sometimes problematic because of the lead times needed to find related or unrelated matched bone marrow stem cell donors. The use of allogeneic stem cells also presents a risk of graft failure and the development of acute and chronic graft versus host disease (GvHD). These complications can lead to death and are increased in incidence when unrelated donors are utilized as a source for allogeneic hematopoietic stem cells.

Another source of ALDP replacement is the use of matched are, more typically, partially matched cord blood cell transplants. The use of cord blood stem cells (CBSCs) is problematic, with a risk of graft failure and prolonged time to engraftment requiring extended transfusion support. Indeed, all forms of allogeneic HCT involve a 10-15% risk of transplant related mortality, and up to a 30% risk of chronic graft versus host disease.

Thus, there is a need in the art for safer and more efficient adrenoleukodystrophy therapies. The present invention provides solutions to these and other problems.

BRIEF SUMMARY

Thus, the appearances of the phrases "in one ~~embodiment~~example" or "in an ~~embodiment~~example" in various places throughout this specification are not necessarily all referring to the same ~~embodiment~~example. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more ~~embodiments~~examples.

In one aspect, there is provided a vector comprising from 5' to 3': (a) a left (5') retroviral LTR; (b) a central polypurine tract/DNA flap (cPPT/FLAP); (c) a retroviral export element; (d) a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide; and (e) a right (3') retroviral LTR; wherein the vector does not comprise a post-transcriptional regulatory element.

In various ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a vector comprising: a left (5') retroviral LTR; a central polypurine tract/DNA flap (cPPT/FLAP); a retroviral export element; a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide; and a right (3') retroviral LTR; wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

In particular ~~embodiments~~examples, the vector is a lentivirus vector.

In related ~~embodiments~~examples, the lentivirus is HIV.

In more particular ~~embodiments~~examples, the lentivirus is HIV-1.

In certain ~~embodiments~~examples, the promoter of the 5' LTR is replaced with a heterologous promoter.

In additional ~~embodiments~~examples, the heterologous promoter is a cytomegalovirus (CMV) promoter, a Rous Sarcoma Virus (RSV)promoter, or an Simian Virus 40 (SV40) promoter.

In additional particular ~~embodiments~~examples, the heterologous promoter is a CMV promoter.

In further ~~embodiments~~examples, the 5' LTR or 3' LTR is a lentivirus LTR.

In other ~~embodiments~~examples, the 5' LTR and 3' LTR are lentivirus LTRs.

In certain particular ~~embodiments~~examples, the lentivirus is HIV-1.

In particular ~~embodiments~~examples, the 3' LTR comprises one or more modifications.

In certain particular ~~embodiments~~examples, the 3' LTR comprises one or more deletions.

In additional ~~embodiments~~examples, the 3' LTR is a self-inactivating (SIN) LTR.

In some ~~embodiments~~examples, the retroviral export element is a rev response element (RRE).

In further ~~embodiments~~examples, the cPPT/FLAP is from HIV-1.

In certain ~~embodiments~~examples, the promoter comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter or transcriptionally active fragment thereof.

In particular ~~embodiments~~examples, the polynucleotide encoding ABCD1 polypeptide is a cDNA.

In related ~~embodiments~~examples, the cDNA comprises an optimized Kozak sequence.

In certain ~~embodiments~~examples, the optimal Kozak sequence is (GCC)RCCATGG, wherein R is a purine (A or G).

In particular ~~embodiments~~examples, the polynucleotide encodes a human ABCD1 polypeptide.

In another aspect, there is provided a lentiviral vector comprising from 5' to 3': (a) a left (5') LTR; (b) a cPPT/FLAP; (c) an RRE; (d) a MND promoter operably linked to a polynucleotide encoding a human ABCD1 polypeptide; (e) a right (3') LTR; and (f) a polyadenylation sequence; wherein the vector does not comprise a post-transcriptional regulatory element.

In various ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a lentiviral vector comprising: a left (5') LTR; a cPPT/FLAP; an RRE; a MND promoter operably linked to a polynucleotide encoding a human ABCD1 polypeptide; a right (3')

LTR; and a polyadenylation sequence; wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

In particular ~~embodiments~~examples, the lentiviral vector comprises a Psi (Ψ) packaging signal.

In certain ~~embodiments~~examples, the polyadenylation sequence is a bovine growth hormone polyadenylation or signal rabbit β-globin polyadenylation sequence.

In certain particular ~~embodiments~~examples, the lentiviral vector comprises a 5' LTR or 3' LTR from HIV-1.

