Claims

1. A method for inducing cardiomyocyte differentiation comprising the steps of:

(i) providing at least one pluripotent stem cell;

(ii) contacting the pluripotent stem cell(s) with:

(a) at least one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor;

(b) at least one immunosuppressant; and

(c) at least one Wnt modulator; in the order listed.

~~2. The method according to claim 1, comprising contacting the pluripotent stem cell(s) with the p38-MAPK inhibitor; the immunosuppressant and the Wnt modulator sequentially, in any order.~~

~~3. The method according to claim 1 or 2, comprising contacting the pluripotent stem cell(s) with the p38-MAPK inhibitor; the immunosuppressant and the Wnt modulator in the order listed.~~

~~4. The method according to claim 1, comprising contacting the pluripotent stem cell(s) with the p38-MAPK inhibitor, the immunosuppressant and the Wnt modulator concurrently.~~

~~5. The method according to claim 4, comprising contacting the pluripotent stem cell(s) with a composition comprising at least one p38-MAPK inhibitor, at least one immunosuppressant and at least one Wnt modulator.~~

~~6~~2. The method according to ~~any one of the preceding~~ claims 1, wherein the pluripotent stem cell(s) comprises isolated pluripotent stem cell(s).

~~7~~3. The method according to ~~any one of the preceding~~ claims 1 or 2, wherein the pluripotent stem cell(s) comprises induced pluripotent stem cell(s) or embryonic stem cell(s).

~~8~~4. The method according to any one of the preceding claims, wherein the pluripotent stem cell(s) is part of an embryoid body.

~~9~~5. The method according to claim ~~8~~4, comprising the steps of:

(i) inducing pluripotent stem cells to form at least one embryoid body;

(ii) contacting the embryoid body with:

(a) at least one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor;

(b) at least one immunosuppressant and

(c) at least one Wnt modulator.

~~10~~6. ~~The method according to claim 1 or 8~~ A method for inducing cardiomyocyte differentiation, comprising (i) contacting ~~the~~ pluripotent stem cell(s) or a embryoid body with a first composition comprising a p38-MAPK inhibitor, (ii) contacting the pluripotent stem cell(s) or embryoid body with a second composition comprising a p38-MAPK inhibitor and an immunosuppressant and (iii) contacting the pluripotent stem cell(s) or embryoid body with a third composition comprising a Wnt inhibitor.

~~11~~7. ~~The method according to claim 1 or 8~~ A method for inducing cardiomyocyte differentiation, comprising (i) contacting ~~the~~ pluripotent stem cell(s) or a embryoid body with a composition B comprising a p38-MAPK inhibitor, (ii) contacting the pluripotent stem cell(s) or embryoid body with a composition C comprising a p38-MAPK inhibitor, an immunosuppressant and a Wnt inhibitor and (iii) contacting the

pluripotent stem cell(s) or embryoid body with a composition D comprising a p38-MAPK inhibitor, an immunosuppressant and a Wnt inhibitor.

~~12~~8. The method according to claim ~~11~~7, wherein the composition B additionally comprises ascorbic acid, bone morphogenetic protein (BMP), activin A and/or fibroblast growth factor (FBF).

~~13~~9. The method according to claim ~~11~~7 or ~~12~~8, wherein the composition C additionally comprises ascorbic acid, Noggin, a transforming growth factor β (TGF-β) type I receptor activin receptor-like kinase 5 (ALK5) kinase inhibitor and vascular endothelial growth factor (VEGF).

140. The method according to claim ~~13~~9, wherein the composition C additionally comprises an epidermal growth factor receptor kinase inhibitor.

~~15~~1. The method according to any one of claims ~~11~~7 to 130, wherein the composition D additionally comprises ascorbic acid and vascular endothelial growth factor (VEGF).

~~16~~2. The method according to claim ~~15~~1, wherein the composition D additionally comprises an epidermal growth factor receptor kinase inhibitor.

~~17~~3. The method according to any one of the preceding claims, wherein the p38-MAPK modulator comprises SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), BIRB 796 (1-[5-tert-butyl-2-(4-methylphenyl)pyrazol-3-yl]-3-[4-(2-morpholin-4-ylethoxy)naphthalen-1-yl]urea), SB 202190 (4-[4-(4-fluorophenyl)-5-pyridin-4-yl-1,3-dihydroimidazol-2-ylidene]cyclohexa-2,5-dien-1-one, VX-702 6-(N-carbamoyl-2,6-difluoroanilino)-2-(2,4-difluorophenyl)pyridine-3-carboxamide), LY2228820 (5-[2-tert-butyl-4-(4-fluorophenyl)-1H-

imidazol-5-yl]-3-(2,2-dimethylpropyl)imidazo[4,5-b]pyridin-2-amine; methanesulfonic acid), VX-745 (5-(2,6-dichlorophenyl)-2-(2,4-difluorophenylthio)-6H-pyrimido[1,6-b]pyridazin-6-one) or PH-797804 (3-[3-bromo-4-[(2,4-difluorophenyl)methoxy]-6-methyl-2-oxopyridin-1-yl]-N,4-dimethylbenzamide).

