CLAIMS

1. A pharmaceutical composition comprising cells committed to the generation of heart tissue, in which the cells show:

(a) an observed increase in expression level as compared to a reference of MEF2C and optionally at least one gene further selected from the group consisting of Nkx2.5, Tbx5, GATA4, GATA6, Mesp1, FOG1 FOG2, Flk1 and homologues thereof; and/or

(b) an observed presence of at least one polypeptide species chosen from the group consisting of Nkx2.5, Tbx5, GATA4, GATA6, Mesp1, FOG1 FOG2, Flk1 and homologues thereof, as compared to a reference and nuclear translocation to the nuclei of the cell of one or more of Nkx2.5 and MEF2C;

and at least one pharmaceutically acceptable excipient, in which the at least one pharmaceutically acceptable excipient is a preservation solution which comprises dimethylsufoxide (DMSO), ions, pH buffers, impermeants, colloids, and metabolites.

2. A pharmaceutical composition according to Claim 1, in which the identity of the cells committed to the generation of heart tissue corresponds to an observed increase in expression level of at least one gene by a minimum of 2-fold as determined by qPCR, as compared to a reference.

3. A pharmaceutical composition according to any preceding claim, in which the preservation solution is chosen in the group comprising preservation solutions which allow cryopreservation at temperatures between -196°C and 0°C and preservation solutions which allow preservation at temperatures between 0°C and +40°C.

4. A pharmaceutical composition according to any preceding claim, in which the preservation solution is a preservation solution which may further contain glycerol, sucrose, serum albumin, trehalose, or any combination thereof.

5. A pharmaceutical composition according to Claim 1, in which the ions are selected from the group consisting of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and combinations of the ions.

6. A pharmaceutical composition according to Claim 1, in which the pH buffers are selected from the group consisting in $H_2PO_4^-$, HCO_3^-, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) and mixtures thereof.

7. A pharmaceutical composition according to Claim 1, in which the impermeants are selected from the group consisting of lactobionate, sucrose, mannitol, glucose and combinations thereof.

8. A pharmaceutical composition according to Claim 1, in which the colloid is Dextran-40.

9. A pharmaceutical composition according to Claim 1, in which the metabolites are selected from the group consisting of adenosine, glutathione, and combinations thereof.

10. A pharmaceutical composition according to any preceding claim, in which the at least one pharmaceutically acceptable excipient comprises at least one component selected from the group consisting of growth factors, cytokines, proteins involved in organogenesis signalling, pharmaceuticals, platelet lysate, serum, isotopes, means for tracing cells in vivo, diluents, lubricants, matrix or scaffold materials, and combinations thereof.

11. A pharmaceutical composition according to any preceding claim, in which the cells committed to the generation of heart tissue are stem cells (provided their production implies no human embryo destruction).

12. A pharmaceutical composition according to any preceding claim, in which the cells committed to the generation of heart tissue are derived from stem cells (provided their production implies no human embryo destruction).

13. A pharmaceutical composition according to Claim 11 or 12, in which the stem cells are selected in the group consisting of adult stem, including mesenchymal stem cells; embryonic stem cells (provided their production implies no human embryo destruction); induced pluripotent stem cells (IPS); marrow-isolated adult multilineage inducible cells (MIAMI); resident cardiac stem cells; vegetal stem cells; and combinations thereof.

14. A pharmaceutical composition according to any preceding claim, in which the cells committed to the generation of heart tissue are mammalian cells.

15. A pharmaceutical composition according to any preceding claim, in which the cells committed to the generation of heart tissue are cardiopoietic cells which can be derived from human adult mesenchymal stem cells, human embryonic stem cells (provided their production

implies no human embryo destruction), embryonic-like stem cells, inducible pluripotent stem cells, cardiac resident stem cells or any other adapted source or combination thereof.

16. A pharmaceutical composition according to Claim 15, in which between 0% and 50% of the total number of cells, preferably between 0% and 15% of the total number of cells are non-cardiopoietic cells, including cardiomyocytes, hematopoietic cells, endothelial progenitor cells, adipoblasts, adipocytes, chondroblasts, chondrocytes, osteoblasts osteocytes, neuroblasts and neurocytes.