In additional ~~embodiments~~examples, the 3' LTR is a SIN LTR.

In another aspect, there is provided a lentiviral vector comprising from 5' to 3': (a) a left (5') HIV-1 LTR; (b) a Psi (Ψ) packaging signal; (c) a cPPT/FLAP; (d) an RRE; (e) a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide; (f) a right (3') self-inactivating (SIN) HIV-1 LTR; and (g) a rabbit .beta.-globin polyadenylation sequence; wherein the vector does not comprise a post transcriptional regulatory element.

In various ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a lentiviral vector comprising: a left (5') HIV-1 LTR; a Psi (Ψ) packaging signal; a cPPT/FLAP; an RRE; a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide; a right (3') self-inactivating (SIN) HIV-1 LTR; and a rabbit β-globin polyadenylation sequence; wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

In various ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a mammalian cell comprising a vector according to any one of preceding vectors described herein.

In another aspect, there is provided a mammalian cell comprising a vector as defined above.

In particular ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a packaging cell comprising: a first polynucleotide encoding gag, a second polynucleotide encoding pol, a third polynucleotide encoding env, and a vector according to any one of preceding vectors described herein.

In another aspect, there is provided a producer cell comprising: a first polynucleotide encoding gag, a second polynucleotide encoding pol, a third polynucleotide encoding env, and a vector as defined above.

In certain ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a producer cell comprising a vector according to any one of preceding vectors described herein.

In additional ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, vector particles produced by the producer cell.

In another aspect, there is provided a vector particle comprising a vector as defined above.

In another aspect, there is provided a vector comprising from 5' to 3': (a) a left (5') retroviral LTR; (b) a central polypurine tract/DNA flap (cPPT/FLAP); (c) a retroviral export element; (d) a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide; (e) a right (3') retroviral LTR; (f) a polyadenylation (polyA) sequence; and wherein the vector does not comprise post-transcriptional regulatory element.

In various particular ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a vector comprising: at least one LTR; a central polypurine tract/DNA flap (cPPT/FLAP); a retroviral export element; and a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D,

member 1 (ABCD1) polypeptide; wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

In various certain ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a vector comprising: at least one LTR; a cPPT/FLAP; an RRE; a MND promoter operably linked to a polynucleotide encoding a human ABCD1 polypeptide; and a polyadenylation sequence; wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

In various additional ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a vector comprising: at least one SIN HIV-1 LTR; a Psi (Ψ) packaging signal; a cPPT/FLAP; an RRE; a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide; and a rabbit β-globin polyadenylation sequence, wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

In another aspect, there is provided a lentiviral vector comprising from 5' to 3': (a) a left (5') HIV-1 LTR; (b) a Psi (.PSI.) packaging signal; (c) a cPPT/FLAP; (d) an RRE; (e) a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide; (f) a right (3') self-inactivating (SIN) HIV-1 LTR; and (g) a polyadenylation (polyA) sequence; wherein the vector does not comprise post-transcriptional regulatory element.

In various ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a host cell transduced with the vector. In particular ~~embodiments~~examples, the host cell is an embryonic stem cell, a somatic stem cell, or a progenitor cell.

In certain ~~embodiments~~examples, the cell is a hematopoietic stem cell.

In particular ~~embodiments~~examples, the present ~~invention~~disclosure further contemplates, in part use of the viral vectors of the ~~invention~~disclosure in gene therapy.

In preferred ~~embodiments~~examples, the gene therapy treats or prevents adrenoleukodystrophy or adrenomyeloneuropathy.

In various other ~~embodiments~~examples, the present ~~invention~~disclosure contemplates, in part, a method of treating adrenoleukodystrophy or adrenomyeloneuropathy, comprising administering to a subject a cell transduced with a vector of the ~~invention~~disclosure.

In particular ~~embodiments~~examples, the cell is an embryonic stem cell, a somatic stem cell, or a progenitor cell.

In certain ~~embodiments~~examples, the cell is a hematopoietic stem cell.

BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is a cDNA sequence that encodes human ABCD1.

SEQ ID NO: 2 is a cDNA sequence that encodes human ABCD1.

SEQ ID NO: 3 is a MND promoter polynucleotide sequence.

