SUMMARY

[0012] Based on the necessity of having time-limited expression of a gene of interest (GOI), a transient gene product delivery system is required, where a nucleic acid or protein can be delivered to or expressed in specific cells, *in vitro* or *in vivo*, where the amount of nucleic acid/protein is sufficient to have a biologically meaningful effect, and where transient expression can be turned off permanently after achieving the biologically meaningful effect. In order to meet these and other needs, the present disclosure relates to methods for transiently inducing an activity of a temperature-sensitive agent (ts-agent) such as a therapeutic ts-agent, either in a subject (*in vivo*) or in cells in culture (*ex vivo*). In some ~~embodiment~~examples, the therapeutic ts-agent is used in combination with mild therapeutic hypothermia. In other ~~embodiment~~examples, the therapeutic ts-agent is used in combination with mild therapeutic hyperthermia, or a localized application of heat. In some ~~embodiment~~examples, the ts-agent is a ts-RNA molecule or ts-protein molecule. In some ~~embodiment~~examples, the ts-agent is encoded by a heterologous nucleic acid inserted in a temperature-sensitive viral vector or a self-replicating RNA. In some ~~embodiment~~examples, the viral vector is selected from but not limited to a Sendai virus vector, a retrovirus vector, an adeno virus vector, an adeno-associated virus vector, and an Alpha virus vector. In some ~~embodiment~~examples, the self-replicating RNA comprises an Alphavirus replicon lacking a viral structural protein coding region. In some ~~embodiment~~examples, the Alphavirus is selected from but not limited to a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus. In some ~~embodiment~~examples, the gene product of interest is not ZSCAN4.

[0012a] In one aspect, the present disclosure refers to a temperature-sensitive agent (ts-agent), wherein the ts-agent is a temperature-sensitive self-replicating RNA comprising an Alphavirus replicon lacking a viral structural protein coding region, and wherein the ts-agent 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.

[0012b] In one aspect, the present disclosure refers to a composition comprising a temperature-sensitive agent (ts-agent) encoding an antigen, wherein the ts-agent is a temperature-sensitive Sendai viral vector, the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

[0012c] In one aspect, the present disclosure refers to a composition comprising a temperature-sensitive agent (ts-agent) encoding an antigen, wherein the ts-agent is a temperature-sensitive self-replicating RNA comprising an Alphavirus replicon lacking a viral structural protein coding region, the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

[0012d] In one aspect, the present disclosure refers to use of a composition comprising a temperature-sensitive agent (ts-agent) encoding an antigen in the manufacture of a medicament for stimulating an immune response against the antigen in a mammalian subject, wherein the ts-agent is a temperature-sensitive Sendai viral vector, the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

[0012e] In one aspect, the present disclosure refers to use of a composition comprising a temperature-sensitive agent (ts-agent) encoding antigen in the manufacture of a medicament for stimulating an immune response against the antigen in a mammalian subject, wherein the ts-agent is a temperature-sensitive self-replicating RNA comprising an Alphavirus replicon lacking a viral structural protein coding region, the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

[0013] The foregoing and other objects and features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **FIGS. 1A – 1D** depict the structure of the Venezuelan Equine Encephalitis Virus (VEEV) genome and locations of mutated regions. **FIG. 1A** shows a schematic representation of a wild type VEEV genome (TC-83 strain: complete genome 11,446 bp linear RNA: NCBI Accession: L01443.1 GI: 323714). Genes of nonstructural proteins (nsP1, nsP2, nsP3, nsP4) encode RNA-dependent RNA polymerase and genes of structural proteins encode viral envelope proteins (C, E1, E2). 5'-UTR (5'-untranslated region) and 3'-UTR (3'-untranslated region). The

CLAIMS

What is claimed:

~~1. An immunogenic composition for stimulating an immune response against a pathogen in a subject, comprising an excipient and a temperature-sensitive agent (ts-agent), wherein the ts-agent is a temperature-sensitive viral vector or a temperature-sensitive self-replicating RNA encoding an antigen of the pathogen, and wherein the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature.~~

