

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 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°C ± 0.5°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°C ± 0.5°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.

**[0012b]** 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 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+ cells from a sample of peripheral blood mononuclear cells obtained from the subject;
- iii) contacting the isolated CD34+ cells with the medicament;
- 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.

**[0012c]** In one aspect, the present disclosure refers to 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.

**[0012d]** In one aspect, the present disclosure refers to 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.

**[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)