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, an ascorbic acid or a combination thereof;
    c. culturing the AFE cells and differentiating the anterior foregut cells 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, a TGFβ inhibitor or a combination thereof; and
        ii. contacting or incubating the AFE cells in a second VPE medium comprising a Noggin, a WNT activator, an FGF, a retinoic acid, an ascorbic acid or a combination thereof; and
    d. culturing the VPE cells and differentiating the VPE cells into thymic cells, by contacting or incubating the VPE cells with an ascorbic acid, an FGF, a BMP, a WNT activator or a combination thereof;

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

2. The method of claim 1, wherein the TEPs are further differentiated into thymic epithelial cells (TECs) by contacting or incubating the TEPs with an Interleukin, a WNT activator, a RANKL, an FGF, a BMP, an ascorbic acid, or a combination thereof; optionally wherein the TEPs are differentiated to TECs for about 4 days.

3. The method of claim 1 or claim 2, 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.

4. The method of any one of claims 1 to 3, 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.

5. 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.

6. The method of any one of claims 2 to 5, wherein the interleukin is IL22.

7. The method of any one of claims 1 to 6, wherein the pluripotent stem cells, the DE cells, the AFE cells, the VPE cells or thymic cells are cultured in 3D culture; optionally cultured as aggregates in suspension.

8. The method of any one of claims 1-7, wherein the method is performed for from about 15 days to 30 days; or from about 18 days to 25 days.

9. The method of any one of claims 1-8, 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 about 2 days to 3 days;
   iii. the AFE cells are cultured in the first VPE media for about 2 to 4 days;
   iv. the AFE cells are cultured in the second VPE media for about 2 to 3 days; and/or
   v. the VPE cells are differentiated to thymic cells for about 3 to 6 days.

10. A population of thymic cells prepared according to the method of any one of claims 1-9.

11. A pharmaceutical composition comprising a population of thymic cells of claim 10 and at least one excipient.

12. The pharmaceutical composition of claim 11 for use in treating or preventing a condition in a subject.

13. The pharmaceutical composition of claim 12, wherein

i. the condition is a condition associated with an absence, decline or aberrant functioning of the thymus of the subject, an immunodeficiency, a cancer, an autoimmune disease, an infectious disease, or graft versus host disease (GvHD)

ii. the pharmaceutical composition is to be administered to the subject via a parenteral route; and/or

iii. the pharmaceutical composition is to be implanted or injected into the subject.

14. A method of increasing FOXN1 expression in a population of thymic cells, the method comprising

a. freezing the population of thymic cells;
b. thawing the population of thymic cells; and
c. measuring and comparing the expression of FOXN1 in the population of thymic cells prior to freezing and comparing with FOXN1 expression after thawing the population of thymic cells.

15. The method of claim14, wherein

i. the FOXN1 expression is increased by from about 10 fold to 100 fold;

ii. the population of thymic cells are cultured in suspension after thawing; optionally cultured as aggregates in suspension; and/or

iii. the thymic cells comprise thymic epithelial progenitors (TEPs), thymic epithelial cells (TECs) or a combination thereof.

16. The method of any one of claims 1-9, wherein culturing the VPE cells and differentiating the VPE cells into thymic cells, comprises contacting or incubating the VPE cells with at least one of BMP4, FGF8b, EGF, SANT-1 (SHH antagonist), CHIR99021, ascorbic acid, or a combination thereof.