~~2. The composition of claim 1, wherein the pathogen comprises one or more of a virus, a bacterium, a protozoan, and a fungus.~~

~~3. The composition of claim 2, wherein the antigen comprises a surface protein or fragment thereof of the pathogen.~~

~~4. The composition of claim 3, wherein the pathogen is a virus, and the virus is different than the viral vector.~~

~~5. The composition of claim 4, wherein the virus is a coronavirus and the antigen comprises a spike protein or fragment thereof of the coronavirus.~~

~~6. The composition of claim 5, wherein the coronavirus is 2019-nCoV and the antigen comprises a receptor-binding domain (RBD) of the 2019-nCoV.~~

~~7. The composition of claim 6, wherein the amino acid sequence of the RBD comprises SEQ ID NO:44, or the amino acid sequence at least 75%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO:44.~~

~~8. The composition of claim 5, wherein the coronavirus is 2019-nCoV and the antigen comprises an extracellular region of the spike protein comprising the amino acid sequence of residues 16-1213 of SEQ ID NO:41, or the amino acid sequence at least 75%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO:41.~~

~~9. The composition of any one of claims 1-8, wherein the permissive temperature is from 30ºC to 36ºC, or 31ºC to 35ºC, or 32ºC to 34ºC, or 33ºC ± 0.5ºC, and the non-permissive temperature is 37ºC ± 0.5ºC.~~

~~10. The composition of claim 9, wherein the ts-agent is a temperature-sensitive self-replicating RNA.~~

~~11. The composition of claim 10, wherein the self-replicating RNA comprises an Alphavirus replicon lacking a viral structural protein coding region.~~

~~12. The composition of claim 11, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.~~

~~13. The composition of claim 11, wherein the Alphavirus is a Venezuelan equine encephalitis virus.~~

~~14. The composition of claim 9, wherein the ts-agent is a temperature-sensitive viral vector.~~

~~15. The composition of claim 14, wherein the viral vector is selected from the group consisting of a Sendai virus, an Adeno virus, an Adeno-associated virus, a Retrovirus, and an Alphavirus.~~

~~16. The composition of claim 15, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.~~

~~17. The composition of claim 15, wherein the viral vector is a Sendai virus.~~

~~18. The composition of claim 9, wherein the subject is a human and the pathogen is a human pathogen.~~

~~19. A method for stimulating an immune response against a pathogen in a mammalian subject, comprising administering the immunogenic composition of claim 18 to a mammalian subject so as to stimulate an immune response against the antigen in the mammalian subject, wherein the mammalian subject is a human subject.~~

~~20. The method of claim 19, wherein the immunogenic composition is administered:~~
~~i) intradermally or subcutaneously; or~~
~~ii) intramuscularly.~~

~~21. The method of claim 19, wherein the immunogenic composition is administered intranasally.~~

~~22~~1. A temperature-sensitive agent (ts-agent), wherein the ts-agent is ~~a temperature-sensitive viral vector or~~ a temperature-sensitive self-replicating RNA comprising an Alphavirus replicon lacking a viral structural protein coding region, and wherein the ts-agent comprises a nonstructural protein coding region with an insertion of 12-18 nucleotides~~, wherein the insertion results~~ resulting 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~~2. The temperature-sensitive agent of claim ~~22~~1, wherein the additional amino acids comprise a~~one~~ sequence selected from the group consisting of SEQ ID NO:38 (GCGRT), SEQ ID NO:39 (TGAAA), and SEQ ID NO:40 (LRPHP).

~~24~~3. The temperature-sensitive agent of claim ~~22~~1, wherein the additional amino acids comprise the sequence of SEQ ID NO:39 (TGAAA).

~~25~~4. The temperature-sensitive agent of claim ~~24~~1, wherein the amino acid sequence of the ~~N~~nsP2 comprises a~~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~~5. The temperature-sensitive agent of any one of claims 1-4~~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~~6. The temperature-sensitive agent of any one of claims 1-4~~claim 26 or claim 27~~, wherein the Alphavirus is a Venezuelan equine encephalitis virus.

