

particular, control of transcription and translation of RNA vectors and self-replicating RNA is desirable.

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 tools 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 examples, the therapeutic ts-agent is used in combination with mild therapeutic hypothermia. In other examples, the therapeutic ts-agent is used in combination with mild therapeutic hyperthermia, or a localized application of heat. In some examples, the ts-agent is a ts-RNA molecule or ts-protein molecule. In some 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 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 examples, the self-replicating RNA comprises an Alphavirus replicon lacking a viral structural protein coding region. In some examples, the Alphavirus is selected from but not limited to a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus. A gene product of particular interest is ZSCAN4, particularly human ZSCAN4.

[0012a] In one aspect, the present disclosure refers to 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 comprising bone marrow failure, the use comprising:

i) isolating CD34⁺ cells from a sample of peripheral blood mononuclear cells obtained from a human subject suffering from the disease after hematopoietic stem cells were mobilized from bone marrow to peripheral blood of the subject;

ii) contacting the isolated CD34⁺ cells with the medicament; and

iii) incubating the contacted CD34⁺ cells at a permissive temperature of 33°C ± 0.5°C for a period of about 12 hours to about 24 hours, wherein replication and transcription of the temperature-sensitive Sendai viral vector occurs at the permissive temperature leading to increased expression of human ZSCAN4;

wherein the contacted CD34⁺ cells are to be infused into the subject under conditions suitable for engrafting the cells to treat the disease.

[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)

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 comprising bone marrow failure, the use comprising:

i) isolating CD34⁺ cells from a sample of peripheral blood mononuclear cells obtained from a human subject suffering from the disease after hematopoietic stem cells were mobilized from bone marrow to peripheral blood of the subject;

ii) contacting the isolated CD34⁺ cells with the medicament; and

iii) incubating the contacted CD34⁺ cells at a permissive temperature of $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for a period of about 12 hours to about 24 hours, wherein replication and transcription of the temperature-sensitive Sendai viral vector occurs at the permissive temperature leading to increased expression of human ZSCAN4; wherein the contacted CD34⁺ cells are to be infused into the subject under conditions suitable for engrafting the cells to treat the disease.

2. The use of claim 1, further comprising after step iii) incubating the contacted CD34⁺ cells at a 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.

3. The use of claim 2, wherein the contacted CD34⁺ cells are incubated at the non-permissive temperature of $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for a period of about 30 minutes to about 180 minutes.

4. The use of any one of claims 1-3, wherein the hematopoietic stem cells were mobilized by administration of one or both of granulocyte-colony stimulation factor and plerixafor to the subject.

5. The use of any one of claims 1-4, wherein the CD34⁺ cells are isolated from the peripheral blood mononuclear cells by positive selection using an anti-CD34 antibody and magnetic beads.

6. The use of any one of claims 1-5, wherein the contacted CD34⁺ cells are washed and resuspended in a sterile, isotonic aqueous solution prior to infusion.
7. The use of any one of claims 1-6, wherein the contacted CD34⁺ cells are to be intravenously infused at a dose of about 1.0×10^5 cells/kg to about 1.0×10^7 cells/kg.