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.

[0004b] In another aspect, the present disclosure refers to an isolated population of engineered BMP4+E4ORF1+ non-thymic endothelial cells (ntECs).

[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.

[0007] In one example the present disclosure provides a population of engineered ntECs that express BMP4 (i.e. BMP4+ ntECs). In another example, the present disclosure provides a population of engineered ntECs that express an adenovirus E4ORF1 polypeptide (i.e. E4ORF1+ ntECs). In another example, the present disclosure provides a population of engineered ntECs that express BMP4 and an adenovirus E4ORF1 polypeptide (i.e. BMP4+E4ORF1+ ntECs). In some examples these populations of engineered ntECs are isolated cell populations. In some examples the populations of engineered ntECs are substantially pure cell populations. In some examples the populations of engineered ntECs are present *in vitro*, for example in cell culture. In some examples the populations of engineered ntECs are present *ex vivo.* In some examples the populations of engineered ntECs are present *in vivo*. In some examples the populations of engineered ntECs are present in a composition, such as a therapeutic composition suitable for administration to a living subject. For example, in some examples the present disclosure provides a therapeutic composition comprising a population of engineered ntECs and a physiological saline suitable for administration to a living subject. Similarly, in some examples the present disclosure provides a therapeutic composition comprising a population of engineered ntECs and a biocompatible matrix material, such as a liquid biocompatible matrix material (e.g. Matrigel) or a solid biocompatible matrix material.

[0008] In some examples the engineered ntECs provided herein, and/or compositions comprising these engineered ntECS, may be used in various therapeutic applications, including, methods for enhancing thymic regeneration and/or T-cell reconstitution in a living subject, such as a subject that has a deficiency in thymic tissue mass, thymic function, or T-cell production, or that is otherwise immunocompromised. In some examples such subjects are of advanced age, have a viral infection (e.g. an HIV infection), have been exposed to radiation (e.g. radiation therapy), have been treated with chemotherapy, or have been treated with myeloablative conditioning agents – for example in preparation for an organ transplant such as a bone marrow or hematopoietic stem cell transplant (HSCT).

[0009] For example, in some examples the present disclosure provides a method for enhancing thymic regeneration and/or T-cell reconstitution in a subject, the method comprising administering to a subject in need thereof an effective amount of ntECs engineered to express E4ORF1 or both E4ORF1 and BMP4, or a therapeutic composition

comprising such ntECs, thereby stimulating thymic regeneration and/or T-cell reconstitution in the subject.

[0010] The endothelial cells (ECs) can be from any non-thymic source. Examples of suitable sources of the ECs (ntECs) include, but are not limited to, adipose tissue (i.e. adipose ECs), skin tissue (i.e. skin ECs), cardiac tissue (i.e. cardiac ECs), kidney tissue (i.e. kidney ECs), lung tissue (i.e. lung ECs), liver tissue (i.e. liver ECs), bone marrow tissue (i.e. bone marrow ECs), umbilical vein (i.e. umbilical vein ECs – or "UVECs"), and the like.

[0011] In some examples the ntECs are adult ECs. In some examples the ntECs are juvenile ECs. In some examples the ntECs are fetal ECs. In some examples the ntECs are embryonic ECs. In some examples the ntECs are differentiated ECs. In some examples the ntECs are derived from endothelial progenitor cells. In some examples the ntECs are derived from stem cells. In some examples the ntECs are from primary tissue cultures. In some examples the ntECs are ECs of an endothelial cell line. In cases where the ntECS are to be used in a treatment method as described herein, in some examples the ntECs are autologous to the subject to whom the cells are to be administered, while in other examples the ntECs are allogeneic to the subject to whom the cells are to be administered.

[0012] In some examples the ntECs comprise a recombinant nucleic acid molecule that comprises a nucleotide sequence that encodes the recited molecule or molecules - for example a nucleotide sequence that encodes BMP4 and/or a nucleotide sequence that encodes an adenovirus E4ORF1 polypeptide. In examples where both BMP4 and E4ORF1 are used, the nucleotide sequence that encodes the BMP4 and the nucleotide sequence that encodes the E4ORF1 polypeptide may be provided in the same recombinant nucleic acid molecule or in different recombinant nucleic acid molecules. In some examples the nucleotide sequence that encodes the BMP4 and/or the nucleotide sequence that encodes the E4ORF1 polypeptide is operatively linked to a heterologous promoter. In some embodiments the recombinant nucleic acid molecule is a plasmid vector. In some examples the recombinant nucleic acid molecule is an expression vector. In some examples the recombinant nucleic acid molecule is a viral vector, such as, for example, a lentiviral vector. In some examples the nucleotide sequence that encodes the E4ORF1 and/or BMP4 polypeptide is transiently expressed in the ntECs. In some examples the nucleotide

sequence that encodes the BMP4 and/or E4ORF1 polypeptide is stably expressed in the ntECs. In some examples the nucleotide sequence that encodes the E4ORF1 and/or BMP4 polypeptide is integrated into the genome of the ntECs.

