

CLAIMS

WHAT IS CLAIMED IS:

1. A population of mortal pluripotent stem cells (MPSCs), wherein the population of MPSCs express HLA-G and insulin, and wherein the population of MPSCs is capable of reaching at least 89 population doublings within about 90 days from a start of culturing the MPSCs.

2. The population of MPSCs of claim 1, wherein the population of MPSCs are capable of reaching: from about 25 to about 30 population doublings within about 12 days, from about 50 to about 55 population doublings within about 30 days, and/or from about 75 to about 80 population doublings within about 63 days, from a start of culturing the MPSCs.

3. The population of MPSCs of claim 1 or 2, that are capable of doubling in about 25 hours or from about 22 to about 27 hours.

~~4. The population of MPSCs of claim 3, that are capable of doubling in about 25 hours.~~

~~5.4. A~~ The population of ~~mortal pluripotent stem cells (MPSCs)~~ of any preceding claim, wherein the population of MPSCs expresses HLA-G and insulin, and wherein the population of MPSCs are free from a pathogen.

~~6.5. The population of MPSCs of any preceding claim 4, wherein the MPSCs are free from pathogen is a bacterium, a virus, or a cytomegalovirus.~~

~~7. The population of MPSCs of any preceding claim, wherein the MPSCs are free from a virus.~~

~~8. The population of MPSCs of any preceding claim, wherein the MPSCs are free from a cytomegalovirus.~~

~~9.6. The population of MPSCs of any preceding claim claim 4 or 5, wherein the MPSCs are free from a pathogen that is an EBV (Epstein-Barr virus), a human adenovirus (HAdV), a human cytomegalovirus (HCMV), a Hepatitis virus, a human immunodeficiency virus (HIV), a human papillomavirus (HPV), a Herpes Simplex Virus (HSV), a human T-lymphotropic virus (HTLV), a varicella virus (VZV), a *Corynebacterium*, a Hantavirus, a lymphocytic choriomeningitis virus (LCMV), a *Mycoplasma*, a *Treponema*, or any combination thereof.~~

~~10.7. The population of MPSCs of claim 95 or 6, wherein the pathogen is a virus and the virus is one or more of:~~ MPSCs are free from the

a. a Hepatitis virus, and wherein the Hepatitis virus comprises Hepatitis A, Hepatitis B, Hepatitis C, or a combination thereof;

- b. a Herpes Simplex Virus (HSV), wherein the Herpes Simplex Virus (HSV) comprises a human herpes virus 6 (HHV 6), a human herpes virus 8 (HHV 8), Herpes Simplex Virus (HSV 1), a Herpes Simplex Virus (HSV 2), or a combination thereof;
- c. a human immunodeficiency virus, wherein the human immunodeficiency virus comprises a human immunodeficiency virus 1 (HIV1), a human immunodeficiency virus 2 (HIV2), or a combination thereof;
- d. a human papillomavirus, wherein the human papillomavirus comprises HPV16, HPV18, or a combination thereof;
- e. a human T-lymphotropic virus, wherein the human T-lymphotropic virus comprises a human T-lymphotropic virus (HTLV 1), a human T-lymphotropic virus (HTLV 2), or a combination thereof; or
- f. a Hantavirus, and wherein the Hantavirus comprises a Hantaan Hantavirus, a Seoul Hantavirus, a Sin Nombre Hantavirus, or a combination thereof.

~~11. — The population of MPSCs of claim 9, wherein the MPSCs are free from the Herpes Simplex Virus (HSV), and wherein the Herpes Simplex Virus (HSV) comprises a human herpes virus 6 (HHV 6), a human herpes virus 8 (HHV 8), or a combination thereof.~~

~~12. — The population of MPSCs of claim 9, wherein the MPSCs are free from the human immunodeficiency virus, and wherein the human immunodeficiency virus comprises a human immunodeficiency virus 1 (HIV1), a human immunodeficiency virus 2 (HIV2), or a combination thereof.~~

~~13. — The population of MPSCs of claim 9, wherein the MPSCs are free from the human papillomavirus, and wherein the human papillomavirus comprises HPV16, HPV18, or a combination thereof.~~

