such as TGF β , CD73, CD39, LAG3, IL1R2, ACKR2, TNFRSF22, TNFRSF23, TNFRS10, DAD1, and/or IFN γ R1 d39, or a gene encoding a biologic that acts as an agonist of TGF β , CD73, CD39, LAG3, IL1R2, ACKR2, TNFRSF22, TNFRSF23, TNFRS10, DAD1, and/or IFN γ R1 d39, as described in WO2018/227286, incorporated by reference herein in its entirety, and specifically for the related techniques outlined therein.

5. Suicide Genes

[00148] In some embodiments, the invention provides HIPO- cells that comprise a "suicide gene" or "suicide switch". These are incorporated to function as a "safety switch" that can cause the death of the hypoinmunogenic pluripotent cells should they grow and divide in an undesired manner. The "suicide gene" ablation approach includes a suicide gene in a gene transfer vector

encoding a protein that results in cell killing only when activated by a specific compound. A suicide gene may encode an enzyme that selectively converts a nontoxic compound into highly toxic metabolites. The result is specifically eliminating cells expressing the enzyme. In some embodiments, the suicide gene is the herpesvirus thymidine kinase (HSV-tk) gene and the trigger is ganciclovir. In other embodiments, the suicide gene is the *Escherichia coli* cytosine deaminase (EC-CD) gene and the trigger is 5-fluorocytosine (5-FC) (Barese *et al.*, *Mol. Therap.* 20(10):1932-1943 (2012), Xu *et al.*, *Cell Res.* 8:73-8 (1998), both incorporated herein by reference in their entirety.)

[00149] In other embodiments, the suicide gene is an inducible Caspase protein. An inducible Caspase protein comprises at least a portion of a Caspase protein capable of inducing apoptosis. In one embodiment, the portion of the Caspase protein is exemplified in SEQ ID NO:6. In preferred embodiments, the inducible Caspase protein is iCasp9. It comprises the sequence of the human FK506-binding protein, FKBP12, with an F36V mutation, connected through a series of amino acids to the gene encoding human caspase 9. FKBP12-F36V binds with high affinity to a small-molecule dimerizing agent, AP1903. Thus, the suicide function of iCasp9 in the instant invention is triggered by the administration of a chemical inducer of dimerization (CID). In some embodiments, the CID is the small molecule drug AP1903. Dimerization causes the rapid induction of apoptosis. (See WO2011146862; Stasi *et al.*, *N. Engl. J. Med* 365;18 (2011); Tey *et al.*, *Biol. Blood Marrow Transplant.* 13:913-924 (2007), each of which are incorporated by reference herein in their entirety.)

6. Assays for HIPO- Phenotypes and Retention of Pluripotency

[00150] Once the HIPO- cells have been generated, they may be assayed for their hypoimmunogenicity and/or retention of pluripotency as is generally described herein and in WO 2018/132783, incorporated herein by reference.

[00151] For example, hypoimmunogenicity may be assayed using a number of techniques. These techniques include transplantation into allogeneic hosts and monitoring for HIPO- cell growth (*e.g.* teratomas) that escape the host immune system. HIPO- derivatives are transduced to express luciferase and can then be followed using bioluminescence imaging. Similarly, the T cell and/or B cell response of the host animal to the HIP cells can be tested to confirm that the HIP cells do not cause an immune reaction in the host animal. T cell function can be assessed by Elispot, Elisa, FACS, PCR, or mass cytometry (CYTOF). B cell response or antibody response can be assessed using FACS or luminex. Additionally or alternatively, the cells may be assayed for their ability to avoid innate immune responses, *e.g.* NK cell killing. K cell lytolytic activity can be assessed *in vitro* or *in vivo*.

[00152] Similarly, the retention of pluripotency can be tested in a number of ways. In one embodiment, pluripotency is assayed by the expression of certain pluripotency-specific factors as generally described herein and in WO 2018/0132783. Additionally or alternatively, the HIPO- cells may be differentiated into one or more cell types as an indication of pluripotency.

E. Embodiments of the Invention

[00153] The iPSCO-, ESCO-, and HIPO- cells, or derivatives thereof, of the invention may be used to treat, for example, Type 1 diabetes, cardiac diseases, neurological diseases, cancer, blindness, vascular diseases, and other diseases/disorders that respond to regenerative medicine therapies. In particular, the invention contemplates using the HIPO- cells for differentiation into any cell type. Thus, provided herein are iPSCO-, ESCO-, and HIPO- cells, or derivatives thereof, that exhibit pluripotency but do not result in a host immune response when transplanted into an allogeneic host such as a human patient, either as the iPSCO-, ESCO-, and HIPO- cells, or as the differentiated products of the those cells.

[00154] In one aspect, the present invention provides an isolated iPSCO-, ESCO-, and HIPO- cell, or derivative thereof, comprising a nucleic acid encoding a chimeric antigen receptor (CAR), wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated and CD47 expression has been increased. The CAR can comprise an extracellular domain, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the extracellular domain binds to an antigen selected from the group consisting of CD19, CD20, CD22, CD38, CD123, CS1, CD171, BCMA, MUC16, ROR1, and WT1. In certain embodiments, the extracellular domain comprises a single chain variable fragment (scFv). In some embodiments, the transmembrane domain comprises CD3 ζ , CD4, CD8 α , CD28, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, and BTLA. In certain embodiments, the intracellular signaling domain comprises CD3 ζ , CD28, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, and BTLA.

[00155] In certain embodiments, the CAR comprises an anti-CD19 scFv domain, a CD28 transmembrane domain, and a CD3 zeta signaling intracellular domain. In some embodiments, the CAR comprises an anti-CD19 scFv domain, a CD28 transmembrane domain, a 4-1BB signaling intracellular domain, and a CD3 zeta signaling intracellular domain.

[00156] In another aspect of the invention, provided is an isolated O- CAR-T (O-CAR-T) or hypimmune O- CAR-T (HIPO-CAR-T) cell produced by *in vitro* differentiation of any one of the

iPSCO-, ESCO-, and HIPO- cells, or derivatives thereof, described herein. In some embodiments, the HIPO-CAR-T cell is a cytotoxic HIPO- CAR-T cell.

[00157] In various embodiments, the *in vitro* differentiation comprises culturing the iPSCO-, ESCO-, and HIPO- cell, or derivative thereof, carrying a CAR construct in a culture media comprising one or more growth factors or cytokines selected from the group consisting of bFGF, EPO, Flt3L, IGF, IL-3, IL-6, IL-15, GM-CSF, SCF, and VEGF. In some embodiments, the culture media further comprises one or more growth factors or cytokines selected from the group consisting of a BMP activator, a GSK3 inhibitor, a ROCK inhibitor, a TGF β receptor/ALK inhibitor, and a NOTCH activator.

[00158] In particular embodiments, the isolated O-CAR-T or HIPO-CAR-T cells produced by *in vitro* differentiation of any one of the iPSCO-, ESCO-, and HIPO- cell- carrying the CAR-T constructs may be used in the treatment of cancer.

[00159] In another aspect of the invention, provided is a method of treating a patient with cancer by administering a composition comprising a therapeutically effective amount of any of the isolated O-CAR-T or HIPO-CAR-T cells described herein. In some embodiments, the composition further comprises a therapeutically effective carrier.

[00160] In some embodiments, the administration step comprises intravenous administration, subcutaneous administration, intranodal administration, intratumoral administration, intrathecal administration, intrapleural administration, and intraperitoneal administration. In certain instances, the administration further comprises a bolus or by continuous perfusion.

[00161] In some embodiments, the cancer is a blood cancer selected from the group consisting of leukemia, lymphoma, and myeloma. In various embodiments, the cancer is a solid tumor cancer or a liquid tumor cancer.

[00162] In another aspect, the present invention provides a method of making any one of the isolated O-CAR-T or HIPO-CAR-T cells described herein. The method includes *in vitro* differentiating of any one of the iPSCO-, ESCO-, or HIPO- cells of the invention wherein *in vitro* differentiating comprises culturing the iPSCO-, ESCO-, or HIPO- cell in a culture media comprising one or more growth factors or cytokines selected from the group consisting of bFGF, EPO, Flt3L, IGF, IL-2, IL-3, IL-6, IL-7, IL-15, GM-CSF, SCF, and VEGF. In some embodiments, the culture media further comprises one or more growth factors or cytokines selected from the group consisting of a BMP activator, a GSK3 inhibitor, a ROCK inhibitor, a TGF β receptor/ALK inhibitor, and a NOTCH activator.

[00163] In some embodiments, the *in vitro* differentiating comprises culturing the iPSCO-, ESCO-, or HIPO-cells on feeder cells. In various embodiments, the *in vitro* differentiating comprises culturing in simulated microgravity. In certain instances, the culturing in simulated microgravity is for at least 72 hours.

[00164] In some aspects, provided herein is an isolated, engineered hypimmune cardiac cell (hypimmunogenic cardiac cell), for example a cardiomyocyte, differentiated from an iPSCO-, ESCO-, or HIPO- cell.

[00165] Cardiomyocytes were previously thought to lack ABO blood group antigens. Differentiation of an ABO blood group type B human embryonic stem cell line into cardiomyocyte-like cells was observed to result in the loss of the B antigen, suggesting that loss of these antigens may occur early during human embryogenesis. *See, e.g., Mölne et al., Transplantation*. 86(10):1407-13 (2008), incorporated by reference herein in its entirety. Other studies also reported that differentiation of induced human pluripotent stem cells into cardiomyocyte-like cells caused the progressive loss of the ABO blood group type A antigen in these cells. *See, e.g., Säljö et al., Scientific Reports*. 13072: 1-14 (2017). Surprisingly, however, the inventors determined that cardiomyocytes express ABO blood group antigens that can cause rejection of such cells to an unmatched recipient.

[00166] Accordingly, in some aspects, provided herein is a method of treating a patient suffering from a heart condition or disease. The method comprises administering a composition comprising a therapeutically effective amount of a population of any one of the isolated, engineered O- or hypimmune O- cardiac cells derived from iPSCO-, ESCO-, or HIPO- cells as described herein. In some embodiments, the composition further comprises a therapeutically effective carrier.

