CLAIMS

1. A method of differentiating pluripotent stem cells to thymic cells, comprising:

a. differentiating the pluripotent stem cells into definitive endoderm (DE) cells;

b. culturing the DE cells and differentiating the DE cells into anterior foregut endoderm (AFE) cells by contacting or incubating the DE cells with a BMP inhibitor, a TGFβ inhibitor, an FGF, and an ascorbic acid;

c. culturing the AFE cells and differentiating the into ventral pharyngeal endoderm (VPE) cells comprising:

i. contacting or incubating the AFE cells in a first VPE medium comprising ascorbic acid, a retinoic acid, an FGF, and a TGFβ inhibitor; and

ii. contacting or incubating the AFE cells in a second VPE medium comprising a Noggin, a WNT activator, an FGF, a retinoic acid, and an ascorbic acid;

d. culturing the VPE cells and differentiating the VPE cells into spheroid of thymic epithelial progenitor (TEP) cells, by contacting or incubating the VPE cells with an ascorbic acid, an FGF, a BMP, and a WNT activator;

e. culturing the TEP cells and differentiating the TEP cells into thymic epithelial cell (TEC), by contacting or incubating the TEP cells with a WNT activator, a RANKL, a FGF, a BMP, and an ascorbic acid; and

f. culturing a single cell suspension of the TECs of e. into 3D suspension, in a culture medium comprising an ascorbic acid, an FGF, a BMP, and a WNT activator, optionally after freezing and thawing the single cell suspension of TECs, and

wherein the pluripotent stem cells, the DE cells, the AFE cells, the VPE cells TEP cells or TEC cells are cultured in 3D culture; optionally cultured as aggregates or spheroids in suspension

wherein the thymic cells are thymic epithelial progenitor (TEP) cells.

2. The method of claim 1, wherein

i. the first VPE media in step c.i. further comprises a WNT inhibitor; optionally wherein the WNT inhibitor is IWR-1; and/or

ii. the second VPE media in step c.ii. further comprises a BMP inhibitor, an SHH inhibitor, or a combination thereof; optionally wherein the BMP inhibitor is LDN193189; and/or the SHH inhibitor is SANT-1.

3. The method of claim 1 or 2, wherein the differentiation of pluripotent stem cells to DE cells comprises

a. contacting or culturing the pluripotent stem cells in a first growth medium, said first growth medium comprising Activin A, PI-103, CHIR99021 or a combination thereof;

b. culturing the pluripotent stem cells in a second growth medium to generate definitive endoderm cells, wherein the second growth medium comprises an, Activin A, a BMP inhibitor, PI-103, CHIR99021 or a combination thereof;

4. The method of any one of claims 1-4, wherein

i. the BMP inhibitor is LDN193189;

ii. the TGFβ inhibitor is SB431542;

iii. the FGF is FGF8b, FGF7, FGF10, FGF1, bFGF or a combination thereof;

iv. the WNT activator is CHIR99021; and/or

v. the BMP is BMP2, BMP4 or a combination thereof.

5. The method of any one of claims 1 to 4, wherein the interleukin is IL22.

6. The method of any one of claims 1-5, wherein the method is performed for from 15 days to 30 days; or from 18 days to 25 days.

7. The method of any one of claims 1-6, wherein

i. the pluripotent stem cells are differentiated into definitive endoderm cells for about 5 days;

ii. the DE cells are differentiated into AFE cells for from 2 days to 3 days;

iii. the AFE cells are cultured in the first VPE media for 2 to 4 days;

iv. the AFE cells are cultured in the second VPE media for 2 to 3 days; and/or

v. the VPE cells are differentiated to thymic cells for 3 to 6 days.

8. A population of thymic cells prepared according to the method of any one of claims 1-

7.

9. A pharmaceutical composition comprising a population of thymic cells of claim 8 and at least one excipient.

10. A pharmaceutical composition of claim 9 for use in therapy.

11. Use of the pharmaceutical composition of claim 9, in the manufacture of a medicament for treating a condition associated with an absence, decline or aberrant functioning of the thymus of a subject, wherein the condition is selected from the group consisting of: Di George syndrome, thymoma, CHARGE syndrome, FOXN1 deficiency, PAX1 deficiency, TBX1 deficiency, thymus cancer, thymic atrophy, thymic cyst, thymic hyperplasia, thymic hypoplasia, thymic aplasia, thymic dysplasia, thymic irradiation, myasthenia gravis, thymic carcinoma, thymic hyperplasia, thymic irradiation, and age- or infection-associated decline in thymic function.

12. The use of claim 11, wherein the medicament is to be administered to the subject via a parenteral route.

13. The use of claim 11, wherein the medicament is to be implanted or injected into one or more lymph nodes of the subject.

14. The method of any one of claims 1-7, wherein culturing the VPE cells and differentiating the VPE cells into thymic cells, comprises contacting or incubating the VPE cells with BMP4, FGF8b, EGF, SANT-1 (SHH antagonist), CHIR99021, ascorbic acid, or a combination thereof.