~~14. — The population of MPSCs of claim 9, wherein the MPSCs are free from the Herpes Simplex Virus, and wherein the Herpes Simplex Virus comprises a Herpes Simplex Virus (HSV 1), a Herpes Simplex Virus (HSV 2), or a combination thereof.~~

~~15. — The population of MPSCs of claim 9, wherein the MPSCs are free from the human T-lymphotropic virus, and wherein the human T-lymphotropic virus comprises a human T-lymphotropic virus (HTLV 1), a human T-lymphotropic virus (HTLV 2), or a combination thereof.~~

~~16.8.~~ The population of MPSCs of claim ~~96~~, wherein the MPSCs are free from the *Corynebacterium*, and wherein the *Corynebacterium* comprises *Corynebacterium bovis*, *Corynebacterium sp.* (HAC2), or a combination thereof.

~~17. — The population of MPSCs of claim 9, wherein the MPSCs are free from the~~

~~Hantavirus, and wherein the Hantavirus comprises a Hantaan Hantavirus, a Seoul Hantavirus, a Sin Nombre Hantavirus, or a combination thereof.~~

~~18.9.~~ 18.9. The population of MPSCs of any preceding claim, wherein the population of the MPSCs further express one or more proteins of:

~~a. b-HCG, HSP90, CDX2, FGFR1, pAKT, pCREB1, HLA-A, HLA-B, HLA-C, or any combination thereof; or~~

~~b. KIR2DL4, Flt3L, NKp46, TCR, ILT-4, CD49f, CD8, CD10, CD11b, CD16, CD38, CD44, CD56, CD90/Thy-1, CD105, CD141, CD146, CD166, CD107a, or any combination thereof.~~

~~19. The population of MPSCs of any preceding claim, wherein the population of the MPSCs further express one or more proteins of KIR2DL4, Flt3L, NKp46, TCR, ILT-4, CD49f, CD3, CD4, CD8, CD10, CD11b, CD14, CD16, CD19, CD34, CD38, CD44, CD56, CD90/Thy-1, CD105, CD141, CD146, CD166, CD107a, or any combination thereof.~~

~~20. The population of MPSCs of any preceding claim, wherein the population of the MPSCs further express one or more proteins of IL-6, IL-8, MCP-1, CLXL2, PDGF-AA, VEGF, PAI-1, IL-10, or any combination thereof.~~

~~21.10.~~ 24.10. The population of MPSCs of any preceding claim, wherein at least some of the MPSCs do not express one or more proteins of (a) Ki-67, HSP70, p53, Syncytin, or a combination thereof; or (b) CD19, CD45, HLA-DR, or a combination thereof.

~~22.11.~~ 22.11. The population of MPSCs of any preceding claim, wherein the population of the MPSCs express one or more proteins of CD44, CD90, CD105, CD146, CD166, HLA-A, HLA-B, HLA-C, or a combination thereof.

~~23. The population of MPSCs of any preceding claim, wherein at least some of the MPSCs do not express one or more proteins of CD19, CD45, HLA-DR, or a combination thereof.~~

~~24.12.~~ 24.12. The population of MPSCs of any preceding claim, wherein more than 96% of the MPSCs do not express one or more proteins of (a) CD19, CD45, HLA-DR, or a combination thereof; or (b) do not express CD3.

~~25. The population of MPSCs of any preceding claim, that expresses CD16, CD56, or a combination thereof.~~

~~26. The population of MPSCs of any preceding claim, wherein at least some of the MPSCs do not express CD3.~~

~~27. The population of MPSCs of any preceding claim, wherein more than 96% of the MPSCs do not express CD3.~~

~~28-13.~~ The population of MPSCs of any preceding claim, wherein at least 65% of the population of the MPSCs express a HLA-G, wherein the HLA-G comprises HLA-G1, HLA-G2, HLA-G3, HLA-G4, HLA-G5, HLA-G6, HLA-G7, or any combination thereof.