184. The method according to any one of the preceding claims, wherein the immunosuppressant comprises cyclosporin-A (CSA), tacrolimus or sirolimus.

195. The method according to any one of the preceding claims, wherein the Wnt modulator comprises IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP3 (2-(3-(4-fluorophenyl)-3,4,6,7-tetrahydro-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP2 (N-(6-Methyl-2-benzothiazolyl)-2-[(3,4,6,7-tetrahydro-4-oxo-3-phenylthieno[3,2-d]pyrimidin-2-yl)thio]-acetamide), pyrvinium (2-[(E)-2-(2,5-dimethyl-1-phenylpyrrol-3-yl)ethenyl]-N,N,1-trimethylquinolin-1-ium-6-amine, BIO ((3Z)-6-bromo-3-[3-(hydroxyamino)indol-2-ylidene]-1H-indol-2-one), CHIR99021 (6-[2-[[4-(2,4-dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)pyrimidin-2-yl]amino]ethylamino]pyridine-3-carbonitrile) or XAV939 (2-[4-(trifluoromethyl)phenyl]-1,5,7,8-tetrahydrothiopyrano[4,3-d]pyrimidin-4-one).

2016. A method for inducing cardiomyocyte differentiation~~The method according to any one of the preceding claims~~, comprising contacting ~~the~~ pluripotent stem cell(s) or embryoid body with

(a) the p38-MAPK inhibitor 4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine (SB 203580);

(b) the immunosuppressant cyclosporin-A (CSA); and

(c) the Wnt modulator IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide).

~~21~~17. The method according to any one of the preceding claims; comprising additionally contacting the pluripotent stem cell(s) or embryoid body with at least one Reactive Oxygen Species (ROS) activator.

~~22. The method according to claim 21 when dependent on claim 4 and/or claim 8, comprising contacting the pluripotent stem cell(s) or embryoid body with the p38-MAPK inhibitor, the immunosuppressant, the Wnt modulator and the ROS activator concurrently.~~

~~23. The method according to claim 22, comprising contacting the pluripotent stem cell(s) or embryoid body with a composition comprising at least one p38-MAPK inhibitor, at least one immunosuppressant, at least one Wnt modulator and at least one ROS activator.~~

~~24~~18. The method according to ~~any one of~~ claims ~~21~~7 ~~to 23~~; wherein the ROS activator comprises hydrogen peroxide (H_2O_2)

~~25. A combination and/or kit comprising at least one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor; at least one immunosuppressant; and at least one Wnt modulator; for use in inducing cardiomyocyte differentiation.~~

~~26. A combination and/or kit comprising at least one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor; at least one immunosuppressant; at least one Wnt modulator; and at least one ROS activator; for use in inducing cardiomyocyte differentiation.~~

~~27. A combination and/or kit comprising at least two components from the group consisting of the following components: a p38 mitogen-activated~~

protein kinase (p38-MAPK) modulator; an immunomodulator; and a Wnt modulator.

28. A composition comprising at least one one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor; at least one immunosuppressant; and at least one Wnt modulator; for use in inducing cardiomyocyte differentiation.

29. A composition comprising at least two components from the group consisting of the following components: a p38 mitogen-activated protein kinase (p38-MAPK) modulator; an immunomodulator; and a Wnt modulator.

30. The composition according to claim 28 or 29, further comprising at least one ROS activator.

31. The combination and/or kit or composition according to any one of claims 25 to 30, wherein the p38-MAPK inhibitor comprises SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), BIRB 796 (1-[5-tert-butyl-2-(4-methylphenyl)pyrazol-3-yl]-3-[4-(2-morpholin-4-ylethoxy)naphthalen-1-yl]urea), SB 202190 (4-[4-(4-fluorophenyl)-5-pyridin-4-yl-1,3-dihydroimidazol-2-ylidene]cyclohexa-2,5-dien-1-one), VX-702 6-(N-carbamoyl-2,6-difluoroanilino)-2-(2,4-difluorophenyl)pyridine-3-carboxamide), LY2228820 (5-[2-tert-butyl-4-(4-fluorophenyl)-1H-imidazol-5-yl]-3-(2,2-dimethylpropyl)imidazo[4,5-b]pyridin-2-amine; methanesulfonic acid) and VX-745 (5-(2,6-dichlorophenyl)-2-(2,4-difluorophenylthio)-6H-pyrimido[1,6-b]pyridazin-6-one) or PH-797804 (3-[3-bromo-4-[(2,4-difluorophenyl)methoxy]-6-methyl-2-oxopyridin-1-yl]-N,4-dimethylbenzamide).

32. The combination and/or kit or composition according to any one of claims 25 to 31, wherein the immunosuppressant comprises cyclosporin-A (CSA), tacrolimus or sirolimus.