CLAIMS

1. A vector comprising:

(a) a left (5') retroviral LTR;

(b) a central polypurine tract/DNA flap (cPPT/FLAP);

(c) a retroviral export element;

(e) a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide; and

(f) a right (3') retroviral LTR;

wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

2. The vector of claim 1, wherein the vector is a lentivirus vector.

3. The vector of claim 2, wherein the lentivirus is HIV.

4. The vector of claim 2, wherein the lentivirus is HIV-1.

5. The vector of claim 1, wherein the promoter of the 5' LTR is replaced with a heterologous promoter.

6. The vector of claim 5, wherein the heterologous promoter is a cytomegalovirus (CMV) promoter, a Rous Sarcoma Virus (RSV)promoter, or an Simian Virus 40 (SV40) promoter.

7. The vector of claim 5, wherein the heterologous promoter is a CMV promoter.

8. The vector claim 1, wherein the 5' LTR or 3' LTR is a lentivirus LTR.

9. The vector claim 1, wherein the 5' LTR and 3' LTR are lentivirus LTRs.

10. The vector of claim 8 or claim 9, wherein the lentivirus is HIV-1.

11. The vector of claim 10, wherein the 3' LTR comprises one or more modifications.

12. The vector of claim 10, wherein the 3' LTR comprises one or more deletions.

13. The vector of claim 10, wherein the 3' LTR is a self-inactivating (SIN) LTR.

14. The vector claim 1, wherein the retroviral export element is a rev response element (RRE).

15. The vector of claim 1, wherein the cPPT/FLAP is from HIV-1.

16. The vector of claim 1, wherein the promoter comprises a myeloproliferative sarcoma virus enhancer,negative control region deleted, dl587rev primer-binding site substituted (MND) promoter or transcriptionally active fragment thereof.

17. The vector of claim 1, wherein the polynucleotide encoding ABCD1 polypeptide is a cDNA.

18. The vector of claim 17, wherein the cDNA comprises an optimized Kozak sequence.

19. The vector of claim 18, wherein the optimal Kozak sequence is (GCC)RCCATGG, wherein R is a purine (A or G).

20. The vector of claim 1, wherein the polynucleotide encodes a human ABCD1 polypeptide.

21. A lentiviral vector comprising:

(a) a left (5') LTR;

(b) a cPPT/FLAP;

(c) an RRE;

(d) a MND promoter operably linked to a polynucleotide encoding a human ABCD1 polypeptide;

(e) a right (3') LTR; and

(f) a polyadenylation sequence;

wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

22. The lentiviral vector of claim 21, comprising a Psi (Ψ) packaging signal.

23. The lentiviral vector of claim 21, wherein the polyadenylation sequence is a bovine growth hormone polyadenylation or signal rabbit β-globin polyadenylation sequence.

24. The lentiviral vector claim 21, wherein the 5' LTR or 3' LTR are from HIV-1.

25. The lentiviral vector of claim 21, wherein the 3' LTR is a SIN LTR.

26. A lentiviral vector comprising:

(a) a left (5') HIV-1 LTR;

(b) a Psi (Ψ) packaging signal;

(c) a cPPT/FLAP;

(d) an RRE;

(e) a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide;

(f) a right (3') self-inactivating (SIN) HIV-1 LTR; and

(g) a rabbit β-globin polyadenylation sequence;

wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

27. A mammalian cell comprising a vector according to any one of claims 1-26.

28. A packaging cell comprising: a first polynucleotide encoding gag, a second polynucleotide encoding pol, a third polynucleotide encoding env, and a vector according to any one of claims 1-26.

29. A producer cell comprising a vector according to any one of claims 1-26.

30. A vector particle produced by the producer cell of claim 29.

31. A vector comprising:

(a) at least one LTR;

(b) a central polypurine tract/DNA flap (cPPT/FLAP);

(c) a retroviral export element; and

(d) a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide;

wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

32. A vector comprising:

(a) at least one LTR;

(b) a cPPT/FLAP;

(c) an RRE;

(d) a MND promoter operably linked to a polynucleotide encoding a human ABCD1 polypeptide; and

(e) a polyadenylation sequence;

wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

33. A vector comprising:

(a) at least one SIN HIV-1 LTR;

(b) a Psi (Ψ) packaging signal;

(c) a cPPT/FLAP;

(d) an RRE;

(e) a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide; and

(f) a rabbit β-globin polyadenylation sequence;

wherein the vector does not comprise a woodchuck post-transcriptional regulatory element (WPRE).

34. A host cell transduced with the vector according to any one of claims 31-33.

35. The transduced host cell of claim 34, wherein the cell is an embryonic stem cell, a somatic stem cell, or a progenitor cell.