~~29.—The population of MPSCs of claim 28, wherein the HLA-G comprises HLA-G1, HLA-G2, HLA-G3, HLA-G4, HLA-G5, HLA-G6, HLA-G7, or any combination thereof.~~

~~30.—The population of MPSCs of claim 28 or 29, wherein the HLA-G comprises HLA-G2, HLA-G4, HLA-G6, HLA-G7, or any combination thereof.~~

~~31.—The population of MPSCs of any one of claims 28-30, wherein the HLA-G comprises HLA-G6, HLA-G7, or a combination thereof.~~

~~32-14.~~ The population of MPSCs of any one of preceding claims 1-13, wherein less than 15% of the population of the MPSCs express HLA-G1.

~~33.—The population of MPSCs of any preceding claim, wherein at least 10% of the population of MPSCs are monoclonal.~~

~~34.—The population of MPSCs of claim 25, wherein from about 13% to about 15% of the population of MPSCs are monoclonal.~~

~~35-15.~~ The population of MPSCs of any preceding claim, that comprises at least 1×10^6 MPSCs.

~~36.—The population of MPSCs of any preceding claim, wherein the MPSCs have a stable karyotype as measured by an array-based whole-genome assay.~~

~~37-16.~~ The population of MPSCs of any preceding claim, wherein the MPSCs exhibit one or more of:

~~a. a stable karyotype as measured by an array-based whole-genome assay;~~

~~b. no chromosomal aberration from population doublings as measured by an array-based whole-genome assay; or-~~

~~c. no substantial chromosomal aberration from freezing and thawing, as measured by an array-based whole-genome assay.~~

~~38.—The population of MPSCs of any preceding claim, wherein the MPSCs exhibit no substantial chromosomal aberration from freezing and thawing, as measured by an array-based whole-genome assay.~~

~~39-17.~~ An in vitro method of growing a population of mortal pluripotent stem cells (MPSCs) of any one of claims 1-16, comprising seeding a subculture of the MPSCs at a density of from ~~about~~ 1,000 to ~~about~~ 5,000 cells/cm² in a culture medium, and culturing the cells, wherein the population of MPSCs express a HLA-G and insulin.

~~40.—A method of growing a population of mortal pluripotent stem cells (MPSCs),~~

comprising seeding a subculture of the MPSCs at a density from about 1,000 to about 5,000 cells/cm² in a culture medium, and culturing the cells, wherein the population of MPSCs express a HLA-G and insulin.

41.18. The method of claim ~~39 or 40~~17, wherein the culture medium is free from an animal component, a serum, or a fetal bovine serum.

~~42. The method of any one of claims 39-41, wherein the culture medium is free from serum.~~

~~43. The method of claim 38, wherein the culture medium is free from fetal bovine serum.~~

44.19. The method of ~~any one of claims 39-43~~claim 17 or 18, wherein the MPSCs are cultured for about 3 or 4 days.

~~45. The method of any one of claims 39-44, wherein the MPSCs are cultured for about 4 days.~~

~~46. The method of any one of claims 39-45, wherein the subculture of the MPSCs is seeded at a density of from about 2,000 to about 4,000 cells/cm².~~

~~47. A population of cells produced by the method of any one of claims 39-46.~~

48.20. ~~A method of producing cells,~~ The method of any one of claims 17-19, further comprising contacting the population of MPSCs ~~of any one of claims 1-38~~ with one or more inducing agents, whereby a population of produced cells are obtained by the method.

49.21. The method of claim ~~48~~20, wherein the produced cells are ectodermal cells, mesodermal cells, endodermal cells, pancreatic cells, pancreatic progenitor cells (PPCs), neural cells (NCS), neural progenitor cells, hepatic cells, hepatic progenitor cells, natural killer cells, adipocytes, chondrocytes, or osteocytes.

~~50. The method of claim 48, wherein the produced cells are mesodermal cells.~~

~~51. The method of claim 48, wherein the produced cells are endodermal cells.~~

22. The method of claim 21, wherein the produced cells are the neural cells (NCS) or the neural progenitor cells.

52.23. The method of claim ~~48~~21, wherein the produced cells are the pancreatic cells or the pancreatic progenitor cells (PPCs).