33. The combination and/or kit or composition according to any one of claims 25 to 32, wherein the Wnt modulator comprises IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP3 (2-(3-(4-fluorophenyl)-3,4,6,7-tetrahydro-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP2 (N-(6-Methyl-2-benzothiazolyl)-2-[(3,4,6,7-tetrahydro-4-oxo-3-phenylthieno[3,2-d]pyrimidin-2-yl)thio]-acetamide), pyrvinium (2-[(E)-2-(2,5-dimethyl-1-phenylpyrrol-3-yl)ethenyl]-N,N,1-trimethylquinolin-1-ium-6-amine, BIO ((3Z)-6-bromo-3-[3-(hydroxyamino)indol-2-ylidene]-1H-indol-2-one, CHIR99021 ((6-[2-[[4-(2,4-dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)pyrimidin-2-yl]amino]ethylamino]pyridine-3-carbonitrile) or XAV939 (2-[4-(trifluoromethyl)phenyl]-1,5,7,8-tetrahydrothiopyrano[4,3-d]pyrimidin-4-one).

34. The combination and/or kit according to claim 26 or the composition according to claim 30; wherein the ROS activator comprises H_2O_2.

35. The combination and/or kit or composition according to any one of claims 25 to 34; for use in inducing cardiomyocyte differentiaton from at least one pluripotent stem cell.

36. Use of at least one one p38 mitogen-activated protein kinase (p38-MAPK) inhibitor; at least one immunosuppressant; and at least one Wnt modulator for the preparation of a combination or composition for inducing cardiomyocyte differentiation.

37. Use of at least one p38-MAPK inhibitor; at least one immunosuppressant; at least one Wnt modulator and at least one ROS activator for the preparation of a combination or composition for inducing cardiomyocyte differentiation.

38. Use of at least two components selected from the group consisting of the following components: a p38-MAPK inhibitor, an immunosuppressant and a Wnt modularor for the preparation of a combination or composition for inducing cardiomyocyte differentiation.

39. Use according to any one of claims 36 to 38; wherein the p38-MAPK inhibitor comprises SB 203580 (4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1H-imidazol-5-yl]pyridine), BIRB 796 (1-[5-tert-butyl-2-(4-methylphenyl)pyrazol-3-yl]-3-[4-(2-morpholin-4-ylethoxy)naphthalen-1-yl]urea), SB 202190 (4-[4-(4-fluorophenyl)-5-pyridin-4-yl-1,3-dihydroimidazol-2-ylidene]cyclohexa-2,5-dien-1-one, VX-702 6-(N-carbamoyl-2,6-difluoroanilino)-2-(2,4-difluorophenyl)pyridine-3-carboxamide), LY2228820 (5-[2-tert-butyl-4-(4-fluorophenyl)-1H-imidazol-5-yl]-3-(2,2-dimethylpropyl)imidazo[4,5-b]pyridin-2-amine; methanesulfonic acid) and VX-745 (5-(2,6-dichlorophenyl)-2-(2,4-difluorophenylthio)-6H-pyrimido[1,6-b]pyridazin-6-one) or PH-797804 3-[3-bromo-4-[(2,4-difluorophenyl)methoxy]-6-methyl-2-oxopyridin-1-yl]-N,4-dimethylbenzamide).

40. Use according to any one of claims 36 to 39; wherein the immunosuppressant comprises cyclosporin-A (CSA), tacrolimus or sirolimus.

41. Use according to any one of claims 36 to 40; wherein the Wnt modulator comprises IWP4 (2-(3,4,6,7-tetrahydro-3-(2-methoxyphenyl)-4-oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP3 (2-(3-(4-fluorophenyl)-3,4,6,7-tetrahydro-4-

~~oxothieno[3,2-d]pyrimidin-2-ylthio)-N-(6-methylbenzo[d]thiazol-2-yl)acetamide), IWP2 (N-(6-Methyl-2-benzothiazolyl)-2-[(3,4,6,7-tetrahydro-4-oxo-3-phenylthieno[3,2-d]pyrimidin-2-yl)thio]-acetamide), pyrvinium (2-[(E)-2-(2,5-dimethyl-1-phenylpyrrol-3-yl)ethenyl]-N,N,1-trimethylquinolin-1-ium-6-amine, BIO ((3Z)-6-bromo-3-[3-(hydroxyamino)indol-2-ylidene]-1H-indol-2-one), CHIR99021 6-[2-[[4-(2,4-dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)pyrimidin-2-yl]amino]ethylamino]pyridine-3-carbonitrile) or XAV939 (2-[4-(trifluoromethyl)phenyl]-1,5,7,8-tetrahydrothiopyrano[4,3-d]pyrimidin-4-one).~~

~~42. Use according to claim 35; wherein the ROS activator comprises H_2O_2.~~

~~43. Use according to any one of claims 34 to 40; wherein the combination or composition is for inducing cardiomyocyte differentiation from at least one pluripotent stem cell.~~