

CLAIMS

What is claimed:

1. A method of treating a disease of blood or a blood-forming organ, comprising:
 - i) mobilizing hematopoietic stem cells from bone marrow to peripheral blood of a human subject suffering from the disease;
 - ii) isolating CD34+ cells from a sample of peripheral blood mononuclear cells obtained from the subject;
 - iii) incubating the isolated CD34+ cells at a temperature of $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$;
 - iv) contacting the incubated CD34+ cells with a temperature-sensitive Sendai viral vector comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4;
 - v) maintaining the contacted CD34+ 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+ cells into the subject under conditions suitable for engrafting the cells to treat the disease.
2. A method of treating a disease of blood or a blood-forming organ, comprising:
 - i) mobilizing hematopoietic stem cells from bone marrow cell to peripheral blood of a human subject suffering from the disease;
 - ii) isolating CD34+ cells from a sample of peripheral blood mononuclear cells obtained from the subject;
 - iii) contacting the isolated CD34+ cells with a temperature-sensitive Sendai viral vector comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4;
 - iv) incubating the contacted CD34+ 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+ cells into the subject under conditions suitable for engrafting the cells to treat the disease.

3. The method of claim 1, further comprising after step v) incubating the contacted CD34+ cells at a non-permissive temperature of $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ prior to infusing the contacted CD34+ 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 method of claim 2, further comprising after step iv) incubating the contacted CD34+ cells at a non-permissive temperature of $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ prior to infusing the contacted CD34+ 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 method of claim 3 or claim 4, wherein the contacted CD34+ 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, optionally for about 30-180 minutes.
6. The method of claim 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 method of claim 6, wherein the peripheral blood mononuclear cells are obtained from the subject by apheresis.
8. The method of claim 7, wherein the CD34+ cells are isolated from the peripheral blood mononuclear cells by positive selection using an anti-CD34 antibody and magnetic beads.
9. The method of claim 8, wherein the contacted CD34+ cells are washed and resuspended in a sterile, isotonic aqueous solution prior to infusion.
10. The method of claim 9, wherein the contacted CD34+ cells are intravenously infused at a dose of about 1.0×10^5 cells/kg to about 1.0×10^7 cells/kg, optionally about $2.0\text{-}8.0 \times 10^6$ cells/kg.
11. A method of treating a disease of blood or a blood-forming organ, comprising:
 - i) administering to a human subject suffering from the disease a temperature-sensitive Sendai viral vector comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4;

ii) lowering the subject's core body temperature to a permissive temperature of $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$;
iii) maintaining the subject's core body temperature at the permissive temperature for a period of from about 12 hours to about 7 days, or for 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

iv) allowing the subject's core body temperature to return to a normal, non-permissive temperature of $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, wherein replication and transcription of the temperature-sensitive Sendai viral vector and expression of human ZSCAN4 ceases at the non-permissive temperature.

12. A method of treating a disease of blood or a blood-forming organ, comprising:

i) lowering the core body temperature of a subject suffering from the disease to a permissive temperature of $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$;

ii) administering to the subject a temperature-sensitive Sendai viral vector comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4;

iii) maintaining the subject's core body temperature at the permissive temperature for a period of from about 12 hours to about 7 days, or for 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

iv) allowing the subject's core body temperature to return to a normal, non-permissive temperature of $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, wherein replication and transcription of the temperature-sensitive Sendai viral vector and expression of human ZSCAN4 ceases at the non-permissive temperature.

13. The method of claim 11 or claim 12, wherein the subject's core body temperature is lowered using a targeted temperature management (TTM) procedure, wherein the TTM procedure comprises application to the subject of one of the group consisting of a cooling catheter, a cooling blanket, and ice.

14. The method of claim 13, wherein the human subject is diagnosed with bone marrow failure prior to treatment, optionally wherein the bone marrow failure comprises one or more of neutropenia, thrombocytopenia, and anemia.