7. The temperature-sensitive agent of any one of claims 1-6, wherein the ts-agent encodes an antigen of a pathogen, and the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature.

8. The temperature-sensitive agent of claim 7, wherein the antigen is a viral antigen and the viral antigen is not an Alphavirus antigen.

9. The temperature-sensitive agent of any one of claims 1-6, wherein the ts-agent encodes a pharmaceutical agent, and wherein the ts-agent is capable of expressing the pharmaceutical agent at a permissive temperature but not at a non-permissive temperature.

10. The temperature-sensitive agent of claim 9, wherein the pharmaceutical agent comprises a protein.

11. The temperature-sensitive agent of claim 9, wherein the pharmaceutical agent is selected from the group consisting of a non-coding RNA, a siRNA, a shRNA, and an endonuclease editing system.

12. The temperature-sensitive agent of any one of claims 1-11, wherein the permissive temperature is from 31ºC to 35ºC, and the non-permissive temperature is 37ºC ± 0.5ºC.

13. A composition comprising a temperature-sensitive agent (ts-agent) encoding an antigen, wherein the ts-agent is a temperature-sensitive Sendai viral vector, the ts-agent is capable of

expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

14. The composition of claim 13, wherein the coronavirus is 2019-nCoV and the antigen comprises a receptor-binding domain (RBD) of the 2019-nCoV spike protein.

15. The composition of claim 13 or claim 14, wherein the permissive temperature is from 31ºC to 35ºC, and the non-permissive temperature is 37ºC ± 0.5ºC.

16. A composition comprising a temperature-sensitive agent (ts-agent) encoding an antigen, wherein the ts-agent is a temperature-sensitive self-replicating RNA comprising an Alphavirus replicon lacking a viral structural protein coding region, the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

17. The composition of claim 16, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.

18. The composition of claim 16, wherein the Alphavirus is a Venezuelan equine encephalitis virus.

19. The composition of any one of claims 16-18, wherein the coronavirus is 2019-nCoV and the antigen comprises a receptor-binding domain (RBD) of the 2019-nCoV spike protein.

20. The composition of any one of claims 16-19, wherein the permissive temperature is from 31ºC to 35ºC, and the non-permissive temperature is 37ºC ± 0.5ºC.

21. Use of a composition comprising a temperature-sensitive agent (ts-agent) encoding an antigen in the manufacture of a medicament for stimulating an immune response against the antigen in a mammalian subject, wherein the ts-agent is a temperature-sensitive Sendai viral vector, the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

22. The use of claim 21, wherein the coronavirus is 2019-nCoV and the antigen comprises a receptor-binding domain (RBD) of the 2019-nCoV spike protein.

23. The use of claim 21 or claim 22, wherein the permissive temperature is from 31ºC to 35ºC, and the non-permissive temperature is 37ºC ± 0.5ºC.

24. Use of a composition comprising a temperature-sensitive agent (ts-agent) encoding antigen in the manufacture of a medicament for stimulating an immune response against the antigen in a mammalian subject, wherein the ts-agent is a temperature-sensitive self-replicating RNA comprising an Alphavirus replicon lacking a viral structural protein coding region, the ts-agent is capable of expressing the antigen at a permissive temperature but not at a non-permissive temperature, and the antigen is a spike protein or fragment thereof of a coronavirus.

25. The use of claim 24, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.

26. The use of claim 24, wherein the Alphavirus is a Venezuelan equine encephalitis virus.

27. The use of claim any one of clams 24-26, wherein the coronavirus is 2019-nCoV and the antigen comprises a receptor-binding domain (RBD) of the 2019-nCoV spike protein.

28. The use of any one of claims 24-27, wherein the permissive temperature is from 31ºC to 35ºC, and the non-permissive temperature is 37ºC ± 0.5ºC.