[00167] In some embodiments, the administration comprises implantation into the patient's heart tissue, intravenous injection, intraarterial injection, intracoronary injection, intramuscular injection, intraperitoneal injection, intramyocardial injection, trans-endocardial injection, trans-epicardial injection, or infusion.

[00168] In some embodiments, the heart condition or disease is selected from the group consisting of pediatric cardiomyopathy, age-related cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, chronic ischemic cardiomyopathy, peripartum cardiomyopathy, inflammatory cardiomyopathy, other cardiomyopathy, myocarditis, myocardial ischemic reperfusion injury, ventricular dysfunction, heart failure, congestive heart

failure, coronary artery disease, end stage heart disease, atherosclerosis, ischemia, hypertension, restenosis, angina pectoris, rheumatic heart, arterial inflammation, or cardiovascular disease.

[00169] In some aspects, provided herein is a method of producing a population of O-hypoimmune cardiac cells from a population of HIPO- cells by *in vitro* differentiation, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated and CD47 expression has been increased as compared to an unmodified iPSCO- cells. The method comprises: (a) culturing a population of HIPO- cells in a culture medium comprising a GSK inhibitor; (b) culturing the population of HIPO- cells in a culture medium comprising a WNT antagonist to produce a population of pre-cardiac cells; and (c) culturing the population of pre-cardiac cells in a culture medium comprising insulin to produce a population of O-hypoimmune cardiac cells. In some embodiments, the GSK inhibitor is CHIR-99021, a derivative thereof, or a variant thereof. In some instances, the GSK inhibitor is at a concentration ranging from about 2 μ M to about 10 μ M. In some embodiments, the WNT antagonist is IWR1, a derivative thereof, or a variant thereof. In some instances, the WNT antagonist is at a concentration ranging from about 2 μ M to about 10 μ M.

[00170] In some aspects, provided herein is an isolated, engineered O- or O-hypoimmune endothelial cell differentiated from iPSCO-, ESCO-, or HIPO- cells. In other aspects, the isolated, engineered O- or O-hypoimmune endothelial cell is selected from the group consisting of a capillary endothelial cell, vascular endothelial cell, aortic endothelial cell, brain endothelial cell, and renal endothelial cell.

[00171] In some aspects, provided herein is a method of treating a patient suffering from a vascular condition or disease. In some embodiments, the method comprises administering a composition comprising a therapeutically effective amount of a population of isolated, engineered O- or O-hypoimmune endothelial cells.

[00172] In some embodiments, the method comprises administering a composition comprising a therapeutically effective amount of a population of any one of the isolated, engineered O- or O-hypoimmune endothelial cells described herein. In some embodiments, the composition further comprises a therapeutically effective carrier. In some embodiments, the administration comprises implantation into the patient's heart tissue, intravenous injection, intraarterial injection, intracoronary injection, intramuscular injection, intraperitoneal injection, intramyocardial injection, trans-endocardial injection, trans-epicardial injection, or infusion.

[00173] In some embodiments, the vascular condition or disease is selected from the group consisting of, vascular injury, cardiovascular disease, vascular disease, ischemic disease, myocardial infarction, congestive heart failure, hypertension, ischemic tissue injury, limb ischemia, stroke, neuropathy, and cerebrovascular disease.

[00174] In some aspects, provided herein is a method of producing a population of O-hypimmune endothelial cells from a population of HIPO- cells by *in vitro* differentiation, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated and CD47 expression has been increased in the HIPO-cells. The method comprises: (a) culturing a population of HIPO-cells in a first culture medium comprising a GSK inhibitor; (b) culturing the population of HIPO-cells in a second culture medium comprising VEGF and bFGF to produce a population of pre-endothelial cells; and (c) culturing the population of pre-endothelial cells in a third culture medium comprising a ROCK inhibitor and an ALK inhibitor to produce a population of hypimmune endothelial cells.

[00175] In some embodiments, the GSK inhibitor is CHIR-99021, a derivative thereof, or a variant thereof. In some instances, the GSK inhibitor is at a concentration ranging from about 1 μ M to about 10 μ M. In some embodiments, the ROCK inhibitor is Y-27632, a derivative thereof, or a variant thereof. In some instances, the ROCK inhibitor is at a concentration ranging from about 1 μ M to about 20 μ M. In some embodiments, the ALK inhibitor is SB-431542, a derivative thereof, or a variant thereof. In some instances, the ALK inhibitor is at a concentration ranging from about 0.5 μ M to about 10 μ M.

[00176] In some embodiments, the first culture medium comprises from 2 μ M to about 10 μ M of CHIR-99021. In some embodiments, the second culture medium comprises 50 ng/ml VEGF and 10 ng/ml bFGF. In other embodiments, the second culture medium further comprises Y-27632 and SB-431542. In various embodiments, the third culture medium comprises 10 μ M Y-27632 and 1 μ M SB-431542. In certain embodiments, the third culture medium further comprises VEGF and bFGF. In particular instances, the first culture medium and/or the second medium is absent of insulin.

[00177] In some aspects, provided herein is an isolated, engineered O- hypimmune dopaminergic neuron (DN) differentiated from a HIPO- cell, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated, CD47 expression has been increased, and the neuron is blood type O and Rh-.

[00178] In some embodiments, the isolated O- hypoimmune dopaminergic neuron is selected from the group consisting of a neuronal stem cell, neuronal progenitor cell, immature dopaminergic neuron, and mature dopaminergic neuron.

[00179] In some aspects, provided herein is a method of treating a patient suffering from a neurodegenerative disease or condition. In some embodiments, the method comprises administering a composition comprising a therapeutically effective amount of a population of any one of the isolated hypoimmune dopaminergic neurons. In some embodiments, the composition further comprises a therapeutically effective carrier. In some embodiments, the population of the isolated hypoimmune dopaminergic neurons is on a biodegradable scaffold. In some embodiments, the administration may comprise transplantation or injection. In some embodiments, the neurodegenerative disease or condition is selected from the group consisting of Parkinson's disease, Huntington disease, and multiple sclerosis.

[00180] In some aspects, provided herein is a method of producing a population of O- hypoimmune dopaminergic neurons from a population of HIPO- cells by *in vitro* differentiation, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated, CD47 expression has been increased, the blood group is O and Rh- in the HIPO-cells. In some embodiments, the method comprises (a) culturing the population of HIPO-cells in a first culture medium comprising one or more factors selected from the group consisting of sonic hedgehog (SHH), BDNF, EGF, bFGF, FGF8, WNT1, retinoic acid, a GSK3 β inhibitor, an ALK inhibitor, and a ROCK inhibitor to produce a population of immature dopaminergic neurons; and (b) culturing the population of immature dopaminergic neurons in a second culture medium that is different than the first culture medium to produce a population of dopaminergic neurons.

[00181] In some embodiments, the GSK β inhibitor is CHIR-99021, a derivative thereof, or a variant thereof. In some instances, the GSK β inhibitor is at a concentration ranging from about 2 μ M to about 10 μ M. In some embodiments, the ALK inhibitor is SB-431542, a derivative thereof, or a variant thereof. In some instances, the ALK inhibitor is at a concentration ranging from about 1 μ M to about 10 μ M. In some embodiments, the first culture medium and/or second culture medium are absent of animal serum.

[00182] In some embodiments, the method also comprises isolating the population of hypoimmune dopaminergic neurons from non-dopaminergic neurons. In some embodiments, the

method further comprises cryopreserving the isolated population of hypoimmune dopaminergic neurons.

[00183] In some aspects, provided herein is an isolated engineered O- hypoimmune pancreatic islet cell differentiated from a HIPO- cell, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated, CD47 expression has been increased, the blood type is O and Rh-.

[00184] In some embodiments, the isolated O- hypoimmune pancreatic islet cell is selected from the group consisting of a pancreatic islet progenitor cell, immature pancreatic islet cell, and mature pancreatic islet cell.

[00185] In some aspects, provided herein is a method of treating a patient suffering from diabetes. The method comprises administering a composition comprising a therapeutically effective amount of a population of any one of the isolated O- hypoimmune pancreatic islet cells described herein. In some embodiments, the composition further comprises a therapeutically effective carrier. In some embodiments, the population of the isolated hypoimmune pancreatic islet cells is on a biodegradable scaffold. In some instances, the administration comprises transplantation or injection.

[00186] In some aspects, provided herein is a method of producing a population of O- hypoimmune pancreatic islet cells from a population of HIPO- cells by *in vitro* differentiation, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated, CD47 expression has been increased, the blood type is O and Rh- in the HIPO- cells. The method comprises: (a) culturing the population of HIPO-cells in a first culture medium comprising one or more factors selected from the group consisting insulin-like growth factor (IGF), transforming growth factor (TGF), fibroblast growth factor (EGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), sonic hedgehog (SHH), and vascular endothelial growth factor (VEGF), transforming growth factor- β (TGF β) superfamily, bone morphogenic protein-2 (BMP2), bone morphogenic protein-7 (BMP7), a GSK3 β inhibitor, an ALK inhibitor, a BMP type 1 receptor inhibitor, and retinoic acid to produce a population of immature pancreatic islet cells; and (b) culturing the population of immature pancreatic islet cells in a second culture medium that is different than the first culture medium to produce a population of hypoimmune pancreatic islet cells.

[00187] In some embodiments, the GSK inhibitor is CHIR-99021, a derivative thereof, or a variant thereof. In some instances, the GSK inhibitor is at a concentration ranging from about 2 μ M to about 10 μ M. In some embodiments, the ALK inhibitor is SB-431542, a derivative thereof, or a variant thereof. In some instances, the ALK inhibitor is at a concentration ranging from about 1 μ M to about 10 μ M. In some embodiments, the first culture medium and/or second culture medium are absent of animal serum.

[00188] In some embodiments, the method also comprises isolating the population of hypimmune pancreatic islet cells from non-pancreatic islet cells. In some embodiments, the method further comprises cryopreserving the isolated population of hypimmune pancreatic islet cells.

[00189] In some aspects, provided herein is an isolated, engineered O- hypimmune retinal pigmented epithelium (RPE) cell differentiated from a HIPO- cell, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated, CD47 expression has been increased, the blood type is O and Rh-.