15. The method of claim 14, wherein the subject does not have cancer.

16. The method of claim 15, wherein the disease is a telomere biology disorder.
17. The method of claim 16, where the telomere biology disorder is selected from the group consisting of dyskeratosis congenita, Hoyeraal-Hreidarsson syndrome, Revesz syndrome, Coats plus syndrome, idiopathic pulmonary fibrosis, and cirrhosis.
18. The method of claim 16, wherein the telomere biology disorder is defined by one or both of:
- i) age-adjusted mean telomere length of less than 1 percentile in one or more of peripheral blood lymphocytes, B-cells, and naïve T-cells; and
 - ii) a pathogenic mutation in a gene selected from the group consisting of DKC1, TERC, TERT, NOP10, NHP2, TINF2, CTC1, PARN, RTEL1, ACD, USB1, and WRAP53.
19. The method of claim 13, wherein the disease is a bone marrow failure syndrome.
20. The method of claim 19, wherein the bone marrow failure syndrome is selected from the group consisting of Fanconi anemia, amegakaryocytic thrombocytopenia, aplastic anemia, Diamond Blackfan anemia, paroxysmal nocturnal hemoglobinuria, Pearson syndrome, Shwachman Diamond syndrome, and myelodysplastic syndrome.
21. The method of claim 14, wherein the disease is associated with a karyotype abnormality.
22. A temperature-sensitive agent, wherein the agent is a temperature-sensitive viral vector or a temperature-sensitive self-replicating RNA comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4, and a nonstructural protein coding region with an insertion of 12-18 nucleotides, wherein the insertion results in expression of a nonstructural Protein 2 (nsP2 = helicase proteinase) comprising from 4 to 6 additional amino acids between beta sheet 5 and beta sheet 6 of the nsP2, optionally wherein the additional amino acids result in temperature-sensitivity of the viral vector or the self-replicating RNA.
23. The temperature-sensitive agent of claim 22, wherein the additional amino acids comprise one sequence selected from the group consisting of SEQ ID NO:43 (GCGRT), SEQ ID NO:44 (TGAAA), and SEQ ID NO:45 (LRPHP).

24. The temperature-sensitive agent of claim 22, wherein the additional amino acids comprise the sequence of SEQ ID NO:44 (TGAAA).
25. The temperature-sensitive agent of claim 24, wherein the amino acid sequence of the NsP2 comprises one sequence selected from the group consisting of SEQ ID NOs:29-36.
26. The temperature-sensitive agent of claim 22, wherein the agent is a temperature-sensitive Alphavirus vector.
27. The temperature-sensitive agent of claim 22, wherein the agent is a temperature-sensitive self-replicating RNA comprising an Alphavirus replicon lacking a viral structural protein coding region.
28. The temperature-sensitive agent of claim 26 or claim 27, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.
29. The temperature-sensitive agent of claim 26 or claim 27, wherein the Alphavirus is a Venezuelan equine encephalitis virus.
30. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent (ts-agent) in a subject, wherein the ts-agent is a temperature-sensitive viral vector or a temperature-sensitive self-replicating RNA comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4, wherein one or more cells at or near the surface of the subject's body comprise the ts-agent, wherein the temperature-sensitive activity of the ts-agent comprises expression of human ZSCAN4 at a permissive temperature, and wherein the permissive temperature is the surface body temperature of the subject, comprising:
- i) maintaining the surface body temperature of the subject at the permissive temperature for a period of time sufficient for the temperature-sensitive activity to induce an effect in the subject; and
 - ii) increasing the surface body temperature of the subject to a non-permissive temperature for a period of time sufficient for the temperature-sensitive activity to cease in the subject.

31. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent (ts-agent) in a subject, wherein the ts-agent is a temperature-sensitive viral vector or a temperature-sensitive self-replicating RNA comprising a heterologous nucleic acid comprising a coding region of human ZSCAN4, wherein the temperature-sensitive activity of the ts-agent comprises expression of human ZSCAN4 at a permissive temperature, and wherein the permissive temperature is the surface body temperature of the subject, comprising:

- i) administering the ts-agent to one or more cells at or near the surface of the subject's body; and
- ii) maintaining the surface body temperature of the subject at the permissive temperature for a period of time sufficient for the temperature-sensitive activity to induce an effect in the subject.