~~30. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent (ts-agent) in a subject, 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 is induced 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 temperature-sensitive activity of the ts-agent is induced 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 30ºC to 36ºC, or 31ºC to 35ºC, or 32ºC to 34ºC, or 33ºC ± 0.5ºC, and the non-permissive temperature is 37ºC ± 0.5ºC.~~

~~36. The method of claim 35, wherein a pharmaceutical agent is encoded by a coding region of the ts-agent or the ts-agent comprises a pharmaceutical agent, and the effect comprises a pharmaceutical effect, optionally wherein the pharmaceutical agent is a therapeutic agent and the pharmaceutical effect is a therapeutic effect, or wherein the pharmaceutical agent is a prophylactic agent and the pharmaceutical effect is a prophylactic effect.~~

~~37. The method claim 35, wherein the ts-agent is a temperature-sensitive viral vector and the temperature-sensitive activity 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, 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 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 pharmaceutical agent is selected from the group consisting of a non-coding RNA, a siRNA, a shRNA, and an endonuclease-editing system.~~

~~46. The method of claim 36, wherein the pharmaceutical agent comprises a protein.~~

~~47. The method of claim 46, wherein the protein comprises an antigen of a pathogen.~~

~~48. The method of claim 47, wherein the pathogen comprises one or more of a virus, a bacterium, a protozoan, and a fungus.~~

~~49. The method of claim 47, wherein the antigen comprises a surface protein or fragment thereof of the pathogen.~~

50. 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.

51. 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.

52. 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 and the effect comprises a therapeutic effect.

53. The method of claim 52, further comprising before step i): contacting the one or more cells with the temperature-sensitive agent.

54. The method of claim 53, wherein the one or more cells are at the permissive temperature when contacted with the temperature-sensitive agent.

55. The method of claim 52, further comprising administering the one or more cells to a subject in need of the therapeutic effect.

56. The method of claim 52, 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.

57. The method of claim 56, 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

~~58. The method of claim 52, wherein the one or more cells are embryonic stem cells or induced pluripotent stem cells.~~

~~59. The method of claim 58, wherein the therapeutic effect comprises the differentiation of said cells into a desired cell type.~~

~~60. The method of claim 59, wherein the desired cell type is selected from the group consisting of a neuron, a glia cell, and an endothelial cell.~~

~~61. The method of claim 53, wherein the one or more cells were isolated from the subject before contacting the one or more cells with the temperature-sensitive agent.~~

~~62. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent in a 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) increasing the body temperature of the subject back to normal body temperature.~~

~~63. A method for transiently inducing a temperature-sensitive activity of a temperature-sensitive agent in a 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) increasing the body temperature of the subject back to normal body temperature, wherein step (i) is performed before, after, or simultaneously with step (ii).~~

~~64. The method of claim 63, wherein the temperature-sensitive agent is administered systemically.~~

~~65. The method of claim 64, wherein the temperature-sensitive agent is administered intravenously.~~

~~66. The method of claim 63, wherein the temperature-sensitive agent is administered to a specific tissue or organ of the subject.~~

~~67. The method of claim 66, wherein the temperature-sensitive agent is administered to the brain and spinal cord by epidural injection.~~

~~68. The method of claim 66, wherein the temperature-sensitive agent is administered by percutaneous injection into a target organ.~~

~~69. The method of claim 66, wherein the temperature-sensitive agent is administered by endoscopy with an injection needle catheter into a target organ.~~

~~70. The method of claim 66, wherein the temperature-sensitive agent is administered by angiocatheter into a target organ.~~

~~71. The method of claim 68, 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.~~

~~72. The method of claim 63, wherein the temperature-sensitive agent is administered by inhalation.~~

~~73. The method of claim 63, wherein altering 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.~~

~~74. The method of claim 63, wherein the subject is a human.~~

~~75. The method of claim 63, wherein the temperature-sensitive agent comprises a therapeutic agent, and the effect comprises a therapeutic effect.~~

~~76. The method claim 75, wherein the therapeutic agent is encoded by a coding region of a heterologous nucleic acid of a temperature-sensitive viral vector.~~

~~77. The method of claim 76, wherein said 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.~~