[00190] In some embodiments, the isolated O- hypimmune RPE cell is selected from the group consisting of an RPE progenitor cell, immature RPE cell, mature RPE cell, and functional RPE cell.

[00191] In some aspects, provided herein is a method of treating a patient suffering from an ocular condition. The method comprises administering a composition comprising a therapeutically effective amount of a population of any one of a population of the isolated hypimmune RPE cells described herein. In some embodiments, the composition further comprises a therapeutically effective carrier. In some embodiments, the population of the isolated hypimmune RPE cells is on a biodegradable scaffold. In some embodiments, the administration comprises transplantation or injection to the patient's retina. In some embodiments, the ocular condition is selected from the group consisting of wet macular degeneration, dry macular degeneration, juvenile macular degeneration, Leber's Congenital Ameurosis, retinitis pigmentosa, and retinal detachment.

[00192] In some aspects, provided herein is a method of producing a population of O- hypimmune retinal pigmented epithelium (RPE) cells from a population of HIPO- cells) by *in vitro* differentiation, wherein endogenous β -2 microglobulin (B2M) gene activity and endogenous class II transactivator (CIITA) gene activity have been eliminated and CD47 expression has been increased in the HIPO-cells. The method comprises: (a) culturing the population of HIPO-cells in a first culture

medium comprising any one of the factors selected from the group consisting of activin A, bFGF, BMP4/7, DKK1, IGF1, noggin, a BMP inhibitor, an ALK inhibitor, a ROCK inhibitor, and a VEGFR inhibitor to produce a population of pre-RPE cells; and (b) culturing the population of pre-RPE cells in a second culture medium that is different than the first culture medium to produce a population of hypimmune RPE cells.

[00193] In some embodiments, the ALK inhibitor is SB-431542, a derivative thereof, or a variant thereof. In some instances, the ALK inhibitor is at a concentration ranging from about 2 μ M to about 10 μ M. In some embodiments, the ROCK inhibitor is Y-27632, a derivative thereof, or a variant thereof. In some instances, the ROCK inhibitor is at a concentration ranging from about 1 μ M to about 10 μ M.

[00194] In some embodiments, the first culture medium and/or second culture medium are absent of animal serum.

[00195] In some embodiments, the method further comprises isolating the population of O-hypimmune RPE cells from non-RPE cells. In some embodiments, the method further comprises cryopreserving the isolated population of hypimmune RPE cells.

[00196] In one aspect, human pluripotent stem cells (hiPSCOs) are rendered hypimmunogenic by a) the disruption of the B2M gene at each allele (e.g. B2M $-/-$), b) the disruption of the CIITA gene at each allele (e.g. CIITA $-/-$), and c) by the overexpression of the CD47 gene (CD47⁺, e.g. through introducing one or more additional copies of the CD47 gene or activating the genomic gene). This renders the hiPSCO- population B2M $-/-$ CIITA $-/-$ CD47tg. In a preferred aspect, the cells are non-immunogenic. In another embodiment, the HIPO- cells are rendered non-immunogenic B2M $-/-$ CIITA $-/-$ CD47tg as described above but are further modified by including an inducible suicide gene that is induced to kill the cells *in vivo* when required. In other aspects, HIPO- cells are created when the HIP cells are rendered blood type O by by knocking out the ABO gene Exon 7 or silencing the SLC14A1 (JK) gene and the cells are rendered Rh- by knocking out the C and E antigens of the Rh blood group system (RH), K in the Kell system (KEL), Fya and Fy3 in the Duffy system (FY), Jkb in the Kidd system (JK), or U and S in the MNS blood group system.

F. Maintenance of O- Pluripotent Cells

[00197] Once generated, the iPSCO-, ESCO-, and HIPO- cells of the invention can be maintained in an undifferentiated state as is known for maintaining iPSCs. For example, HIPO- cells

can be cultured on Matrigel using culture media, which prevents differentiation and maintains pluripotency.

G. Differentiation of O- Pluripotent Cells

[00198] The invention provides iPSCO-, ESCO-, and HIPO- cells that are differentiated into different cell types for subsequent transplantation into subjects. As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. The cells are differentiated in suspension and then put into a gel matrix form, such as matrigel, gelatin, or fibrin/thrombin forms to facilitate cell survival. Differentiation is assayed as is known in the art, generally by evaluating the presence of cell-specific markers.

[00199] In some embodiments, the iPSCO-, ESCO-, and HIPO- cells of the invention are differentiated into hepatocytes to address loss of the hepatocyte functioning or cirrhosis of the liver. There are a number of techniques that can be used to differentiate iPSCO-, ESCO-, and HIPO- cells into hepatocytes; see for example Pettinato *et al.*, doi:10.1038/spre32888, Snykers *et al.*, *Methods Mol Biol* 698:305-314 (2011), Si-Tayeb *et al.*, *Hepatology* 51:297-305 (2010) and Asgari *et al.*, *Stem Cell Rev* (:493-504 (2013), all of which are hereby expressly incorporated by reference in their entirety and specifically for the methodologies and reagents for differentiation. Differentiation is assayed as is known in the art, generally by evaluating the presence of hepatocyte associated and/or specific markers, including, but not limited to, albumin, alpha fetoprotein, and fibrinogen. Differentiation can also be measured functionally, such as the metabolization of ammonia, LDL storage and uptake, ICG uptake and release and glycogen storage.

[00200] In some embodiments, the iPSCO-, ESCO-, and HIPO- cells are differentiated into beta-like cells or islet organoids for transplantation to address type I diabetes mellitus (T1DM). Cell systems are a promising way to address T1DM, see, e.g., Ellis *et al.*, doi/10.1038/nrgastro.2017.93, incorporated herein by reference. Additionally, Pagliuca *et al.* reports on the successful differentiation of β -cells from hiPSCs (see doi/10.106/j.cell.2014.09.040, hereby incorporated by reference in its entirety and in particular for the methods and reagents outlined there for the large-scale production of functional human β cells from human pluripotent stem cells). Furthermore, Vegas *et al.* shows the production of human β cells from human pluripotent stem cells followed by encapsulation to avoid immune rejection by the host; (doi:10.1038/nm.4030, hereby incorporated by reference in its entirety and in particular for the methods and reagents outlined there for the large-scale production of functional human β cells from human pluripotent stem cells).

[00201] Differentiation is assayed as is known in the art, generally by evaluating the presence of β cell associated or specific markers, including but not limited to, insulin. Differentiation can also be measured functionally, such as measuring glucose metabolism, see generally Muraro et al, doi:10.1016/j.cels.2016.09.002, hereby incorporated by reference in its entirety, and specifically for the biomarkers outlined there.

[00202] Once the differentiated iPSCO-, ESCO-, and HIPO- beta cells are generated, they can be transplanted (either as a cell suspension or within a gel matrix as discussed herein) into the portal vein/liver, the omentum, the gastrointestinal mucosa, the bone marrow, a muscle, or subcutaneous pouches.

[00203] In some embodiments, the iPSCO-, ESCO-, and HIPO- cells are differentiated into retinal pigment epithelium (RPE) to address sight-threatening diseases of the eye. Human pluripotent stem cells have been differentiated into RPE cells using the techniques outlined in Kamao et al., *Stem Cell Reports* 2014:2:205-18, hereby incorporated by reference in its entirety and in particular for the methods and reagents outlined there for the differentiation techniques and reagents; see also Mandai et al., doi:10.1056/NEJMoa1608368, also incorporated in its entirety for techniques for generating sheets of RPE cells and transplantation into patients.

[00204] Differentiation can be assayed as is known in the art, generally by evaluating the presence of RPE associated and/or specific markers or by measuring functionally. See for example Kamao et al., doi:10.1016/j.stemcr.2013.12.007, hereby incorporated by reference in its entirety and specifically for the markers outlined in the first paragraph of the results section.

[00205] In some embodiments, the iPSCO-, ESCO-, and HIPO- cells of the invention are differentiated into cardiomyocytes to address cardiovascular diseases. Techniques are known in the art for the differentiation of hiPSCs to cardiomyocytes and discussed in the Examples. Differentiation can be assayed as is known in the art, generally by evaluating the presence of cardiomyocyte associated or specific markers or by measuring functionally; see for example Loh et al., doi:10.1016/j.cell.2016.06.001, hereby incorporated by reference in its entirety and specifically for the methods of differentiating stem cells including cardiomyocytes.

[00206] In some embodiments, the iPSCO-, ESCO-, and HIPO- cells are differentiated into endothelial colony forming cells (ECFCs) to form new blood vessels to address peripheral arterial disease. Techniques to differentiate endothelial cells are known. See, e.g., Prasain et al., doi:10.1038/nbt.3048, incorporated by reference in its entirety and specifically for the methods and

reagents for the generation of endothelial cells from human pluripotent stem cells, and also for transplantation techniques. Differentiation can be assayed as is known in the art, generally by evaluating the presence of endothelial cell associated or specific markers or by measuring functionally.

[00207] In some embodiments, the iPSCO-, ESCO-, and HIPO- cells are differentiated into thyroid progenitor cells and thyroid follicular organoids that can secrete thyroid hormones to address autoimmune thyroiditis. Techniques to differentiate thyroid cells are known the art. *See, e.g. Kurmann et al.*, doi:10.106/j.stem.2015.09.004, hereby expressly incorporated by reference in its entirety and specifically for the methods and reagents for the generation of thyroid cells from human pluripotent stem cells, and also for transplantation techniques. Differentiation can be assayed as is known in the art, generally by evaluating the presence of thyroid cell associated or specific markers or by measuring functionally.

H. Transplantation of Differentiated HIPO- Cells

[00208] As will be appreciated by those in the art, the differentiated HIPO- derivatives are transplanted using techniques known in the art that depends on both the cell type and the ultimate use of these cells. In general, the differentiated iPSCO-, ESCO-, and HIPO- cells of the invention are transplanted either intravenously or by injection at particular locations in the patient. When transplanted at particular locations, the cells may be suspended in a gel matrix to prevent dispersion while they take hold.

