

## CLAIMS

What is claimed:

1. Use of a temperature-sensitive Sendai viral vector comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4 in the manufacture of a medicament for treating a disease of blood or a blood-forming organ, the use comprising:

- i) mobilizing hematopoietic stem cells from bone marrow to peripheral blood of a human subject suffering from the disease;
- ii) isolating CD34<sup>+</sup> cells from a sample of peripheral blood mononuclear cells obtained from the subject;
- iii) incubating the isolated CD34<sup>+</sup> cells at a temperature of  $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ ;
- iv) contacting the incubated CD34<sup>+</sup> cells with the medicament;
- v) maintaining the contacted CD34<sup>+</sup> cells at a permissive temperature of  $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for a period of at least about 12-72 hours, wherein replication and transcription of the temperature-sensitive Sendai viral vector occurs at the permissive temperature leading to increased expression of human ZSCAN4; and
- vi) infusing the contacted CD34<sup>+</sup> cells into the subject under conditions suitable for engrafting the cells to treat the disease.

2. Use of a temperature-sensitive Sendai viral vector comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4 in the manufacture of a medicament for treating a disease of blood or a blood-forming organ, the use comprising:

- i) mobilizing hematopoietic stem cells from bone marrow cell to peripheral blood of a human subject suffering from the disease;
- ii) isolating CD34<sup>+</sup> cells from a sample of peripheral blood mononuclear cells obtained from the subject;
- iii) contacting the isolated CD34<sup>+</sup> cells with the medicament;
- iv) incubating the contacted CD34<sup>+</sup> cells at a permissive temperature of  $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for a period of at least about 12-72 hours, wherein replication and transcription of the temperature-sensitive Sendai viral vector occurs at the permissive temperature leading to increased expression of human ZSCAN4; and

v) infusing the contacted CD34<sup>+</sup> cells into the subject under conditions suitable for engrafting the cells to treat the disease.

3. The use of claim 1, further comprising after step v) incubating the contacted CD34<sup>+</sup> cells at a non-permissive temperature of  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  prior to infusing the contacted CD34<sup>+</sup> cells into the subject, wherein replication and transcription of the temperature-sensitive Sendai viral vector and expression of human ZSCAN4 ceases at the non-permissive temperature.

4. The use of claim 2, further comprising after step iv) incubating the contacted CD34<sup>+</sup> cells at a non-permissive temperature of  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  prior to infusing the contacted CD34<sup>+</sup> cells into the subject, wherein replication and transcription of the temperature-sensitive Sendai viral vector and expression of human ZSCAN4 ceases at the non-permissive temperature.

5. The use of claim 3 or claim 4, wherein the contacted CD34<sup>+</sup> cells are incubated at the non-permissive temperature of  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for about 30 minutes to about 10 days.

6. The use of any one of claims 1-5, wherein the hematopoietic stem cells are mobilized by administration of one or both of granulocyte-colony stimulation factor and plerixafor to the subject.

7. The use of any one of claims 1-6, wherein the peripheral blood mononuclear cells are obtained from the subject by apheresis.

8. The use of any one of claims 1-7, wherein the CD34<sup>+</sup> cells are isolated from the peripheral blood mononuclear cells by positive selection using an anti-CD34 antibody and magnetic beads.

9. The use of any one of claims 1-8, wherein the contacted CD34<sup>+</sup> cells are washed and resuspended in a sterile, isotonic aqueous solution prior to infusion.

10. The use of any one of claims 1-9, wherein the contacted CD34<sup>+</sup> cells are intravenously infused at a dose of about  $1.0 \times 10^5$  cells/kg to about  $1.0 \times 10^7$  cells/kg.

11. Use of a temperature-sensitive Sendai viral vector or a temperature-sensitive self-replicating RNA, each of which comprises a nucleic acid comprising a coding region of human

ZSCAN4 in the manufacture of a medicament for treating a disease of blood or a blood-forming organ, the use comprising:

- (i) administering to a human subject suffering from the disease a cytokine to mobilize bone marrow cells from bone marrow to peripheral blood of the human subject;
- (ii) isolating a plurality of the bone marrow cells present peripheral blood of the subject;
- (iii) contacting the isolated bone marrow cells with the medicament;
- (iv) maintaining the contacted bone marrow cells at a permissive temperature to permit replication and transcription of the temperature-sensitive Sendai viral vector or the temperature-sensitive self-replicating RNA at the permissive temperature to produce bone marrow cells expressing human ZSCAN4; and
- (v) infusing, within two, three, or four days of completion of step (i), the bone marrow cells expressing human ZSCAN4 into the subject under conditions suitable for engrafting the cells to treat the disease.

12. The use of claim 11, wherein the infusing of the bone marrow cells is performed after 36 hours of the completion of step (i).

13. The use of claim 11 or claim 12, further comprising maintaining the isolated bone marrow cells at the permissive temperature prior to initiation of step (iii).

14. The use of any one of claims 11-13, wherein the permissive temperature is from 31°C to 35°C.

15. The use of any one of claims 11-14, wherein the cytokine is granulocyte-colony stimulation factor.

16. The use of claim 15, wherein the granulocyte-colony stimulation factor is to be administered to the subject at a concentration of about 0.1µg/kg to about 100µg/kg, or about 1.0µg/kg to about 10µg/kg.

17. The use of any one of claims 11-16, wherein the isolated bone marrow cells are obtained from the subject by apheresis.

18. The use of any one of claims 11-17, wherein plerixafor is to be further administered to the subject after completion of step (ii) to further mobilize bone marrow cells from the bone marrow to the peripheral blood of the subject.
19. The use of claim 18, wherein the plerixafor is to be administered to the subject at a concentration of about 1µg/kg to about 1000µg/kg, or about 75µg/kg to about 500µg/kg.
20. The use of any one of claims 11-19, further comprising repeating the mobilizing step and the isolating step prior to the contacting step.
21. A composition comprising a temperature-sensitive self-replicating RNA comprising:  
a viral replicon lacking a viral structural protein coding region; and  
a nucleic acid comprising a coding region of human ZSCAN4,  
wherein the temperature-sensitive self-replicating RNA is capable of expressing the human ZSCAN4 at a permissive temperature but not at a non-permissive temperature.
22. The composition of claim 21 wherein the viral replicon is an Alphavirus replicon.
23. The composition of claim 22, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.
24. The composition of claim 22, wherein the Alphavirus is a Venezuelan equine encephalitis virus.
25. The composition of any one of claims 21-24, wherein the permissive temperature is from 31°C to 35°C.
26. The composition of any one of claims 21-25, wherein the non-permissive temperature is above 36°C.
27. The composition of any one of claims 21-26, wherein the viral replicon comprises a nonstructural protein coding region with an insertion of 12-18 nucleotides resulting in expression of a nonstructural Protein 2 (nsP2) comprising from 4 to 6 additional amino acids between beta sheet 5 and beta sheet 6 of the nsP2.

28. The composition of claim 27, wherein the additional amino acids comprise one sequence selected from the group consisting of SEQ ID NO:38 (GCGRT), SEQ ID NO:39 (TGAAA), and SEQ ID NO:40 (LRPHP).

29. The composition of claim 27, wherein the amino acid sequence of the nsP2 comprises one sequence selected from the group consisting of SEQ ID NOs:29-36.

30. The composition of any one of claims 21-29, wherein the amino acid sequence of the human ZSCAN4 comprises:

- (i) SEQ ID NO:38 or is at least 95% identical to SEQ ID NO:38; or
- (ii) one of the group consisting of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42, or is at least 95% identical to one of the group consisting of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42.