17. A pharmaceutical composition according any of Claims 1 to 16 for use for the treatment of ischemic cardiomyopathy, acute myocardial infarction, chronic myocardial infarction, heart failure of non-ischemic origin, heart failure of ischemic origin, congenital cardiomyopathy, or combination thereof.

18. A process for the manufacture of a pharmaceutical composition according to any preceding claim comprising the following steps:

(a) obtaining cells from which cells committed to the generation of heart tissue can derive;

(b) culturing the cells in conditions which allow to obtain cells committed to the generation of heart tissue;

(c) harvesting the committed cells; and

(d) adding to the committed cells at least one pharmaceutically acceptable excipient in which the at least one pharmaceutically acceptable excipient is a preservation solution which comprises dimethylsulfoxide (DMSO), ions, pH buffers, impermeants, colloids, and metabolites.

19. A process according to Claim 18, in which the cells from which cells committed to the generation of heart tissue can derive are put into contact with a cardiogenic composition composed of one or more cardiogenic substances chosen amongst the group consisting of: Activin A, α thrombin, angiopoietin, Bone morphogenetic proteins (BMP) such as BMP-1, BMP-2, BMP-4, BMP-5, BMP-6, Cardiotrophin 1, Cardiogenol C, Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factors (FGF) such as FGF-1, FGF-2, FGF 4, FGF 5, FGF 12, FGF 13, FGF 15, FGF 20, Granulocyte-colony stimulating factor (G-CSF), Granulocyte-macrophage colony stimulating factor (GM-CSF), Growth differentiation factor-

9 (GDF-9), Hepatocyte growth factor (HGF), Insulin-like growth factor (IGF) such as IGF-1, IGF-2, Myostatin (GDF-8), Neurotrophins such as NT-3, NT-4, NT-1 and Nerve growth factor (NGF), Platelet-derived growth factor (PDGF) such as PDGF-beta, PDGF-AA, PDGF-BB, Thrombopoietin (TPO), TGF- (Transforming growth factor alpha), Transforming growth factors β (TGF- β) such as TGF β1, TGF β2, TGF-β3, VEGF (Vascular endothelial growth factor) such as VEGF A, VEGF C, TNF α, Leukemia Inhibitory Factor (LIF), interleukin 6 (IL-6), retinoic acid, C SDF-1 (stromal cell-derived factor-1), BDNF (brain-derived neurotrophic factor), Periostin, Angiotensin II, Flt3 Ligand, Glial-Derived Neurotrophic Factor, Insulin-Like Growth Factor Binding Protein-3, Insulin-Like Growth Factor Binding Protein-5, Interleukin-3, Interleukin-8, Midkine, Progesterone, Putrescine, Stem Cell Factor, TGF-alpha, Wntl, Wnt3a, Wnt5a, caspase-4, chemokine ligand 1, chemokine ligand 2, chemokine ligand 5, chemokine ligand 7, chemokine ligand 11, chemokine ligand 20, haptoglobin, lectin, cholesterol 25-hydroxylase, syntaxin-8, syntaxin-11, ceruloplasmin, complement component 1, complement component 3, integrin alpha 6, lysosomal acid lipase 1, β-2 microglobulin, ubiquitin, macrophage migration inhibitory factor, cofilin, cyclophillin A, FKBP12, NDPK, profilin 1, cystatin C, calcyclin, stanniocalcin-1, PGE-2, mpCCL2, IDO, iNOS, HLA-G5, M-CSF, , PIGF, MCP-1, extracellular matrix molecules, CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL7 (MCP-3), CCL20 (MIP-3α), CCL26 (eotaxin-3), CX3CL1 (fractalkine), CXCL5 (ENA-78), CXCL11 (i-TAC), CXCL1 (GROα), CXCL2 (GROβ), CXCL8 (IL-8), CCL10 (IP-10), and combinations thereof.