[00209] In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

VIII. EXAMPLES

Example 1: Generation of Human iPSCs

[00210] The Human Episomal iPSC Line was derived from CD34+ cord blood (Cat. No. A33124, Thermo Fisher Scientific) using a three-plasmid, seven-factor (SOKMNLT; SOX2, OCT4 (POU5F1), KLF4, MYC, NANOG, LIN28, and SV40L T antigen) EBNA-based episomal system from ThermoFisher. This iPSC line is considered to have a zero footprint as there was no integration into the genome from the reprogramming event. It has been shown to be free of all reprogramming genes. The iPSCs have a normal XX karyotype and endogenous expression of pluripotent markers like OCT4, SOX2, NANOG (as shown by RT-PCR) OCT4, SSEA4, TRA-1-60 and TRA-1-81 (as shown by

ICC). In directed differentiation and teratoma analyses, these hiPSCs retained their differentiation potential for the ectodermal, endodermal, and mesodermal lineages. In addition, vascular, endothelial, and cardiac lineages were derived with robust efficiencies.

[00211] Several gene-delivery vehicles for iPSC generation were successfully used, including retroviral vectors, adenoviral vectors, Sendai virus as well as virus-free reprogramming methods (using episomal vectors, piggyBac transposon, synthetic mRNAs, microRNAs, recombinant proteins, and small molecule drugs, etc).

[00212] Different factors were successfully used for re-programming, such as the first reported combination of *OCT3/4*, *SOX2*, *KLF4*, and *C-MYC*, known as the Yamanaka factors. In one embodiment, only three of these factors were successfully combined and omitted *C-MYC*, although with reduced reprogramming efficiency.

[00213] In one embodiment, *L-MYC* or *GLIS1* instead of *C-MYC* showed improved reprogramming efficiency. In another embodiment, reprogramming factors are not limited to genes associated with pluripotency.

[00214] All data are expressed as mean $\pm$ SD or in box blot graphs showing the median and the minimum to maximum range. Intergroup differences were appropriately assessed by either the unpaired Student's t test or the one-way analysis of variance (ANOVA) with Bonferroni's postHoc test. * $p < 0.05$, ** $p < 0.01$.

Example 2: Generation of human HIP cells

[00215] Hypoimmune pluripotent (HIP) cells were generated as disclosed in WO2018/132783 and U.S. Prov. App. Nos. 62/698,941, 62/698,965, 62/698,973, 62/698,978, 62/698,981, and 62/698,984, each of which are incorporated by reference herein in their entirety.

[00216] Human Cas9 iPSC underwent 2 gene-editing steps. In the first step, CRISPR technology was performed by a combined targeting of the coding sequence of human beta-2-microglobulin (B2M) gene with the CRISPR sequence 5'-CGTGAGTAAACCTGAATCTT-3' and the coding sequence of human CIITA gene with the CRISPR sequence 5'-GATATTGGCATAAGCCTCCC-3'. Linearized CRISPR sequence with T7 promoter was used to synthesize gRNA as per the kit's instructions (MEGashortscript T7 Transcription Kit, Thermo Fisher). The collected in-vitro transcription (IVT) gRNA was then purified via the MEGAclean Transcription Clean-Up Kit. For IVT gRNA delivery, singularized cells were electroporated with 300ng IVT gRNA using a Neon electroporation system. After electroporation, edited Cas9 iPSCs were expanded for single cell

seeding: iPSC cultures were dissociated to single cells using TrypLE (Gibco) and stained with Tra1-60 Alexa Fluor® 488 and propidium iodide (PI). FACS Aria cell sorter (BD Biosciences) was used for the sorting and doublets and debris were excluded from seeding by selective gating on forward and side scatter emission. Viable pluripotent cells were selected on the absence of PI and presence of Tra1-60 Alexa Fluor 488 staining. Single cells were then expanded into full size colonies, after which the colonies were tested for a CRISPR edit. CRISPR mediated cleavage was assessed using the GeneArt Genomic Cleavage Detection Kit (Thermo Fisher). Genomic DNA was isolated from 1×10^6 hiPSCs and the B2M and CIITA genomic DNA regions were PCR amplified using AmpliTaq Gold 360 Master Mix and the primer sets F: 5'-TGGGGCCAAATCATGTAGACTC-3' and R: 5'-TCAGTGGGGGTGAATTCAGTGT-3' for B2M as well as F: 5'-CTTAACAGCGATGCTGACCCC-3' and R: 5'-TGGCCTCCATCTCCCTCTCTT-3' for CIITA. For TIDE analysis, the obtained PCR product was cleaned up (PureLink PCR Purification Kit, Thermo Fisher) and Sanger sequencing was performed for the prediction of indel frequency. After the confirmation of B2M/CIITA knockout, cells were further characterized through karyotype analysis and the TaqMan hPSC Scorecard Panel (Thermo Fisher). The PSC were found to be pluripotent and maintained a normal (46, XX) karyotype during the genome editing process.

[00217] In the second step, the CD47 gene was synthesized and the DNA was cloned into a plasmid lentivirus with an EF1a promoter and puromycin resistance. Cells were transduced with lentiviral stocks of 1×10^7 TU/mL and 6 µg/mL of Polybrene (Thermo Fisher). Media was changed daily after transduction. Three days after transduction, cells were expanded and selected with 0.5 µg/mL of puromycin. After 5 days of antibiotic selection, antibiotic resistant colonies emerged and were further expanded to generate stable pools. Level of CD47 was confirmed by qPCR. Pluripotency assay (TaqMan hPSC Scorecard Panel, Thermo Fisher). and karyotyping were performed again to verify the pluripotent status of the cells.

Example 3: HIP Cell Rejection in Rhesus Macaque Monkeys and Pigs

[00218] 10 million hypoimmunogenic B2M^{-/-} CIITA^{-/-} rhesus CD47 tg iPSC derived endothelial cells (expressing luciferase) were injected subcutaneously into rhesus macaque monkeys and cells were longitudinally followed using bioluminescence imaging; each animal was injected intravenously with 100 mg/ml D-luciferin (PerkinElmer, San Jose, CA) via a peripheral vessel for *in vivo* imaging using a Xenogen IVIS®200 Series imaging system (Caliper Life Sciences, Alameda, CA, Cat. No. 122799). Our pre-transplant screening assays and validation studies predicted that the cells would not be rejected, resulting in stable BLI signals. The BLI-signals decreased, however, on day 6 and were not detected by day 16. In addition, "bumps" were observed on the injection sites (data

not shown). Blood was drawn from the same monkeys and T cell, cytotoxic T cell, NK cell (**Figure 1A**), B cell (DSA; donor specific antibodies), or macrophages activation (**Figure 1B**) was not observed. Blood typing the monkeys by PCR confirmed that all tested monkeys were blood type B. Therefore, the hypoinmunogenic endothelial cell transplantation was ABO mismatched (data not shown). In contrast to these results, transplanting unmodified human iPSC-derived endothelial cells into Macaque Rhesus monkeys resulted in rejection of the cells and the activation of an adaptive immune response (**Figure 2A**). Additionally, transplanting HLA-I deficient / HLA-II deficient B2M^{-/-} CIITA^{-/-} human iPSC-derived endothelial cells into monkeys resulted in rejection of the cells and the activation of an innate immune response (**Figure 2B**).

Example 4: IgM Antibodies Killed Endothelial Cells from hiPSCs

[00219] Blood type rejection was confirmed by incubating human hypoinmunogenic endothelial cells with rhesus macaque serum.

[00220] **Human iPSC differentiation into hiECs.** hiPSC were plated on diluted Matrigel (Corning, Tewksbury, MA, Cat. No. 356231) in six-well plates and maintained in Essential 8 Flex media (Thermo Fisher, Cat. No. A2858501). The differentiation was started at 60% confluency and media was changed to RPMI-1640 containing 2% B-27 minus insulin (both Gibco Thermo Fisher, Cat. No. A1895601) and 5 μ M CHIR-99021 (Selleckchem, Cat. No. S1263). On day 2, the media was changed to reduced media: RPMI-1640 containing 2% B-27 minus insulin and 2 μ M CHIR-99021. From day 4 to 7, cells were exposed to RPMI-1640 EC media (RPMI-1640, 2% B-27 minus insulin, 50 ng ml⁻¹ human vascular endothelial growth factor (VEGF, Peprotech, Rocky Hill, NJ, Cat. No. 100-20), 10 ng ml⁻¹ human fibroblast growth factor basic (FGFb; Peprotech, Cat. No. 100-18B), 10 μ M Y-27632 (Selleckchem, Cat. No. S1049), and 1 μ M SB 431542 (Reagentsdirect, Cat. No. 21-A94).

[00221] Endothelial cell clusters were visible from day 7 and cells were maintained in EGM-2 SingleQuots media (Lonza, Basel, Switzerland, Cat. No. CC-3162) plus 10% Fetal Calf Serum hi (Gibco Thermo Fisher, Cat. No. 10082147), 25 ng ml⁻¹ VEGF, 2 ng ml⁻¹ FGFb, 10 μ M Y-27632 and 1 μ M SB 431542. The differentiation process was completed after 14 days and undifferentiated cells detached during the differentiation process. For purification, cells were treated with 20 μ M PluriSIn-1 (StemCell Technologies, Cambridge, MA, Cat. No. 72824) for 48 hours. The highly purified ECs were cultured in EGM-2 SingleQuots media plus supplements and 10% FCS hi (Gibco). TrypLE Express was used for passaging the cells 1:3 every 3–4 days.

[00222] When human hypoinmunogenic B2M^{-/-} CIITA^{-/-} rhesus CD47 tg endothelial cells (blood type A) were incubated with rhesus macaque serum (blood type B), cells were killed immediately. The antibody type that killed the cells was determined by antibody depletion analyses.

[00223] IgM depletion was done in a working solution of 50mM dithiothreitol (DTT, Millipore Sigma, St. Louis, MO, Cat. No.D0632). 10 µl of DTT were mixed with 90 µl serum.

[00224] IgG depletion was done with Pierce Protein Beads (Thermo Fisher, Waltham, MA, Cat. No. 88803). The beads were washed in the wash buffer and collected magnetically. 0.5mg of the washed beads were combined with 100 µl of the rhesus macaque serum and incubated for 60 minutes at room temperature with gentle inversion every 10 minutes. The beads were separated magnetically, the serum was transferred to a new tube and kept on ice until used.