32. The method of claim 31, further comprising iii) increasing the surface body temperature of the subject to a non-permissive temperature for a period of time sufficient for the temperature-sensitive activity to cease in the subject.

33. The method of claim 31, wherein the temperature-sensitive agent is administered intradermally or subcutaneously.

34. The method of claim 31, wherein the temperature-sensitive agent is administered intramuscularly.

35. The method of any one of claims 30-34, wherein the permissive temperature is from 30°C to 36°C, or 31°C to 35°C, or 32°C to 34°C, or 33°C $\pm$ 0.5°C, and the non-permissive temperature is 37°C $\pm$ 0.5°C.

36. The method of claim 35, wherein the effect of expression of human ZSCAN4 is prophylactic effect or a therapeutic effect.

37. The method of claim 35, wherein the ts-agent is a temperature-sensitive viral vector and the temperature-sensitive activity further comprises replication and transcription of the temperature-sensitive viral vector.

38. The method of claim 37, wherein the temperature-sensitive viral vector is selected from the group consisting of a Sendai virus, an Adeno virus, an Adeno-associated virus, a Retrovirus, and an Alphavirus.
39. The method of claim 37, wherein the temperature-sensitive viral vector is an Alphavirus, optionally wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.
40. The method of claim 37, wherein the temperature-sensitive viral vector is a Sendai virus.
41. The method of claim 35, wherein the ts-agent is a temperature-sensitive self-replicating RNA and the temperature-sensitive activity further comprises one or both of replication and transcription of the temperature-sensitive self-replicating RNA.
42. The method of claim 41, wherein the self-replicating RNA comprises an Alphavirus replicon lacking an Alphavirus viral structural protein coding region.
43. The method of claim 42, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.
44. The method of claim 42, wherein the Alphavirus is a Venezuelan equine encephalitis virus.
45. The method of claim 36, wherein the period of time sufficient for the temperature-sensitive activity to produce an effect ranges from about 12 hours to about 12 weeks, optionally wherein the period of time is from 1 to 7 days.
46. The method of claim 36, wherein the period of time sufficient to induce an effect in the subject is from about 12 hours to about 7 days, optionally wherein the period of time is from about 12 hours to about 72 hours.
47. The method of claim 36, wherein the subject is a mammalian subject, optionally wherein the subject is a human.

48. The method of any one of claims 1-47, wherein the amino acid sequence of human ZSCAN4 comprises SEQ ID NO:38 or is at least 95% identical to SEQ ID NO:38.
49. The method of any one of claims 1-47, wherein the amino acid sequence of human ZSCAN4 comprises 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.
50. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent, comprising:
- i) incubating one or more cells comprising the temperature-sensitive agent at a permissive temperature to induce the temperature-sensitive activity for a period of time sufficient for the temperature-sensitive activity to produce an effect in the one or more cells; and
 - ii) incubating the one or more cells at a non-permissive temperature, wherein the non-permissive temperature reduces the temperature-sensitive activity of the temperature-sensitive agent,
- wherein the temperature-sensitive agent comprises a therapeutic agent comprising a human ZSCAN4 protein, or a nucleic acid comprising a coding region of human ZSCAN4, and the effect comprises a therapeutic effect.
51. The method of claim 50, further comprising before step i): contacting the one or more cells with the temperature-sensitive agent.
52. The method of claim 51, wherein the one or more cells are at the permissive temperature when contacted with the temperature-sensitive agent.
53. The method of claim 50, further comprising administering the one or more cells to a subject in need of the therapeutic effect.
54. The method of claim 50, wherein incubating the one or more cells at a non-permissive temperature comprises administering the one or more cells to a subject in need of the therapeutic effect, wherein the subject's body temperature is the non-permissive temperature.