20. A process according to Claim 18 or 19, in which the cardiogenic composition is diluted in a medium containing compounds selected from the group consisting of foetal calf serum, human serum, platelet lysate, platelet-derived growth factor, and mixtures thereof and compounds selected from:

(a) a first group consisting of: TGFβ-1, BMP-4, α-thrombin, a compound selected from the group consisting of Cardiotrophin 1 and IL 6, and a compound selected from the group consisting of Cardiogenol C and retinoic acid;

(b) a second group consisting of TGFβ-1, BMP-4, α thrombin, Cardiotrophin 1, IL-6, retinoic acid and Cardiogenol C;

(c) a third group consisting of Activin-A, FGF-2, IL 6, IGF-1 and retinoic acid; and

(d) a fourth group consisting of TGF β1, TGF β2, TNF-α, BMP-1, BMP-2, BMP-4, BMP-6, FGF 2, FGF 4, FGF 5, FGF 12, FGF 13, FGF 15, FGF 20, leukaemia inhibitory factor, VEGF A, VEGF C, insulin like growth factor 1, interleukin 6 (IL-6), Activin A, α thrombin, Retinoic acid, Cardiotrophin 1, Cardiogenol C, and combinations thereof.

21. A process according to any of Claims 18 to 20, in which when cells committed to the generation of heart tissue are cardiopoietic cells, identity of the cardiopoietic cells corresponds to an observed increase in expression level of at least one gene in the group consisting of Nkx2.5, Tbx5, MEF2C, GATA4, GATA6, Mesp1, FOG1, FOG2, Flk1 and homologues thereof.

22. A process according to Claim 21, in which the increase of the gene expression is a minimum 2-fold as determined by qPCR method, as compared to a reference.

23. A process according to any of Claims 18 to 22, in which when cells committed to the generation of heart tissue are cardiopoietic cells, identity of the cardiopoietic cells is considered fulfilled by the observed presence of at least one polypeptide species chosen from the group consisting of Nkx2.5, Tbx5, MEF2C, GATA4, GATA6, Mesp1, FOG1, FOG2, Flk1 and homologues thereof, as compared to a reference, and either Nkx2.5 or MEF2C or both being further translocated to the nuclei of the cardiopoietic cells.

24. A process according to any of Claims 18 to 23, in which homogeneity is fulfilled when at least 50%, preferably at least 85%, in a given sample are cardiopoietic cells.

25. A kit for the administration of a pharmaceutical composition according to Claims 1 to 17 which comprises a container containing the pharmaceutical composition.

CLAIMS

1. A pharmaceutical composition comprising cells committed to the generation of heart tissue, in which the cells are cardiopoietic cells, and in which the cardiopoietic cells show:

(a) an observed increase in expression level as compared to a reference of MEF2C and optionally at least one gene further selected from the group consisting of Nkx2.5, Tbx5, GATA4, GATA6, Mesp1, FOG1 FOG2, Flk1 and homologues thereof; and/or

(b) an observed presence of at least one polypeptide species chosen from the group consisting of Nkx2.5, Tbx5, GATA4, GATA6, Mesp1, FOG1 FOG2, Flk1 and homologues thereof, as compared to a reference and nuclear translocation to the nuclei of the cell of one or more of Nkx2.5 and MEF2C;

and at least one pharmaceutically acceptable excipient, in which the at least one pharmaceutically acceptable excipient is a preservation solution which comprises dimethylsufoxide (DMSO), ions, pH buffers, impermeants, colloids, and metabolites.

2. A pharmaceutical composition according to Claim 1, in which the identity of the cells committed to the generation of heart tissue corresponds to an observed increase in expression level of at least one gene by a minimum of 2-fold as determined by qPCR, as compared to a reference.

3. A pharmaceutical composition according to any preceding claim, in which the preservation solution is chosen in the group comprising preservation solutions which allow cryopreservation at temperatures between -196°C and 0°C and preservation solutions which allow preservation at temperatures between 0°C and +40°C.