[00225] Depletion of either IgM or IgG antibodies demonstrated that the ABO-antibodies are from the IgM type (**Figure 3**). Blood type rejection was also similarly confirmed by IgM and IgG depletion using HIP-derived cardiomyocytes and adult cardiac tissue (Celprogen, Torrance, CA, Cat. No. 36044-15-T75, data not shown).

[00226] **Rhesus Monkey Blood Type B Serum Causes Complement-Dependent Cytotoxicity (CDC) of human wt iECs of blood type A.** Human wt iPSCs of blood type A or O were differentiated to wt iECs and incubated with serum from a rhesus monkey of blood type B on the XCelligence Real-Time Cell Analysis (ACEA Biosciences, San Diego, CA) platform. Differentiated iECs of blood type A were quickly killed (**Figure 2C**) whereas iECs of blood type O were unaffected (**Figure 2D**). Since anti-A antibodies had been detected in this rhesus monkey 1 (using a clinically-approved agglutination test), iECs carrying the A blood type antigen were killed and iECs not expressing the A antigen survived.

[00227] **Embryonic stem cell-derived ECs undergo the same ABO blood type-dependent CDC as iPSC-derived iECs.** To confirm that the ABO blood type sensitivity seen in iPSC-derived iECs is not inherent to the iPSC starter cell, the H9 human embryonic stem cell line was used. ECs were differentiated from H9 which has blood type A, Rh+. Similar to the observations with iPSC-derived iECs, H9-derived ECs underwent CDC killing on the XCelligence platform when incubated with human ABO-incompatible serum (**Figure 2E**) and rhesus monkey ABO-incompatible serum (data not shown).

Example 5: Human HIP-derived Cardiomyocytes or mature Cardiomyocytes Survive Blood Group-compatible Xenogeneic serum Exposure

[00228] **The human cells were not rejected by other pre-formed antibodies in xenogeneic transplantation.** Human B2M^{-/-} CIITA^{-/-} rhesus CD47 tg⁻ derived endothelial cells (blood type A) were killed when incubated with ABO-incompatible rhesus macaque serum (blood type B). When compatible serum from rhesus macaque with blood type AB was used, however, the human cells survived (**Figure 4**). Thus, rhesus macaques do not have other pre-existing antibodies against human cells.

[00229] **Human iPSCs were differentiated into hiCMs.** hiPSCs were plated on diluted Matrigel in six-well plates and maintained in Essential 8 Flex media (Thermo Fisher). Differentiation was started at 90% confluency, and media was changed to 5 ml of RPMI-1640 containing 2% B-27 minus Insulin and 6 μ M CHIR-99021. After 2 days, media was changed to RPMI-1640 containing 2% B-27 minus insulin without CHIR. On day 3, 5 μ l IWR1 (Selleckchem, Houston, TX, Cat. No. S7086) was added to the media for two further days. At day 5, the media was changed back to RPMI-1640 containing 2% B-27 minus insulin medium and left for 48 h. At day 7, media was changed to RPMI-1640 containing B27 plus insulin and replaced every 3 days thereafter with the same media. Spontaneous beating of cardiomyocytes was first visible around day 10. Purification of cardiomyocytes was performed on day 10 post-differentiation. Briefly, media was changed to low glucose media and maintained for 3 days. At day 13, media was changed back to RPMI-1640 containing B27 plus insulin. This procedure was repeated on day 14.

[00230] **The human hypoinmunogenic iPSC-derived cardiomyocytes (blood type A) survive when incubated with allogeneic human serum blood type A and AB.** However, serum containing pre-formed antibodies against A (blood type O and B) killed the cells immediately (**Figure 5A**). These results were validated using mature cardiomyocytes. Mature cardiomyocytes (purchased from CELPROGEN) (blood type A) survived when incubated with allogeneic ABO matched human serum (blood type A and AB), but they were killed when incubated with serum containing pre-formed antibodies against A (blood type O and B) (**Figure 5b**).

[00231] Translational analyses were performed by incubating pig serum (blood type A) with human endothelial cells from all blood types. Only those cells with blood type B or AB were killed (data not shown). This confirms that human cells are hyperacutely rejected when transplanted into ABO mismatched pigs.

Example 6: Hepatocytes Fail to Survive ABO Blood Group-incompatible Serum Exposure

[00232] Human hepatocytes (blood type A; Corning, cat. No. 454543) survived when incubated with ABO matched human serum (blood type A and AB) but the cells were killed when incubated with ABO-incompatible human serum (blood type B and O; BioChemed, Winchester, VA), as measured using XCelligence Real-Time Cell Analysis (ACEA Biosciences) to monitor cell survival/killing (**Figure 6**). Similarly, human hepatocytes, which are blood type AB, survived when incubated with ABO matched human serum (blood type AB) but the cells were killed when incubated with ABO-incompatible human serum (blood type A, B, and O) .

[00233] This example demonstrates the importance of ABO matching in mature and differentiated hepatocytes.

Example 7: Relevance of Rhesus Factor in Sensitized Serum

[00234] Endothelial cells (blood type A Rh+ or blood type A Rh- survived when incubated with an ABO matched human serum (blood type A) even when the serum was mismatched for Rh (Rh+ or Rh-), as long as the serum had not been previously sensitized to the Rh factor antigen (**Figure 7A**). However, when endothelial cells which are blood type A Rh+ or blood type B Rh+ were incubated with serum which is ABO-compatible (blood type AB) but Rh mismatched (Rh-), and the serum had been previously sensitized to the Rh antigen factor and contains anti-Rh antibodies, the cells were killed (**Figure 7B**). Hypoimmunogenic endothelial cells (B2M-/- CIITA -/- CD47 tg) (blood type A Rh+) were also killed when incubated with an ABO-compatible serum (blood type AB) which is Rh- and had been previously sensitized to the Rh factor antigen and contains anti-Rh antibodies. Moreover, endothelial cells which are blood type O Rh+ were killed when using O Rh- serum which had been previously sensitized to the Rh factor and contains anti-Rh antibodies (**Figure 7C**). This example demonstrates that use of Rh- cells may be important for transplantation into previously sensitized subjects.

[00235] Embryonic stem cell-derived ECs undergo the same Rh blood type-dependent CDC as iPSC-derived iECs and primary wt ECs. **Figure 7D** shows that when H9-derived ECs of blood type A Rh+ were incubated with ABO-compatible serum containing Rh antibodies, they underwent CDC killing.

[00236] Blood type O Rh- ECs do not undergo ABO- or Rh-mediated CDC. The established EC line HEK293, a cell line derived from human embryonic kidney cells grown in tissue culture, is blood type O Rh-. **Figure 7E** shows that the HEK293 incubated with serum containing Rh antibodies did not undergo killing.

Example 8: Generation of human HIPO- cells

[00237] In some embodiments, HIPO- cells are generated using blood type O Rh- pluripotent stem cells as starter cells and following the HIP cell generation protocols described herein. Therefore, the HIP cells are HIPO- cells.

[00238] In other aspects, a HIPO- cell is generated from a non-universal blood group iPSC, ESC or HIP cell. For example, a blood type B- embryonic or iPSC cell line is transformed into O- by generating a knock-out cell line in the ABO gene using CRISPR technology for targeting of the coding sequence (gene ID: 28; Ensembl:ENSG00000175164 MIM:110300). Therefore, CRISPR guide RNAs targeting the coding sequence of the B gene are ligated into vectors containing the Cas9 expression cassette and subsequently transfected into hiPSCs. Linearized CRISPR sequence with T7 promoter are used to synthesize gRNA as per the kit's instructions (MEGAscript T7 Transcription Kit, Thermo Fisher). The resulting *in vitro* transcription (IVT) gRNA is then purified via the MEGAclear Transcription Clean-Up Kit. For IVT gRNA delivery, cells are electroporated with 300 ng IVT gRNA using a Neon electroporation system using 1,200 V, 30 ms, 1 pulse into hiPSC stably expressing Cas9.

[00239] After electroporation, edited hiPSC are expanded for single cell seeding: hiPSC cultures are dissociated into single cells using TrypLE Express (Gibco) and stained with Alexa Fluor 488-conjugated anti-TRA-160 mAb and propidium iodide. A FACSAria II cell sorter (BD Biosciences) is used for the sorting and doublets and debris are excluded from seeding by selective gating on forward and side light scatter properties. Viable pluripotent cells are selected on the absence of propidium iodide and presence of Tra1-60 staining. Single cells are then expanded into full-size colonies, after which the colonies are tested for CRISPR editing by sequencing.

[00240] CRISPR-mediated cleavage is assessed using the GeneArt Genomic Cleavage Detection Kit (Thermo Fisher) for testing of the initial edited pools. For screening the isolated clones, genomic DNA is isolated from 1×10^6 hiPSCs and the B genomic DNA regions are PCR amplified using AmpliTaq Gold 360 Master Mix. For TIDE analysis, the resulting PCR product is cleaned up (PureLink PCR Purification Kit, Thermo Fisher) and Sanger sequencing is performed for the prediction of indel frequency. After the confirmation of B disruption, cells are further characterized through karyotype analysis and the TaqMan hiPSC Scorecard Panel (Thermo Fisher).

[00241] Another example is using a O rh+ cell line and transforming this into a O rh- cell line by deleting RHAG (Rh-associated glycoprotein; ammonium transport; associated with RhD;

chromosome 6p21-qter) using CRISP/Cas9 technology as described above (RHAG gRNA sequence: CCAGTGGGGCACTATTGTAC).

