

INTERFERING RNAS TARGETING SEVERE ACUTE RESPIRATORY SYNDROME-ASSOCIATED CORONAVIRUS AND USES THEREOF FOR TREATING COVID-19

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of the filing dates of International Application No. PCT/CN2020/133565, filed December 3, 2020 and International Application No. PCT/CN2021/121762, filed September 29, 2021, the entire contents of each of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 Coronaviruses, members of the family Coronaviridae and subfamily Coronavirinae, are enveloped viruses containing single-strand, positive-sense RNA genome ranging from 26 to 32 kilobases in length. Coronaviruses have been identified in several vertebrate hosts including bird, bat, pig, rodent, camel, and human. Human can acquire coronavirus infection from other host of mammals, which may cause detrimental upper respiratory tract illness.

15 Members of the coronaviruses family include virus strains having different phylogenetic origin and causing different severity in mortality and morbidity. As such, treatment for coronavirus infection varies depending on the specific strains, for example, SARS-CoV-2 variants (*e.g.*, the Delta variant) that cause the infection. So far, there is no approved antiviral drug treatment for coronavirus infection. Accordingly, there is a need for developing treatment of coronavirus infection, particularly treatment of infection caused by severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV), *e.g.*, treatment of COVID-19.

SUMMARY

25 The present disclosure is based, at least in part, on the development of interfering RNA molecules targeting the genomic RNA of a SARS-CoV virus, for example, SARS-CoV-1 or SARS-CoV-2. The interfering RNA molecules disclosed herein exhibited high efficiency in inhibiting SARS-CoV-2 replication and production. Accordingly, provided herein are anti-SARS-CoV-2 interfering RNAs (*e.g.*, siRNAs) and uses thereof for inhibiting a SARS-CoV-2 virus and for treating COVID-19.

30 In one aspect, the present disclosure provides a use of a small interfering RNA (siRNA) in the manufacture of a medicament for inhibiting a severe acute respiratory syndrome coronavirus

(SARS-CoV) wherein the siRNA targets a genomic site of a SARS-CoV virus, which optionally is SARS-CoV-1 or SARS-CoV-2. In another aspect, the present disclosure provides a small interfering RNA (siRNA) that targets a SARS-CoV virus, wherein the siRNA comprises a sequence complementary to a genomic site of the SARS-CoV virus, wherein the siRNA is set forth as described herein. In yet another aspect, the present disclosure provides a pharmaceutical composition comprising a small interfering RNA as described herein and a pharmaceutically acceptable carrier. In some examples, the present disclosure provides a method for inhibiting a SARS-CoV-2 virus (e.g., SARS-CoV-1 or SARS-CoV-2), the method comprising: contacting an effective amount of a

small interfering RNA (siRNA) with a cell infected with a SARS-CoV virus, wherein the siRNA targets a genomic site of the SARS-CoV virus. In some examples, the siRNA may target a SARS-CoV (e.g., SARS-CoV-1 or SARS-CoV-2) POL gene, Spike gene, Helicase gene, or Envelop gene. In some examples, the siRNA may target a SARS-CoV genomic site (e.g., a genomic site of SARS-CoV-1 or SARS-CoV-2) comprising one of the following nucleotide sequences (e.g., (vi)-(viii), (x), or (xi)):

- (i) 5'- GAGGCACGUCAACAUCUUA-3' (SEQ ID NO: 2),
- (ii) 5'- CAGCAUUAUAAUCACACUAA-3' (SEQ ID NO: 4),
- (iii) 5'- CGGUGUUUAAACCGUGUUU-3' (SEQ ID NO: 6),
- (iv) 5'- GUGGUACAACUACACUAA-3' (SEQ ID NO: 8),
- (v) 5'- UGGCUUGAUGACGUAGUUU-3' (SEQ ID NO: 10),
- (vi) 5'- CUGUCAAACCCGGUAAUUU-3' (SEQ ID NO: 12),
- (vii) 5'- GCGGUUCACUAUAUGUAA-3' (SEQ ID NO: 14),
- (viii) 5'- GCCACUAGUCUCUAGUCAG-3' (SEQ ID NO: 16),
- (ix) 5'- CUCCUACUUGGCGUGUUUA-3' (SEQ ID NO: 18),
- (x) 5'- CGCACAUUGCUAACUAAGG-3' (SEQ ID NO: 20), and
- (xi) 5'- CAGGUACGUAAUAGUUA-3' (SEQ ID NO: 22).

