response to chemotherapy, radiation exposure, conditioning regimens used before organ transplant (such as bone marrow transplantation), and infection (such as HIV infection). *Id.* Regeneration of thymic tissue can replenish the T cell compartment – restoring immune function. *Id.* While the thymus does have some intrinsic regenerative capacity, the extent and speed of this thymic regeneration is frequently insufficient, leaving patients severely immuno-compromised and at risk from potentially life-threatening infections. As such, there is a need in the art for compositions and methods that can enhance both thymic regeneration and T-cell reconstitution. The present invention addresses these needs.

SUMMARY

[0004a] In one aspect, the present disclosure refers to a use of an effective amount of a therapeutic composition comprising engineered non-thymic endothelial cells (ntECs) in the manufacture of a medicament for enhancing thymic regeneration, wherein the therapeutic composition comprising engineered non-thymic endothelial cells (ntECs) is to be administered to a subject in need of thymic regeneration, wherein the engineered ntECs are either E4ORF1+ or BMP4+E4ORF1+, thereby enhancing thymic regeneration in the subject, wherein the ntECs are human umbilical vein endothelial cells (HUVECs).

[0004b] In another aspect, the present disclosure refers to an isolated population of engineered BMP4+E4ORF1+ non-thymic endothelial cells (ntECs), wherein the ntECs are human umbilical vein endothelial cells (HUVECs) or bone marrow endothelial cells.

[0004c] In another aspect, the present disclosure refers to a composition comprising a population of ntECs as described herein.

[0004d] In yet another aspect, the present disclosure refers to therapeutic composition comprising a population of ntECs as described herein and a solution suitable for administration to a subject.

[0004e] In yet another aspect, the present disclosure refers to a therapeutic composition comprising a population of ntECs as described herein and a biocompatible matrix material.

[0005] The present disclosure is based, in part, upon the surprising discovery that thymic regeneration can be induced *in vivo* by administering to a living subject non-thymic

endothelial cells ("ntECs") engineered to express either an adenovirus E4ORF1 polypeptide or both an adenovirus E4ORF1 polypeptide and BMP4. As shown in the Examples section of this patent disclosure, administration of engineered ntECs expressing either E4ORF1 alone, or E4ORF1 together with BMP4, to living subjects leads to enhanced thymic regeneration and T-cell reconstitution. These effects are particularly surprising given that prior published studies had demonstrated that, while administration of endothelial cells could enhance thymic regeneration, this effect could only be achieved if thymic endothelial cells were used. *See* Wertheimer et al., (2018); *"Production of BMP4 by endothelial cells is crucial for endogenous thymic regeneration;"* Sci. Immunol. 3, 2736. In these prior studies thymic regeneration was not observed when non-thymic endothelial cells were used. *Id*. The discovery that engineered ntECs expressing E4ORF1 alone, or both BMP4 and E4ORF1, can be used to induce thymic regeneration and T-cell reconstitution *in vivo* has several important practical implications, one of which is that it eliminates the need to perform complicated and invasive procedures to obtain and culture endothelial cells from a patient's thymus. Instead, endothelial cells for use in thymic regeneration and T-cell reconstitution protocols can be obtained from much more accessible sources – such as from adipose tissue, skin tissue or umbilical cord tissue – greatly simplifying the applicability of endothelial cell therapy for thymic regeneration.

[0006] Accordingly, the present disclosure provides a variety of novel compositions and methods.

CLAIMS

We claim:

1. Use of an effective amount of a therapeutic composition comprising engineered non-thymic endothelial cells (ntECs) in the manufacture of a medicament for enhancing thymic regeneration, wherein the therapeutic composition comprising engineered non-thymic endothelial cells (ntECs) is to be administered to a subject in need of thymic regeneration, wherein the engineered ntECs are either E4ORF1+ or BMP4+E4ORF1+, thereby enhancing thymic regeneration in the subject, wherein the ntECs are human umbilical vein endothelial cells (HUVECs).

2. The use of claim 1, wherein the thymic regeneration comprises recovery of at least one cell type from among CD45- thymic stromal cells and CD45+ T cells.

3. The use of claim 1, wherein the thymic regeneration comprises recovery of both CD45- thymic stromal cells and CD45+ T cells.