Example 9: Differentiation of human HIPO- cells

1. Differentiation of hHIPO- cells to human cardiomyocytes

[00242] This is done using a protocol adapted from Sharma *et al.*, *J. Vis Exp.* 2015 doi: 10.3791/52628, hereby incorporated by reference in its entirety and specifically for the techniques to differentiate the cells. HIPO- cells are plated on diluted Matrigel (356231, Corning) in 6-well plates and maintained in Essential 8 Flex media (Thermo Fisher). After the cells arrive at 90% confluency, the differentiation is started and media is changed to 5 mL of RPMI1640 containing 2% B-27 minus Insulin (both Gibco) and 6uM CHIR-99021 (Selleck Chem). After 2 days, media is changed to RPMI1640 containing 2% B-27 minus Insulin without CHIR. On day 3, 5uL IWR1 is added to the media for two further days. At day 5, the media is changed back to RPMI 1640 containing 2% B-27 minus insulin medium and incubated for 48 hr. At day 7, media is changed to RPMI 1640 containing B27 plus insulin (Gibco) and replaced every 3 days thereafter with the same media. Spontaneous beating of cardiomyocytes may first be visible at approximately day 10 to day 12. Purification of Cardiomyocytes is performed on day 10 post-differentiation. Briefly, media is changed to low glucose media and maintained for 3 days. At day 13, media is changed back to RPMI 1640 containing B27 plus insulin. This procedure is repeated on day 14. The remaining cells are highly purified cardiomyocytes.

2. Differentiation of HIPO- cells to human endothelial cells

[00243] HIPO- cells are plated on diluted Matrigel (356231, Corning) in 6-well plates and maintained in Essential 8 Flex media (Thermo Fisher). After the cells arrive at 60% confluency, the differentiation is started and media is changed to RPMI1640 containing 2% B-27 minus Insulin (both Gibco) and 5 μ M CHIR-99021 (Selleck Chem). On day 2, the media is changed to reduced media: RPMI1640 containing 2% B-27 minus Insulin (both Gibco) and 2 μ M CHIR-99021 (Selleck Chem). From day 4 to day 7, cells are exposed to RPMI EC media, RPMI1640 containing 2% B-27 minus Insulin plus 50 ng/mL vascular endothelial growth factor (VEGF; R&D Systems, Minneapolis, MN, USA), 10 ng/mL fibroblast growth factor basic (FGFb; R&D Systems), 10 μ M Y-27632 (Sigma-Aldrich, Saint Louis, MO, USA) and 1 μ M SB 431542 (Sigma-Aldrich). Endothelial cell clusters are visible from day 7 and cells are maintained in EGM-2 SingleQuots media (Lonza, Basel, Switzerland) plus 10% FCS hi (Gibco), 25 ng/mL vascular endothelial growth factor (VEGF; R&D Systems, Minneapolis, MN, USA), 2 ng/mL fibroblast growth factor basic (FGFb; R&D Systems), 10 μ M Y-27632 (Sigma-Aldrich,

Saint Louis, MO, USA) and 1 μ M SB 431542 (Sigma-Aldrich). The differentiation process may be completed after 14 days und undifferentiated cells detach during the differentiation process. For purification, cells go through MACS progress according to the manufactures' protocol using CD31 microbeads (Miltenyi, Auburn, CA). The highly purified EC-cells are cultured in EGM-2 SingleQuots media (Lonza, Basel, Switzerland) plus supplements and 10% FCS hi (Gibco). TrypLE was used for passaging the cells 1:3 every 3 to 4 days.

IX. Exemplary sequences:

SEQ ID NO:1 – Human β -2-Microglobulin

MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKD
WSFYLLYYTEFTPTEKDEYACRVNHVTLSPKIVKWDRDI

SEQ ID NO:2 – Human CIITA protein, 160 amino acid N-terminus

MRCLAPRPAGSYLSEPPQGSSQCATMELGPLEGGYLELLNSDADPLCLYHFYDQMDLAGEEEIELYSEPDTDTINCD
QFSRLLCDMEGDEETREAYANIAELDQYVFQDSQLEGLSKDIFKHIGPDEVIGESMEMPAEVGQKSQKRPFPEELP
ADLKHWP

SEQ ID NO:3 – Human CD47

MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVWKFKGRDIYTFDGA LNKS
TVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETIIEKYRVVSWFSPNENILIVIFPIFAILL
WGQFGIKTKYRSGGMDEKTIALLVAGLVITVIVGAILFVPGEYSLKNATGLGLIVTSTGILLHHYVVFSTAIGLTSFV
IALVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLGLVYMKFVE

SEQ ID NO:4 – Herpes Simplex Virus Thymidine Kinase (HSV-tk)

MASYPCHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRLEQKMPPTLLRVYIDGPHGMGKTTTTQLLVALGS
RDDIVYVPEPMYVQVLGASETIANIYTTQHRLDQGEISAGDAAVVMTSAQITMGMPYAVTDAVLAPHVGGEA
GSSHAPPPALTIFDRHPAIAALLCYPARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGALPEDRHIDRLAKRQRPGE
RLDLAMLAARRVYGLLANTVRYLQGGGSWWEDWGQLSGTAVPPQGAEPQSNAGPRPHIGDTLFTLFRAPELLA
PNGDLNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQTHVTPGSIPTICDLARTFAREMGE
AN

SEQ ID NO:5 – *Escherichia coli* Cytosine Deaminase (EC-CD)

MSNNALQTIINARLPGEGLWQIHLQDGKISIDAQSGVMPITENSLDAEQGLVIPPFVEPHIHLDTTQTAGQPNW
NQSGTLFEGIERWAERKALLTHDDVKQRAWQTLKWQIANGIQHVRTHVDVSDATLTALKAMLEVKEVAPWIDL
QIVAFPQEGILSYPNGEALLEEALRLGADVVGAIHPHFETREYGVESLHKTFALAQKYDRIDVHCDEIDDEQSRFVET
VAALAHHEGMGARVTASHTTAMHSYNGAYTSRLFRLLKMSGINLVANPLVNIHLQGRFDTPKRRGITRVKEMLE
SGINVCFGHDDVDFPWYPLGTANMLQVLHMLHVCQLMGYGQINDGLNLITHHSARTLNLQDYGIAAGNSANLI
ILPAENGFDALRRQVPVRYSVRGGKVIASQPAQTTVYLEQPEAIDYKR

SEQ ID NO:6 – Truncated human Caspase 9

GFGDVGALSLRGNADLAYILSMEPCGHCLINNPNFCRESGLRTRTGSNIDCEKLRRRFSSLHFMVEVKGDLTAKK
MVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQAC
GGEQKDHGFEVASTPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYV
ETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNLRKKLFFKTS

[00244] All publications and patent documents disclosed or referred to herein are incorporated by reference in their entirety. The foregoing description has been presented only for purposes of illustration and description. This description is not intended to limit the invention to the precise form disclosed. It is intended that the scope of the invention be defined by the claims appended hereto.

CLAIMS

What is claimed:

1. A modified pluripotent cell or a cell derived therefrom, wherein the cell is ABO blood group type O and Rhesus factor negative (Rh-), wherein the cell has (a) a reduced or eliminated ABO blood group antigen selected from the group consisting of A1, A2, and B; and/or (b) has a reduced or eliminated Rh protein antigen expression selected from the group consisting of Rh C antigen, Rh E antigen, Kell K antigen (KEL), Duffy (FY) Fya antigen, Duffy Fy3 antigen, Kidd (JK) Jkb antigen, MNS antigen U, and MNS antigen S.
2. A modified pluripotent cell or a cell derived therefrom, comprising: (a) a modification that reduces or eliminates antigenicity of one or more ABO blood group antigens selected from the group consisting of A1, A2, and B rendering the cell ABO blood group type O; and/or (b) a modification that reduces or eliminates antigenicity of one or more Rh antigens selected from the group consisting of Rh C antigen, Rh E antigen, Kell K antigen (KEL), Duffy (FY) Fya antigen, Duffy Fy3 antigen, Kidd (JK) Jkb antigen, MNS antigen U, and MNS antigen S rendering the cell Rh-.
3. The modified pluripotent cell of claim 1 or claim 2, wherein the cell is a hypoinmunogenic pluripotent (HIP) cell comprising:
 - a. an endogenous Major Histocompatibility Antigen Class I (HLA-I) function that is reduced when compared to an unmodified pluripotent cell;
 - b. an endogenous Major Histocompatibility Antigen Class II (HLA-II) function that is reduced when compared to an unmodified pluripotent cell; and
 - c. an increased CD47 function that reduces susceptibility to NK cell killing.
4. A modified pluripotent cell or a cell derived therefrom, wherein the cell comprises modulated expression of one or more of HLA-I human leukocyte antigens, HLA-II human leukocyte antigens, CD47, CCR5, CXCR4, NLRC5, CIITA, B2M, HLA-A, HLA-B, HLA-C, HLA-E, HLA-G, PD-L1, CTLA-4-Ig, CD47, CI-inhibitor, IL-35, RFX-5, RFXAP, RFXANK, NFY-A, NFY-B, NFY-C, IRF-1, OX40, GITR, 4-1BB, CD28, B7-1, B7-2, ICOS, CD27, HVEM, SLAM, CD226, PD1, CTL4, LAG3, TIGIT, TIM3, CD160, BTLA, CD244, CD30, TLT, VISTA, B7-H3, PD-L2, LFA-1, CD2, CD58, ICAM-3, TCRA, TCRB, FOXP3, HELIOS, ST2, PCSK9, APOC3, CD200, FASLG, CLC21, MFGE8, SERPIN B9, TGFβ, CD73, CD39, LAG3, IL1R2, ACKR2, TNFRSF22, TNFRSF23, TNFRS10, DAD1, and/or

IFN γ R1 d39 relative to a wild-type stem cell, wherein the cell is ABO blood group type O or Rhesus factor negative (Rh-).