[0013] In some examples, where a recombinant nucleic acid molecule that comprises a nucleotide sequence that encodes an E4ORF1 polypeptide is used, the recombinant nucleic acid molecule is not an adenovirus genome. In some examples, where a recombinant nucleic acid molecule that comprises a nucleotide sequence that encodes an E4ORF1 polypeptide is used, the recombinant nucleic acid molecule is not a naturally occurring adenovirus genome. In some examples, where a recombinant nucleic acid molecule that comprises a nucleotide sequence that encodes an E4ORF1 polypeptide is used, the recombinant nucleic acid molecule is not an adenoviral vector.

[0014] These and other examples of the disclosure are described further in other sections of this patent disclosure. In addition, as will be apparent to those of skill in the art, certain modifications and combinations of the various examples described herein fall within the scope of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **Fig. 1.** In vitro expansion assay of 4 cell lines derived from same umbilical cord. At the first day, a small flask with 25 cm2 surface area was seeded with 500,000 cells of each cell line. All cell lines during expansion grown in the presence of serum. While E4ORF1+ HUVEC and BMP4+ E4ORF1+ HUVEC covered in less than 15 days 4 flask with surface area 225 cm2 (4xT225), HUVEC and BMP4+ HUVEC covered only 1 T225 flask (1xT225) in 16 days.

[0016] **Fig. 2A-C.** BMP4+ E4ORF1+ ntECs stimulate thymic regeneration. Results from experiments described in Example. 2. Each graph shows the numbers of live thymic cells (**Fig 2. A**) live medullar epithelial cells (mTEC) (**Fig. 2B**), and recovered live cortical epithelial cells (cTEC) (**Fig. 2C**) for the following treatment groups: (1) NoRad, No ECs, (2) Rad. No ECs, (3) Rad, Mu Thymic ECs, (4) Rad Mu BMEC, and (5) Rad. Mu BMEC/BMP4. The y axis shows the number of total cells isolated from the thymus of each individual animal. Significant differences between treatment groups are indicated by asterisks where * indicates a P-value <0.05, ** indicates a P-value <0.01, and *** indicates a P-value < 0.001.

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.

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 ntECs are selected from the group consisting of: umbilical vein endothelial cells (UVECs), adipose endothelial cells, skin endothelial cells, lung endothelial cells, heart endothelial cells, kidney endothelial cells and bone marrow endothelial cells.

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

8. The use of any of the preceding claims, wherein the ntECs are human umbilical vein endothelial cells (HUVECs).

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

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

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

12. 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.

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

14. The use of claim 13, wherein the subject has an HIV infection.

15. 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.

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

17. 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.

18. 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.

19. The use of claim 18, wherein engineered ntECs and the HSCs are to be administered concurrently.

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

21. 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.

22. 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.

23. An isolated population of engineered BMP4+E4ORF1+ non-thymic endothelial cells (ntECs).

24. The population of engineered ntECs of claim 23, wherein the population is a substantially pure population.

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

26. The population of ntECs of claim 25, wherein the nucleotide sequence that encodes BMP4 is operatively linked to a heterologous promoter.

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

28. The population of ntECs of claim 27, wherein the nucleotide sequence that encodes E4ORF1 is operatively linked to a heterologous promoter.

29. The population of ntECs of any of claims 25-28, wherein the recombinant nucleic acid molecule is an expression vector.

30. The population of ntECs of claim 29, wherein the expression vector is a viral vector.

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

32. The population of ntECs of claim 29, wherein the expression vector is a retroviral vector.

33. The population of ntECs of any of claims 23-32, wherein the ntECs are selected from the group consisting of: human umbilical vein endothelial cells (HUVECs), adipose

endothelial cells, skin endothelial cells, lung endothelial cells, heart endothelial cells, kidney endothelial cells and bone marrow endothelial cells.

34. The population of ntECs of any of claims 23-32, wherein the ntECs are human umbilical vein endothelial cells (HUVECs).

35. A composition comprising a population of ntECs according to any of claims 23-34.

36. A therapeutic composition comprising a population of ntECs according to any of claims 23-34 and a solution suitable for administration to a subject.

37. A therapeutic composition comprising a population of ntECs according to any of claims 23-34 and a biocompatible matrix material.

38. A therapeutic composition according to claim 37, wherein the biocompatible matrix material is a liquid.

39. A therapeutic composition according to claim 37, wherein the biocompatible matrix material is a solid.