4. The use of claim 2 or claim 3, wherein the CD45- thymic stromal cells are selected from the group consisting of thymic epithelial progenitors (TEPCs), cortical thymic epithelial cells (cTECs), and medullary thymic epithelial cells (mTECs).

5. The use of claim 2 or claim 3, wherein the CD45+ T cells are selected from the group consisting of CD3+ T cells, CD4+ T cells, CD8+ T cells, double-positive T cells (DP), double-negative T cells (DN), double-negative type 1 (DN1) T cells, double-negative type 2 (DN2) T cells, double-negative type 3 (DN3) T cells and double-negative type 4 (DN4) T cells.

6. The use of any of the preceding claims, wherein the subject is a human.

7. The use of any of the preceding claims, wherein the ntECs are autologous to the subject.

8. The use any of claims 1-6, wherein the ntECs are allogeneic to the subject.

9. The use of any of the preceding claims, wherein the ntECs are MHC/HLA-matched to the subject.

10. The use of any of the preceding claims, wherein the subject has previously been treated with chemotherapy, radiation therapy, a pre-transplantation conditioning regimen, or a myeloablative conditioning regimen.

11. The use of any of the preceding claims, wherein the subject has an immunodeficiency.

12. The use of claim 11, wherein the subject has an HIV infection.

13. The use of any of the preceding claims, wherein the subject has an ageing-related deficiency in thymic tissue mass, thymic function, or T-cell production.

14. The use of any of the preceding claims, wherein the engineered ntECs are to be administered to the subject by IV infusion.

15. The use of any of the preceding claims, wherein the engineered ntECs are to be administered to the subject in multiple IV infusions over the course of several days or weeks.

16. The use of any of the preceding claims, wherein a therapeutic composition comprising hematopoietic stem cells (HSCs) is to be further administered to the subject.

17. The use of claim 16, wherein engineered ntECs and the HSCs are to be administered concurrently.

18. The use of claim 16, wherein engineered ntECs and the HSCs are to be administered to the subject in the same IV infusion.

19. The use of any of the preceding claims, further comprising an initial step of genetically modifying ntECs by transducing or transfecting the ntECs *ex vivo* with a nucleic acid molecule encoding E4ORF1 and optionally a nucleic acid molecule encoding BMP4, prior to administration of the ntECs to the subject.

20. The use of any of the preceding claims, further comprising an initial step of genetically modifying autologous ntECs from the subject by transducing or transfecting the ntECs *ex vivo* with a nucleic acid molecule encoding E4ORF1 and optionally a nucleic acid molecule encoding BMP4, prior to administration of the autologous ntECs to the subject.

21. An isolated population of engineered BMP4+E4ORF1+ non-thymic endothelial cells (ntECs), wherein the ntECs are human umbilical vein endothelial cells (HUVECs) or bone marrow endothelial cells.

22. The population of engineered ntECs of claim 21, wherein the population is a substantially pure population.

23. The population of engineered ntECs of claim 21, wherein the ntECs comprise a recombinant nucleic acid molecule that comprises a nucleotide sequence that encodes BMP4.

24. The population of ntECs of claim 23, wherein the nucleotide sequence that encodes BMP4 is operatively linked to a heterologous promoter.

25. The population of ntECs of claim 23, wherein the ntECs comprise a recombinant nucleic acid molecule that comprises a nucleotide sequence that encodes E4ORF1.

26. The population of ntECs of claim 25, wherein the nucleotide sequence that encodes E4ORF1 is operatively linked to a heterologous promoter.

27. The population of ntECs of any of claims 23-26, wherein the recombinant nucleic acid molecule is an expression vector.

28. The population of ntECs of claim 27, wherein the expression vector is a viral vector.

29. The population of ntECs of claim 27, wherein the expression vector is a lentiviral vector.

30. The population of ntECs of claim 27, wherein the expression vector is a retroviral vector.

31. A composition comprising a population of ntECs according to any of claims 21-30.

32. A therapeutic composition comprising a population of ntECs according to any of claims 21-30 and a solution suitable for administration to a subject.

33. A therapeutic composition comprising a population of ntECs according to any of claims 21-30 and a biocompatible matrix material.

34. A therapeutic composition according to claim 33, wherein the biocompatible matrix material is a liquid.

35. A therapeutic composition according to claim 33, wherein the biocompatible matrix material is a solid.