4. A pharmaceutical composition according to any preceding claim, in which the preservation solution is a preservation solution which may further contain glycerol, sucrose, serum albumin, trehalose, or any combination thereof.

5. A pharmaceutical composition according to Claim 1, in which the ions are selected from the group consisting of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and combinations of the ions.

6. A pharmaceutical composition according to Claim 1, in which the pH buffers are selected from the group consisting in $H_2PO_4^-$, HCO_3^-, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) and mixtures thereof.

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9. A pharmaceutical composition according to Claim 1, in which the metabolites are selected from the group consisting of adenosine, glutathione, and combinations thereof.

10. A pharmaceutical composition according to any preceding claim, in which the at least one pharmaceutically acceptable excipient comprises at least one component selected from the group consisting of growth factors, cytokines, proteins involved in organogenesis signalling, pharmaceuticals, platelet lysate, serum, isotopes, means for tracing cells in vivo, diluents, lubricants, matrix or scaffold materials, and combinations thereof.

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16. A pharmaceutical composition according to Claim 15, in which between 0% and 50% of the total number of cells, preferably between 0% and 15% of the total number of cells are non-cardiopoietic cells, including cardiomyocytes, hematopoietic cells, endothelial progenitor cells, adipoblasts, adipocytes, chondroblasts, chondrocytes, osteoblasts osteocytes, neuroblasts and neurocytes.

17. A pharmaceutical composition according any of Claims 1 to 16 for use for the treatment of ischemic cardiomyopathy, acute myocardial infarction, chronic myocardial infarction, heart failure of non-ischemic origin, heart failure of ischemic origin, congenital cardiomyopathy, or combination thereof.

18. A process for the manufacture of a pharmaceutical composition according to any preceding claim comprising the following steps:

(a) obtaining cells from which cells committed to the generation of heart tissue can derive;

(b) culturing the cells in conditions which allow to obtain cells committed to the generation of heart tissue;

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(b) a second group consisting of TGFβ-1, BMP-4, α thrombin, Cardiotrophin 1, IL-6, retinoic acid and Cardiogenol C;

(c) a third group consisting of Activin-A, FGF-2, IL 6, IGF-1 and retinoic acid; and

(d) a fourth group consisting of TGF β1, TGF β2, TNF-α, BMP-1, BMP-2, BMP-4, BMP-6, FGF 2, FGF 4, FGF 5, FGF 12, FGF 13, FGF 15, FGF 20, leukaemia inhibitory factor, VEGF A, VEGF C, insulin like growth factor 1, interleukin 6 (IL-6), Activin A, α thrombin, Retinoic acid, Cardiotrophin 1, Cardiogenol C, and combinations thereof.

21. A process according to any of Claims 18 to 20, in which when cells committed to the generation of heart tissue are cardiopoietic cells, identity of the cardiopoietic cells corresponds to an observed increase in expression level of at least one gene in the group consisting of Nkx2.5, Tbx5, MEF2C, GATA4, GATA6, Mesp1, FOG1, FOG2, Flk1 and homologues thereof.

22. A process according to Claim 21, in which the increase of the gene expression is a minimum 2-fold as determined by qPCR method, as compared to a reference.

23. A process according to any of Claims 18 to 22, in which when cells committed to the generation of heart tissue are cardiopoietic cells, identity of the cardiopoietic cells is considered fulfilled by the observed presence of at least one polypeptide species chosen from the group consisting of Nkx2.5, Tbx5, MEF2C, GATA4, GATA6, Mesp1, FOG1, FOG2, Flk1 and homologues thereof, as compared to a reference, and either Nkx2.5 or MEF2C or both being further translocated to the nuclei of the cardiopoietic cells.

24. A process according to any of Claims 18 to 23, in which homogeneity is fulfilled when at least 50%, preferably at least 85%, in a given sample are cardiopoietic cells.

25. A kit for the administration of a pharmaceutical composition according to Claims 1 to 17 which comprises a container containing the pharmaceutical composition.