55. The method of claim 54, wherein the one or more cells are further incubated at the non-permissive temperature prior to administering the one or more cells to the subject.
56. The method of claim 50, wherein the one or more cells were isolated from the subject before contacting the one or more cells with the temperature-sensitive agent.
57. The method of claim 50, wherein the therapeutic effect comprises increasing telomere length of the one or more cells.
58. The method of claim 50, wherein the one or more cells are mammalian cells.
59. The method of claim 54, wherein the subject is a human subject.
60. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent in a human subject, wherein one or more cells of the subject comprise the temperature-sensitive agent, wherein the temperature-sensitive activity of the temperature-sensitive agent is induced at a permissive temperature, and wherein the permissive temperature is lower than the body temperature of the subject, comprising:
- i) lowering the body temperature of the subject to the permissive temperature;
 - ii) maintaining said lowered body temperature for a period of time sufficient for the temperature-sensitive activity to induce an effect in the subject; and
 - iii) raising the body temperature of the subject back to normal body temperature,
- wherein the temperature-sensitive agent comprises a therapeutic agent comprising a human ZSCAN4 protein, or a nucleic acid comprising a coding region of human ZSCAN4, and the effect is a therapeutic effect.
61. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent in a human subject, wherein the temperature-sensitive activity of the temperature-sensitive agent is induced at a permissive temperature, and wherein the permissive temperature is lower than the body temperature of the subject, comprising:
- i) lowering the body temperature of the subject to the permissive temperature;
 - ii) administering the temperature-sensitive agent to one or more cells of the subject;
 - iii) maintaining said lowered body temperature for a period of time sufficient for the temperature-sensitive activity to induce an effect in the subject; and

iv) raising the body temperature of the subject back to normal body temperature, wherein step (i) is performed before, after, or simultaneously with step (ii), wherein the temperature-sensitive agent comprises a therapeutic agent comprising a human ZSCAN4 protein, or a nucleic acid comprising a coding region of human ZSCAN4, and the effect is a therapeutic effect.

62. The method of claim 61, wherein the temperature-sensitive agent is administered systemically.

63. The method of claim 62, wherein the temperature-sensitive agent is administered intravenously.

64. The method of claim 61, wherein the temperature-sensitive agent is administered to a specific tissue or organ of the subject.

65. The method of claim 64, wherein the temperature-sensitive agent is administered to the brain and spinal cord by epidural injection.

66. The method of claim 64, wherein the temperature-sensitive agent is administered by percutaneous injection into a target organ.

67. The method of claim 64, wherein the temperature-sensitive agent is administered by endoscopy with an injection needle catheter into a target organ.

68. The method of claim 64, wherein the temperature-sensitive agent is administered by angiocatheter into a target organ.

69. The method of claim 66, wherein the target organ is selected from the group consisting of the liver, kidneys, skeletal muscles, cardiac muscles, pancreas, spleen, heart, brain, spinal cord, skin, eye, lung, intestine, thymus, bone marrow, bone, and cartilage.

70. The method of claim 61, wherein the temperature-sensitive agent is administered by inhalation.

71. The method of claim 61, wherein lowering the body temperature of a subject comprises using a targeted temperature management (TTM) procedure, wherein the TTM procedure comprises application to the subject of one of the group consisting of a cooling catheter, a cooling blanket, and ice.
72. The method of claim 61, wherein normal body temperature of the subject is a non-permissive temperature for the temperature-sensitive agent.
73. The method of claim 61, wherein the temperature-sensitive agent comprises a human ZSCAN4 protein.
74. The method of claim 61, wherein the temperature-sensitive agent comprises a nucleic acid comprising a coding region of human ZSCAN4.
75. The method of claim 74, wherein a temperature-sensitive viral vector comprises the nucleic acid comprising the coding region of human ZSCAN4.
76. The method of claim 75, wherein the temperature-sensitive viral vector is selected from the group consisting of a Sendai virus, an Adeno virus, an Adeno-associated virus, a Retrovirus, and an Alphavirus.
77. The method of claim 75, wherein said temperature-sensitive viral vector is an Alphavirus.
78. The method of claim 77, wherein said Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.
79. The method of claim 75, wherein the temperature-sensitive viral vector is a Sendai virus.
80. The method of claim 79, wherein the Sendai virus is SeV18+/TS15ΔF.
81. The method of claim 75, wherein the temperature sensitive activity comprises replication and transcription of the temperature-sensitive viral vector.
82. The method of claim 74, wherein a temperature-sensitive self-replicating RNA comprises the nucleic acid comprising the coding region of human ZSCAN4.