5. A modified pluripotent cell or a cell derived therefrom, wherein the cell is an O-hypoinmunogenic pluripotent (HIPO-) cell comprising:
 - a. an endogenous Major Histocompatibility Antigen Class I (HLA-I) function that is reduced when compared to an unmodified pluripotent cell;
 - b. an endogenous Major Histocompatibility Antigen Class II (HLA-II) function that is reduced when compared to an unmodified pluripotent cell;
 - c. an increased CD47 function that reduces susceptibility to NK cell killing;
 - d. an ABO blood group type O (O); and
 - e. optionally, a Rhesus factor negative (Rh-) blood type.
6. The modified pluripotent cell or cell derived therefrom of any one of claims 1-5, wherein the cell is selected from O- induced pluripotent stem cells (iPSCO-), O- embryonic stem cells (ESCO-), O- endothelial cells, O- cardiomyocytes, O- hepatocytes, O- dopaminergic neurons, O- pancreatic islet cells, O- retinal pigment endothelium cells, and other O- cell types used for transplantation and medical therapies, including O- chimeric antigen receptor (O- CAR) cells.
7. The modified pluripotent cell or cell derived therefrom of claim 6, wherein the O- CAR cell is an O- chimeric antigen receptor T (O- CAR-T) cell.
8. The modified pluripotent cell or cell derived therefrom of any one of claims 1-7, wherein said ABO blood group type O results from a reduced ABO blood group protein expression.
9. The modified pluripotent cell or cell derived therefrom of claim 8, wherein said ABO blood group type O results from a disruption in human Exon 7 of the ABO gene.
10. The modified pluripotent cell or cell derived therefrom any one of claims 1-7, wherein said ABO blood group type O results from an enzymatic modification of an ABO gene product on a surface of said cell.
11. The modified pluripotent cell or cell derived therefrom of claim 10, wherein said enzymatic modification removes a carbohydrate from said ABO gene product.

12. The modified pluripotent cell or cell derived therefrom of claim 10, wherein said enzymatic modification removes a carbohydrate from an ABO A1 antigen, A2 antigen, and/or B antigen.
13. The modified pluripotent cell or cell derived therefrom of any one of claims 1-12, wherein said Rh- results from a reduced Rh protein expression.
14. The modified pluripotent cell or cell derived therefrom of claim 13, wherein said Rh- results from a disruption in an Rh C antigen, an Rh E antigen, a Kell K antigen (KEL), a Duffy (FY) Fya antigen, a Duffy Fy3 antigen, a Kidd (JK) Jkb antigen, or a Kidd SLC14A1 gene.
15. The modified pluripotent cell or cell derived therefrom of any one of claims 1-7, wherein said ABO blood group type is endogenously type O.
16. The modified pluripotent cell or cell derived therefrom of any one of claims 1-7, wherein said Rh- blood type is endogenously type Rh-.
17. The modified pluripotent cell or cell derived therefrom of any one of claims 3-16, wherein said HLA-I function is reduced by a reduction in expression of a β -2 microglobulin protein.
18. The modified pluripotent cell or cell derived therefrom of of claim 17, wherein a gene encoding said β -2 microglobulin protein is knocked out.
19. The modified pluripotent cell or cell derived therefrom of any one of claims 3-16, wherein said HLA-I function is reduced by a reduction in expression of an HLA-A protein.
20. The modified pluripotent cell or cell derived therefrom of claim 19, wherein a gene encoding said HLA-A protein is knocked out.
21. The modified pluripotent cell or cell derived therefrom of any one of claims 3-16, wherein said HLA-I function is reduced by a reduction in expression of an HLA-B.
22. The modified pluripotent cell or cell derived therefrom of claim 21, wherein a gene encoding said HLA-B protein is knocked out.
23. The modified pluripotent cell or cell derived therefrom of any one of claims 3-16, wherein said HLA-I function is reduced by a reduction in expression of an HLA-C protein.

24. The modified pluripotent cell or cell derived therefrom of claim 23, wherein a gene encoding said HLA-C protein is knocked out.
25. The modified pluripotent cell or cell derived therefrom of any one of claims 3-24, wherein said HIPO- cell does not comprise an HLA-I function.
26. The modified pluripotent cell or cell derived therefrom of any one of claims 3-25, wherein said HLA-II function is reduced by a reduction in expression of a CIITA protein.
27. The modified pluripotent cell or cell derived therefrom of claim 26, wherein a gene encoding said CIITA protein is knocked out.
28. The modified pluripotent cell or cell derived therefrom of any one of claims 3-25, wherein said HLA-II function is reduced by a reduction in expression of an HLA-DP protein.
29. The modified pluripotent cell or cell derived therefrom of claim 28, wherein a gene encoding said HLA-DP protein is knocked out.
30. The modified pluripotent cell or cell derived therefrom of any one of claims 3-25, wherein said HLA-II function is reduced by a reduction in expression of an HLA-DR protein.
31. The modified pluripotent cell or cell derived therefrom of claim 30, wherein a gene encoding said HLA-DR protein is knocked out.
32. The modified pluripotent cell or cell derived therefrom of any one of claims 3-25, wherein said HLA-II function is reduced by a reduction in expression of an HLA-DQ protein.
33. The modified pluripotent cell or cell derived therefrom of claim 32, wherein a gene encoding said HLA-DQ protein is knocked out.
34. The modified pluripotent cell or cell derived therefrom of any one of claims 3-33, wherein said hypoimmunogenic pluripotent cell does not comprise an HLA-II function.
35. The modified pluripotent cell or cell derived therefrom of any one of claims 3-34, wherein said reduced susceptibility to NK cell killing is caused by an increased expression of a CD47 protein.
36. The modified pluripotent cell or cell derived therefrom of claim 35, wherein said increased CD47 protein expression results from a modification to an endogenous CD47 gene locus.

37. The modified pluripotent cell or cell derived therefrom of claim 35, wherein said increased CD47 protein expression results from a CD47 transgene.
38. The modified pluripotent cell or cell derived therefrom of any one of claims 35-37, wherein said CD47 protein has at least a 90% sequence identity to SEQ ID NO:3.
39. The modified pluripotent cell or cell derived therefrom of claim 38, wherein said CD47 protein has the sequence of SEQ ID NO:3.
40. The modified pluripotent cell or cell derived therefrom of any one of claims 3-39, further comprising a suicide gene that is activated by a trigger that causes said hypoimmunogenic pluripotent cell to die.
41. The modified pluripotent cell or cell derived therefrom of claim 40, wherein said suicide gene is a herpes simplex virus thymidine kinase gene (HSV-tk) and said trigger is ganciclovir.
42. The modified pluripotent cell or cell derived therefrom of claim 41, wherein said HSV-tk gene encodes a protein comprising at least a 90% sequence identity to SEQ ID NO:4.
43. The modified pluripotent cell or cell derived therefrom of claim 42, wherein said HSV-tk gene encodes a protein comprising the sequence of SEQ ID NO:4.
44. The modified pluripotent cell or cell derived therefrom of claim 40, wherein said suicide gene is an *Escherichia coli* cytosine deaminase gene (EC-CD) and said trigger is 5-fluorocytosine (5-FC).
45. The modified pluripotent cell or cell derived therefrom of claim 44, wherein said EC-CD gene encodes a protein comprising at least a 90% sequence identity to SEQ ID NO:5.
46. The modified pluripotent cell or cell derived therefrom of claim 45, wherein said EC-CD gene encodes a protein comprising the sequence of SEQ ID NO:5.
47. The modified pluripotent cell or cell derived therefrom of claim 40, wherein said suicide gene encodes an inducible Caspase protein and said trigger is a chemical inducer of dimerization (CID).
48. The modified pluripotent cell or cell derived therefrom of claim 47, wherein said gene encodes an inducible Caspase protein comprising at least a 90% sequence identity to SEQ ID NO:6.

49. The modified pluripotent cell or cell derived therefrom of claim 48, wherein said gene encodes an inducible Caspase protein comprising the sequence of SEQ ID NO:6.
50. The modified pluripotent cell or cell derived therefrom of any one of claims 47-49, wherein said CID is AP1903.
51. A cell derived from the modified pluripotent cell of any one of claims 3-50, wherein said cell is selected from the group consisting of an O- chimeric antigen receptor (O- CAR) cell, an endothelial cell, a dopaminergic neuron, a cardiac cell, a pancreatic islet cell, and a retinal pigment endothelium cell.
52. The cell of claim 51, wherein said O- CAR cell is an O- CAR-T cell.
53. The modified pluripotent cell or cell derived therefrom of any one of claims 1-51, wherein the modified pluripotent cell or cell derived therefrom is a cardiomyocyte or cardiomyocyte progenitor cell.
54. The modified pluripotent cell or cell derived therefrom of any one of claims 1-53 further comprising a reduced or eliminated expression of a CCR5 gene or a CXCR4 gene.
55. The modified pluripotent cell or cell derived therefrom of any one of claims 1-54 further comprising a reduced or eliminated expression of an NLRC5 gene.
56. The modified pluripotent cell or cell derived therefrom of any one of claims 1-55 further comprising a modified expression of at least one of gene selected from the group consisting of HLA-A, HLA-B, HLA-C, HLA-E, HLA-G, PD-L1, CTLA-4-Ig, CD47, CI-inhibitor, and IL-35.
57. The modified pluripotent cell or cell derived therefrom of any one of claims 1-56 further comprising a modified expression of at least one gene selected from RFX-5, RFXAP, RFXANK, NFY-A, NFY-B, NFY-C, and IRF-1.
58. The modified pluripotent cell or cell derived therefrom of any one of claims 1-57 further comprising a modified expression of at least one gene selected from the group consisting of OX40, GITR, 4-1BB, CD28, B7-1, B7-2, ICOS, CD27, HVEM, SLAM, CD226, PD1, CTL4, LAG3, TIGIT, TIM3, CD160, BTLA, CD244, CD30, TLT, VISTA, B7-H3, PD-L2, LFA-1, CD2, CD58, ICAM-3, TCRA, TCRB, FOXP3, HELIOS, ST2, PCSK9, CCR5, and APOC3.