36. The transduced host cell of claim 35, wherein the cell is a hematopoietic stem cell.

37. Use of the viral vector according to any one of claims 31-33 in gene therapy.

38. The use of claim 37, wherein the gene therapy treats or prevents adrenoleukodystrophy or adrenomyeloneuropathy.

39. A method of treating adrenoleukodystrophy or adrenomyeloneuropathy, comprising administering to a subject a cell transduced with a vector according to any one of claims 31-33.

40. The method of claim 39, wherein the cell is an embryonic stem cell, a somatic stem cell, or a progenitor cell.

41. The method of claim 39, wherein the cell is a hematopoietic stem cell.

WHAT IS CLAIMED:

1. A vector comprising from 5' to 3': (a) a left (5') retroviral LTR; (b) a central polypurine tract/DNA flap (cPPT/FLAP); (c) a retroviral export element; (d) a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide; and (e) a right (3') retroviral LTR; wherein the vector does not comprise a post-transcriptional regulatory element.

2. The vector of claim 1, wherein the vector is a lentivirus vector.

3. The vector of claim 2, wherein the lentivirus is HIV.

4. The vector of claim 2, wherein the lentivirus is HIV-1.

5. The vector of claim 1, wherein the promoter of the 5' LTR is replaced with a heterologous promoter selected from the group consisting of: a cytomegalovirus (CMV) promoter, a Rous Sarcoma Virus (RSV) promoter, or an Simian Virus 40 (SV40) promoter.

6. The vector of claim 1, wherein the 3' LTR is a self-inactivating (SIN) LTR.

7. The vector of claim 1, wherein the promoter active in a microglial cell comprises a myeloproliferative sarcoma virus enhancer, negative control region deleted, d1587rev primer-binding site substituted (MND) promoter or transcriptionally active fragment thereof.

8. The vector of claim 1, wherein the polynucleotide encoding the ABCD1 polypeptide is a cDNA.

9. The vector of claim 8, wherein the cDNA comprises an optimized Kozak sequence.

10. A lentiviral vector comprising from 5' to 3': (a) a left (5') LTR; (b) a cPPT/FLAP; (c) an RRE; (d) a MND promoter operably linked to a polynucleotide encoding a human ABCD1 polypeptide; (e) a right (3') LTR; and (f) a polyadenylation sequence; wherein the vector does not comprise a post-transcriptional regulatory element.

11. The lentiviral vector of claim 10, further comprising a Psi (Ψ) packaging signal.

12. The lentiviral vector of claim 10, wherein the polyadenylation sequence is a

bovine growth hormone polyadenylation signal or a rabbit β-globin polyadenylation sequence.

13. A lentiviral vector comprising from 5' to 3': (a) a left (5') HIV-1 LTR; (b) a Psi (Ψ) packaging signal; (c) a cPPT/FLAP; (d) an RRE; (e) a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide; (f) a right (3') self-inactivating (SIN) HIV-1 LTR; and (g) a rabbit β-globin polyadenylation sequence; wherein the vector does not comprise a post transcriptional regulatory element.

14. A vector comprising from 5' to 3': (a) a left (5') retroviral LTR; (b) a central polypurine tract/DNA flap (cPPT/FLAP); (c) a retroviral export element; (d) a promoter active in a microglial cell, operably linked to a polynucleotide encoding an ATP-binding cassette, sub-family D, member 1 (ABCD1) polypeptide; (e) a right (3') retroviral LTR; (f) a polyadenylation (polyA) sequence; and wherein the vector does not comprise post-transcriptional regulatory element.

15. A lentiviral vector comprising from 5' to 3': (a) a left (5') HIV-1 LTR; (b) a Psi (Ψ) packaging signal; (c) a cPPT/FLAP; (d) an RRE; (e) a MND promoter, operably linked to a cDNA encoding a human ABCD1 polypeptide; (f) a right (3') self-inactivating (SIN) HIV-1 LTR; and (g) a polyadenylation (polyA) sequence; wherein the vector does not comprise post-transcriptional regulatory element.

16. A mammalian cell comprising a vector according to any one of claims 1, 10, 13, 14 and 15.

17. A producer cell comprising: a first polynucleotide encoding gag, a second polynucleotide encoding pol, a third polynucleotide encoding env, and a vector according to any one of claims 1, 10, 13, 14 and 15.

18. A vector particle comprising a vector according to any one of claims 1, 10, 13, 14, and 15.