83. The method of claim 82, wherein the self-replicating RNA comprises an Alphavirus replicon lacking a viral structural protein coding region.
84. The method of claim 83, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.
85. The method of claim 82, wherein the temperature-sensitive activity comprises one or both of replication and transcription of the temperature-sensitive self-replicating RNA.
86. The method of claim 74, wherein the coding region is operably linked to a promoter.
87. The method of claim 50, wherein the period of time sufficient for the temperature-sensitive activity to produce the therapeutic effect ranges from about 12 hours to about 12 weeks, optionally wherein the period of time is from 1 to 7 days.
88. The method of claim 61, wherein the period of time sufficient to induce the therapeutic effect in the subject is from about 12 hours to about 7 days, optionally wherein the period of time is from about 12 hours to about 72 hours.
89. The method of any one of claims 50-88, wherein the permissive temperature ranges from 30°C to 36°C, or 31°C to 35°C, or 32°C to 34°C.
90. The method of claim 89, wherein the permissive temperature is 33°C $\pm$ 0.5°C.
91. The method of claim 90, wherein the non-permissive temperature is 37°C $\pm$ 0.5°C.
92. The method of claim 50, wherein the one or more cells are human cells.
93. The method of claim 92, wherein the one or more human cells are adult stem cells, tissue stem cells, progenitor cells, embryonic stem cells, or induced pluripotent stem cells.
94. The method of claim 92, wherein the one or more human cells are selected from the group consisting of hematopoietic stem cells, mesenchymal stem cells, endothelial stem cells, adipose stem cells, neuronal stem cells, and germ stem cells.

95. The method of claim 92, wherein the one or more human cells are somatic cells, mature cells, or differentiated cells.
96. The method of claim 95, wherein the one or more human cells are selected from the group consisting of epidermal cells, fibroblasts, lymphocytes, hepatocytes, epithelial cells, myocytes, chondrocytes, osteocytes, adipocytes, cardiomyocytes, pancreatic cells, pancreatic β cells, keratinocytes, erythrocytes, peripheral blood mononuclear cells (PBMCs), neurons, glia cells, neurocytes, astrocytes, germ cells, sperm cells, and oocytes.
97. The method of claim 92, wherein the one or more human cells are human bone marrow cells.
98. The method of claim 97, wherein the human bone marrow cells are CD34⁺ hematopoietic stem cells.
99. The method of claim 98, wherein the human subject suffers from a telomere biology disorder, optionally wherein the subject suffers from bone marrow failure.
100. The method of claim 89, wherein the temperature-sensitive viral vector or the temperature-sensitive self-replicating RNA comprises a nonstructural protein coding region with an insertion of 12-18 nucleotides, wherein the insertion results in expression of a nonstructural Protein 2 (nsP2 = helicase proteinase) comprising from 4 to 6 additional amino acids between beta sheet 5 and beta sheet 6 of the nsP2, optionally wherein the additional amino acids result in temperature-sensitivity of the viral vector or the self-replicating RNA.
101. The method of claim 100, wherein the additional amino acids comprise one sequence selected from the group consisting of SEQ ID NO:43 (GCGRT), SEQ ID NO:44 (TGAAA), and SEQ ID NO:45 (LRPHP).
102. The method of claim 100, wherein the additional amino acids comprise the sequence of SEQ ID NO:44 (TGAAA).
103. The method of claim 102, wherein the amino acid sequence of the NsP2 comprises one sequence selected from the group consisting of SEQ ID NOs:29-36.

104. The method of any one of claims 50-103, wherein the amino acid sequence of human ZSCAN4 comprises SEQ ID NO:38 or is at least 95% identical to SEQ ID NO:38.

105. The method of any one of claims 50-103, wherein the amino acid sequence of human ZSCAN4 comprises 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.