59. The modified pluripotent cell or cell derived therefrom of any one of claims 1-58 further comprising at least one transgene selected from the group consisting of PDL-1, HLA-G, CD47, CD200, FASLG, CLC21, MFGE8, and SERPIN B9, or at least one transgene encoding an agonist of PDL-1, HLA-G, CD47, CD200, FASLG, CLC21, MFGE8, or SERPIN 9.
60. The modified pluripotent cell or cell derived therefrom of any one of claims 1-59 further comprising at least one transgene selected from the group consisting of TGF β , CD73, CD39, LAG3, IL1R2, ACKR2, TNFRSF22, TNFRSF23, TNFRS10, DAD1, and IFN γ R1 d39, or at least one transgene encoding an agonist of TGF β , CD73, CD39, LAG3, IL1R2, ACKR2, TNFRSF22, TNFRSF23, TNFRS10, DAD1, or IFN γ R1 d39.
61. A method of transplanting a cell, comprising administering one or more cells derived from the modified pluripotent cell of any one of claims 1-60 into a subject in need thereof, wherein said subject is a human, cow, pig, chicken, turkey, horse, sheep, goat, donkey, mule, duck, goose, buffalo, camel, yak, llama, alpaca, mouse, rat, dog, cat, hamster, guinea pig.
62. The method of claim 61, wherein said cell derived from the modified pluripotent cell is selected from the group consisting of an O- CAR cell, an O- endothelial cell, an O- dopaminergic neuron, an O- cardiac cell, an O- pancreatic islet cell, and an O- retinal pigment endothelium cell.
63. A method of treating a disease in a patient in need of transplanted cells, comprising administering to the patient a cell derived from the modified pluripotent cell of any one of claims 1-60.
64. The method of claim 63, wherein said cell derived from the modified pluripotent cell is selected from the group consisting of an O- CAR cell, an O- endothelial cell, an O- dopaminergic neuron, an O- cardiac cell, an O- pancreatic islet cell, and an O- retinal pigment endothelium cell.
65. The method of claim 63, wherein said disease is selected from the group consisting of Type I Diabetes, a cardiac disease, a neurological disease, a cancer, an ocular disease, and a vascular disease.
66. A method for generating a modified pluripotent cell that is ABO blood type O and Rh factor negative (Rh-) from a pluripotent cell that is not O-, comprising:

- a. eliminating or reducing the expression of any ABO blood group antigens A1, A2, and/or B to provide an ABO blood type O; and/or
 - b. eliminating or reducing expression of the Rhesus factor (Rh) blood group antigen to provide a blood type Rh negative (-).
67. A method for generating a hypoimmunogenic pluripotent ABO group O Rh factor negative (HIPO-) cell from an O- pluripotent cell comprising:
- a. eliminating the Major Histocompatibility Antigen Class I (HLA-I) function when compared to an unmodified pluripotent cell;
 - b. eliminating the Major Histocompatibility Antigen Class II (HLA-II) function when compared to an unmodified pluripotent cell; and
 - c. increasing the expression of CD47 as compared to an unmodified pluripotent cell.
68. The method of claim 66 or 67, wherein said pluripotent cell is of human, cow, pig, chicken, turkey, horse, sheep, goat, donkey, mule, duck, goose, buffalo, camel, yak, llama, alpaca, mouse, rat, dog, cat, hamster, or guinea pig origin.
69. The method of any one of claims 66-68, wherein said ABO blood group type O results from eliminating an ABO blood group protein expression.
70. The method of any one of claims 66-69, wherein the cell is endogenously type O.
71. The method of any one of claims 66-69, wherein the pluripotent cell is human and wherein the ABO blood group type O results from disrupting a human Exon 7 of the ABO gene.
72. The method of claim 71, wherein disrupting the human Exon 7 of the ABO gene is accomplished by a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 reaction that disrupts both alleles of the Exon 7.
73. The method of any one of claims 66-69, wherein said ABO blood group type O results from enzymatically modifying an ABO gene product on a surface of the pluripotent cell.
74. The method of claim 73, wherein said enzymatic modification removes a carbohydrate from said ABO gene product.
75. The method of claim 74, wherein said enzymatic modification removes a carbohydrate from an ABO A1 antigen, A2 antigen, and/or B antigen.

76. The method of any one of claims 66-69, wherein the cell is endogenously type Rh-.
77. The method of any one of claims 66-69, comprising eliminating an Rh protein expression.
78. The method of claim 77, wherein said type Rh- results from disrupting a gene encoding Rh C antigen, Rh E antigen, Kell K antigen (KEL), Duffy (FY) Fya antigen, Duffy Fy3 antigen, Kidd (JK) Jkb antigen, or Kidd SLC14A1 gene.
79. The method of claim 78, wherein said disruption results from a CRISPR/Cas9 reaction that disrupts both alleles of said gene.
80. The method of any one of claims 67-79, wherein said increased CD47 expression results from introducing at least one copy of a human CD47 gene under the control of a promoter into said parent cell.
81. The method of claim 80, wherein said promoter is a constitutive promoter.
82. The method of any one of claims 67-81, comprising disrupting both alleles of said B2M gene (e.g., using a CRISPR/Cas9 reaction).
83. The method of any one of claims 67-82, wherein said HLA-I function is reduced by reducing expression of a β -2 microglobulin protein.
84. The method of claim 83, wherein expression of the β -2 microglobulin protein is reduced by knocking out a gene encoding said β -2 microglobulin protein.
85. The method of claim 84, wherein said β -2 microglobulin protein has at least a 90% sequence identity to SEQ ID NO:1.
86. The method of claim 85, wherein said β -2 microglobulin protein has the sequence of SEQ ID NO:1.
87. The method of any one of claims 67-81, wherein said HLA-I function is reduced by reducing expression of an HLA-A protein.
88. The method of claim 87, wherein expression of said HLA-A protein is reduced by knocking out a gene encoding said HLA-A protein.

89. The method of any one of claims 67-81, wherein said HLA-I function is reduced by reducing expression of an HLA-B protein.
90. The method of claim 89, wherein expression of said HLA-B protein is reduced by knocking out a gene encoding said HLA-B protein.
91. The method of any one of claims 67-81, wherein said HLA-I function is reduced by reducing expression of an HLA-C protein.
92. The method of claim 91, wherein expression of said HLA-C protein is reduced by knocking out a gene encoding said HLA-C protein.
93. The method of any one of claims 67-92, wherein said hypoinmunogenic pluripotent cell does not comprise an HLA-I function.
94. The method of any one of claims 67-93, wherein said HLA-II function is reduced by reducing expression of a CIITA protein.
95. The method of claim 94, wherein expression of said CIITA protein is reduced by knocking out a gene encoding said CIITA protein.
96. The method of claim 95, wherein said CIITA protein has at least a 90% sequence identity to SEQ ID NO:2.
97. The method of claim 96, wherein said CIITA protein has the sequence of SEQ ID NO:2.
98. The method of any one of claims 67-97, comprising reducing expression of a CIITA protein by a CRISPR reaction that disrupts both alleles of a CIITA gene.
99. The method of any one of claims 67-98, wherein said HLA-II function is reduced by reducing expression of an HLA-DP protein.
100. The method of claim 99, wherein expression of said HLA-DP protein is reduced by knocking out a gene encoding said HLA-DP protein.
101. The method of any one of claims 67-98, wherein said HLA-II function is reduced by reducing expression of an HLA-DR protein.
102. The method of claim 101, wherein expression of said HLA-DR protein is reduced by knocking out a gene encoding said HLA-DR protein.

103. The method of any one of claims 67-98, wherein said HLA-II function is reduced by reducing expression of an HLA-DQ protein.
104. The method of claim 103, wherein expression of said HLA-DQ protein is reduced by knocking out a gene encoding said HLA-DQ protein.
105. The method of any one of claims 67-104, wherein said hypoimmunogenic pluripotent cell does not comprise an HLA-II function.
106. The method of any one of claims 67-105, wherein said increased expression of CD47 results from expression of a transgene that encodes a CD47 protein.
107. The method of claim 106, wherein said CD47 protein has at least a 90% sequence identity to SEQ ID NO:3.
108. The method of claim 107, wherein said CD47 protein has the sequence of SEQ ID NO:3.
109. The method of any one of claims 67-108, further comprising expressing a suicide gene that is activated by a trigger that causes said hypoimmunogenic pluripotent cell to die.
110. The method of claim 109, wherein said suicide gene is a herpes simplex virus thymidine kinase gene (HSV-tk) and said trigger is ganciclovir.
111. The method of claim 110, wherein said HSV-tk gene encodes a protein comprising at least a 90% sequence identity to SEQ ID NO:4.
112. The method of claim 111, wherein said HSV-tk gene encodes a protein comprising the sequence of SEQ ID NO:4.
113. The method of claim 109, wherein said suicide gene is an *Escherichia coli* cytosine deaminase gene (EC-CD) and said trigger is 5-fluorocytosine (5-FC).
114. The method of claim 113, wherein said EC-CD gene encodes a protein comprising at least a 90% sequence identity to SEQ ID NO:5.
115. The method of claim 114, wherein said EC-CD gene encodes a protein comprising the sequence of SEQ ID NO:5.

116. The method of claim 109, wherein said suicide gene encodes an inducible Caspase protein and said trigger is a specific chemical inducer of dimerization (CID).
117. The method of claim 116, wherein said gene encodes an inducible Caspase protein comprising at least a 90% sequence identity to SEQ ID NO:6.
118. The method of claim 117, wherein said gene encodes an inducible Caspase protein comprising the sequence of SEQ ID NO:6.
119. The method of any one of claims 116-118, wherein said CID is AP1903.
120. A method of transplanting a hypoimmunogenic pluripotent (HIP) cell or cell derived therefrom into a subject in need thereof comprising: determining an ABO blood group type of the HIP cell or cell derived therefrom, determining an ABO blood group type of the subject, and transplanting the HIP cell or cell derived therefrom only if the ABO blood group type of the HIP cell or cell derived therefrom matches the ABO blood group type of the subject.
121. The method of claim 120, further comprising: determining a Rhesus (Rh) factor type of the HIP cell or cell derived therefrom, determining an Rh factor type of the subject, and transplanting the HIP cell or cell derived therefrom only if the Rh blood type of the HIP cell or cell derived therefrom matches the Rh blood type of the subject.
122. The method of claim 120 or 121, wherein the HIP cell or cell derived therefrom comprises
- a. an endogenous Major Histocompatibility Antigen Class I (HLA-I) function that is reduced when compared to an unmodified pluripotent cell;
 - b. an endogenous Major Histocompatibility Antigen Class II (HLA-II) function that is reduced when compared to an unmodified pluripotent cell; and
 - c. an increased CD47 function that reduces susceptibility to NK cell killing.
123. The method of any one of claims 119-120, wherein the cell derived from the HIP cell is a cardiomyocyte or cardiomyocyte progenitor.