53.24. The method of claim ~~52~~20, wherein the one or more inducing agents comprise (a) bFGF (basic fibroblast growth factor); (b) 2-mercaptoethanol and nicotinamide; (c) retinoic acid, (d) fibroblast growth factor (FGF), a steroid, or a cytokine; or (e) FGF.

25. Use of the population of MPSCs of any one of claims 1-16 in the manufacture of a medicament for the treatment of a motor neuron disease, a spinal cord injury, a pancreatic

injury, or a disease or disorder caused by a pancreatic injury in a subject in need thereof.

26. The population of MPSCs of any one of claims 1-16, the neural stem cells (NSCs) produced by the method of claim 22, a composition comprising the neural stem cells (NSCs) produced by the method of claim 22, the pancreatic progenitor cells (PPCs) produced by the method of claim 23, or a composition comprising the pancreatic progenitor cells (PPCs) produced by the method of claim 23 for use in therapy.

27. Use of the neural stem cells (NSCs) produced by the method of claim 22 in the manufacture of a medicament for the treatment of a motor neuron disease or a spinal cord injury.

28. Use of a composition comprising the neural stem cells (NSCs) produced by the method of claim 22 in the manufacture of a medicament for the treatment of a motor neuron disease or a spinal cord injury.

29. Use of the pancreatic progenitor cells (PPCs) produced by the method of claim 23 in the manufacture of a medicament for the treatment of a pancreatic injury or a disease or disorder caused by a pancreatic injury.

30. Use of a composition comprising the pancreatic progenitor cells (PPCs) produced by the method of claim 23 in the manufacture of a medicament for the treatment of a pancreatic injury or a disease or disorder caused by a pancreatic injury.

~~54. The method of claim 53, wherein the one or more inducing agents further comprise 2-mercaptoethanol and nicotinamide.~~

~~55. The method of any one of claims 52-54, wherein the PPCs comprise β -HCG, CDX2, HLA-G, or any combination thereof.~~

~~56. The method of claim 55, wherein the PPCs comprise β -HCG and CDX2; β -HCG and HLA-G; CDX2 and HLA-G; or HCG, CDX2, and HLA-G.~~

~~57. The method of claim 55 or 56, wherein the PPCs further comprise PDX1, FOXA2, SOX9, or any combination thereof.~~

~~58. The method of claim 48, wherein the produced cells are neural cells (NCS) or neural progenitor cells.~~

~~59. The method of claim 58, wherein the one or more inducing agents comprise retinoic acid.~~

~~60. The method of claim 58 or 59, wherein the NCS cells comprise RAR- β , CDX2, HLA-G, or any combination thereof.~~

~~61. The method of claim 60, wherein the NCS cells comprise RAR- β and CDX2; RAR- β and HLA-G; CDX2 and HLA-G; or RAR- β , CDX2 and HLA-G.~~

62. — The method of claim 60 or 61, wherein the NCS cells further comprise N-CAD, NESTIN, SOX2, PAX6, or any combination thereof.

63. — The method of claim 48, wherein the produced cells are hepatic cells or hepatic progenitor cells.

64. — The method of claim 63, wherein the one or more inducing agents comprise a fibroblast growth factor (FGF), a steroid, and a cytokine.

65. — The method of claim 48, wherein the produced cells are natural killer cells and the inducing agent comprises an FGF.

66. — The method of claim 65, wherein the natural killer cells are CD16+, CD56+, and CD3-.

67. — The method of claim 66, wherein the natural killer cells further are HLA-G+ and CDX2+.

68. — The method of claim 48, wherein the produced cells comprise adipocytes, chondrocytes, osteocytes, or any combination thereof.

69. — The method of claim 68, wherein the produced cells comprise adipocytes and chondrocytes.

70. — The method of claim 68, wherein the produced cells comprise adipocytes and osteocytes.

71. — The method of claim 68, wherein the produced cells comprise chondrocytes and osteocytes.

72. — The method of claim 68, wherein the produced cells comprise adipocytes, chondrocytes, and osteocytes.

73. — The method of claim 68, wherein the produced cells comprise adipocytes.