~~78. The method of claim 77, wherein said temperature-sensitive viral vector is an Alphavirus.~~

~~79. The method of claim 78, wherein said Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.~~

~~80. The method of claim 77, wherein the temperature-sensitive viral vector is a Sendai virus.~~

~~81. The method of claim 75, wherein the therapeutic agent is selected from the group consisting of a non-coding RNA, a siRNA, a shRNA, and an endonuclease editing system.~~

~~82. The method of claim 75, wherein the therapeutic agent is a protein.~~

~~83. The method of claim 82, wherein the protein is a transcription factor, optionally wherein the transcription factor is selected from the group consisting of human neurogenin-3 (NGN3), and human ETS translocation variant 2 (ETV2).~~

~~84. The method of claim 82, wherein the protein is a growth factor, optionally wherein the growth factor is selected from the group consisting of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF).~~

~~85. The method of claim 76, wherein the temperature-sensitive activity comprises replication and transcription of the temperature-sensitive viral vector.~~

~~86. The method of claim 75, wherein the therapeutic agent is encoded by a coding region of a temperature-sensitive self-replicating RNA.~~

87. The method of claim 86, wherein said self-replicating RNA comprises an Alphavirus replicon lacking a viral structural protein coding region.

88. The method of claim 87, wherein said Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.

89. The method of claim 86, wherein the therapeutic agent is selected from the group consisting of a non-coding RNA, a siRNA, a shRNA, and an endonuclease editing system.

90. The method of claim 86, wherein the therapeutic agent is a protein.

91. The method of claim 90, wherein the protein is a transcription factor, optionally wherein the transcription factor is selected from the group consisting of human neurogenin-3 (NGN3), and human ETS translocation variant 2 (ETV2).

92. The method of claim 90, wherein the protein is a growth factor, optionally wherein the growth factor is selected from the group consisting of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF).

93. The method of claim 86, wherein the temperature-sensitive activity comprises one or both of replication and transcription of the temperature-sensitive self-replicating RNA.

94. The method of claim 76, wherein the coding region is operably linked to a promoter.

95. The method of claim 75, wherein the period of time sufficient for the temperature-sensitive activity to produce a therapeutic effect ranges from about 12 hours to about 12 weeks, optionally wherein the period of time is from 1 to 7 days.

96. The method of claim 75, 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.

97. The method of any one of claims 52-96, wherein the permissive temperature ranges from 30ºC to 36ºC, or 31ºC to 35ºC, or 32ºC to 34ºC.

98. The method of claim 97, wherein the permissive temperature is 33ºC ± 0.5ºC.

99. The method of claim 98, wherein the non-permissive temperature is 37ºC ± 0.5ºC.

100. The method of claim 52, wherein the one or more cells are human cells.

101. The method of claim 100, wherein the one or more human cells are adult stem cells, tissue stem cells, progenitor cells, embryonic stem cells, or induced pluripotent stem cells.

102. The method of claim 101, wherein the one or more human cells are selected from the group consisting of hematopoietic stem cells, mesenchymal stem cells, adipose stem cells, neuronal stem cells, and germ stem cells.

103. The method of claim 100, wherein the one or more human cells are somatic cells, mature cells, or differentiated cells.

104. The method of claim 103, 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 (PBMC), neurons, glia cells, neurocytes, astrocytes, germ cells, sperm cells, and oocytes.

105. The method of claim 100, wherein the one or more human cells are human bone marrow cells.

106. The method of claim 105, wherein the human bone marrow cells are selected from the group consisting of hematopoietic stem cells, mesenchymal stem cells, and endothelial stem cells.

107. The method of claim 106, wherein the hematopoietic stem cells are CD34+.

108. The method of claim 97, 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.

~~109. The method of claim 108, 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).~~

~~110. The method of claim 108, wherein the additional amino acids comprise the sequence of SEQ ID NO:39 (TGAAA).~~

~~111. The method of claim 110, wherein the amino acid sequence of the NsP2 comprises one sequence selected from the group consisting of SEQ ID NOs:29-36.~~