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 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. In some 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. 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.

2. The temperature-sensitive agent of claim 1, wherein the additional amino acids comprise a sequence selected from the group consisting of SEQ ID NO:38 (GCGRT), SEQ ID NO:39 (TGAAA), and SEQ ID NO:40 (LRPHP).

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

4. The temperature-sensitive agent of claim 1, wherein the amino acid sequence of the nsP2 comprises a sequence selected from the group consisting of SEQ ID NOs:29-36.

5. The temperature-sensitive agent of any one of claims 1-4, wherein the Alphavirus is selected from the group consisting of a Venezuelan equine encephalitis virus, a Sindbis virus, and a Semliki Forrest virus.

6. The temperature-sensitive agent of any one of claims 1-4, 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.