74. — The method of claim 68, wherein the adipocytes comprise leptin, HOXC8, HOXC9, Ucp1, CIDEA, PRDM16, Zic1, Lhx8, Eva1, Epsti1, Cd137, Tmem26, Tbx1, Cited1, Shox2, amino acid transporter ASC-1, amino acid transporter PAT2, purinergic receptor P2RX5, ATGL, CAV1, FABP4, COX4, LMNB1, or a combination thereof.

75. — The method of claim 73 or 74, wherein the adipocytes comprise white adipocytes.

76. — The method of claim 75, wherein the white adipocytes comprise leptin, HOXC8, HOXC9, or a combination thereof.

77. — The method of claim 73 or 74, wherein the adipocytes comprise brown adipocytes.

78. — The method of claim 77, wherein the brown adipocytes comprise Ucp1, CIDEA,

PRDM16, Zic1, Lhx8, Eva1, Epsti1, or a combination thereof.

~~79. The method of claim 73 or 74, wherein the adipocytes comprise beige adipocytes.~~

~~80. The method of claim 79, wherein the beige adipocytes comprise Cd137, Tmem26, Tbx1, Cited1, Shox2, or a combination thereof.~~

~~81. The method of claim 73 or 74, wherein the adipocytes comprise beige fat cell precursors.~~

~~82. The method of claim 81, wherein the beige fat cell precursors comprise CD137, TMEM26, or a combination thereof.~~

~~83. The method of claim 68, wherein the produced cells comprise chondrocytes.~~

~~84. The method of claim 83, wherein the chondrocytes comprise Annexin A6, CD44, CD151, ITM2A, FAM20B, FoxC1, FoxC2, SOX5, SOX6, SOX9, Aggrecan, Cathepsin B, CHADL, Chondroadherin, Collagen II, Collagen IV, CRTAC1, DSPG3, IBSP/Sialoprotein II, Matrilin-1, Matrilin-3, Matrilin-4, MIA, Otoraplin/OTOR, URB, or a combination thereof.~~

~~85. The method of claim 68, wherein the produced cells comprise osteocytes.~~

~~86. The method of claim 85, wherein the osteocytes comprise a pre-osteoblast, an osteoblast, embedding osteoblast, osteoid-osteocyte, mineralizing osteocyte, or a mature osteocyte.~~

~~87. The method of claim 85 or 86, wherein the osteocytes comprise RUNX2, OCN, E11, DMP1, PHEX, MEPE, sclerostin, CapG, ORP150, or a combination thereof.~~

~~88. The method of claim 85 or 86, wherein the osteocytes comprise the pre-osteoblast, and wherein the pre-osteoblast comprises RUNX2.~~

~~89. The method of claim 85 or 86, wherein the osteocytes comprise the pre-osteoblast, and wherein the pre-osteoblast comprises RUNX2.~~

~~90. The method of claim 85 or 86, wherein the osteocytes comprise the osteoblast, and wherein the osteoblast comprises RUNX2 and OCN.~~

~~91. The method of claim v, wherein the osteocytes comprise the embedding osteoblast, and wherein the embedding osteoblast comprises OCN, E11, DMP1, PHEX, and CapG.~~

~~92. The method of claim 85 or 86, wherein the osteocytes comprise the osteoid osteocyte or the mineralizing osteocyte, and wherein the osteoid-osteocyte or the mineralizing osteocyte comprises OCN, E11, DMP1, PHEX, MEPE, and CapG.~~

~~93. The method of claim 85 or 86, wherein the osteocytes comprise the mature osteocyte, and wherein the mature osteocyte comprises DMP1, PHEX, MPEP, Sclerostin, CapG, and ORP150.~~

94.—A population of mortal pluripotent stem cells (MPSCs), wherein the population of MPSCs express HLA-G, and wherein the population of MPSCs comprise a phenotype that comprises one or more of: negative for Indoleamine 2-3 deoxygenase (IDO) secretion, negative for kynurenine secretion, and positive for interleukin 2 (IL-2) secretion.

95.—The population of MPSCs of claim 94, wherein the population of MPSCs comprise a phenotype of negative for Indoleamine 2-3 deoxygenase (IDO) secretion, negative for kynurenine secretion, and positive for interleukin 2 (IL-2) secretion.