As used herein, an siRNA targeting a genomic site of a SARS-CoV virus (e.g., SARS-CoV-1 or SARS-CoV-2) means that the siRNA comprises a fragment that is complementary to the genomic site (completely or partially) such that the siRNA can interact with the genomic RNA of the virus or a messenger RNA (mRNA) comprising a region transcribed by the genomic site to exert its inhibitory activity, e.g., inhibiting viral genome replication and/or down-regulating expression of the encoded protein product. In some instances, the siRNA targets a site of an mRNA synthesized by the SARS-CoV virus.

In some examples, the siRNA disclosed herein may target a site within a RNA-dependent RNA polymerase (RdRP) of the SARS-CoV virus (e.g., target a site with the RdRP messenger RNA (mRNA)). Such a siRNA may target a site in the RdRP mRNA within the nucleotide sequence of 5'-UUGCUUUUCAAACUGUCAAACCCGGUAAUUUUAACAAAGA-3' (SEQ ID NO: 23). In some examples, the siRNA may target a site within the nucleotide sequence of 5'-UUUCAAACUGUCAAACCCGGUAAUUUU-3' (SEQ ID NO: 24).

In other examples, provided herein are any of the siRNAs disclosed herein that targets a SARS-CoV-2 virus, for example, C6, C7, C8, C10, or C11, and pharmaceutical compositions comprising such and a pharmaceutically acceptable carrier. In some examples, the siRNAs are modified by, e.g., any pattern disclosed herein. In specific examples, the siRNAs are modified
5 siRNAs of C6G25S, C8G25S, or C10G31A.

Also within the scope of the present disclosure are any of the siRNAs or pharmaceutical compositions comprising such for use in inhibiting SARS-CoV (e.g., SARS-CoV-1 or SARS-CoV-2) infection and/or for treating diseases caused by the infection (e.g., COVID-19), as well as uses of such siRNA or pharmaceutical composition for manufacturing a medicament for use in inhibiting SARS-CoV
10 (e.g., SARS-CoV-1 or SARS-CoV-2) infection and/or for treating diseases caused by the infection, for example, COVID-19.

The details of one or more examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

15 BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific embodiments presented herein.

Figure 1 is a graph illustrating the inhibitory activities of exemplary siRNAs as indicated against
20 SARS-CoV-2 proliferation in Vero cells, as determined by RT-qPCR.

Figure 2 is a graph illustrating the reduction of virus production, as quantified by plaque assay, in the presence of the exemplary siRNAs as indicated.

Figures 3A-3E include diagrams showing the identification of highly potent siRNAs against SARS-CoV-2. **Figure 3A:** a flowchart illustrating the selection strategy used for identifying potent
25 siRNAs targeting SARS-CoV-2. The selection criteria and numbers of hits remaining at the end of each stage were indicated. **Figure 3B:** a graph showing the viral E gene expression in Vero E6 cells. Vero E6 cells were pre-transfected with 10 nM of siRNA and SARS-CoV-2 was added after 24h at a multiplicity of infection (MOI) of 0.1. The numbers of viral RNA copies were quantified with RT-qPCR. The control is scrambled siRNA and is abbreviated as “Ctrl.” C1–C11 are the final candidate
30 sequences after selection. **Figure 3C:** a graph showing the plaque inhibition in Vero E6 cells. Vero E6 cells were pre-transfected with 10 nM of siRNA and SARS-CoV-2 was added after

What Is Claimed Is:

1. Use of a small interfering RNA (siRNA) in the manufacture of a medicament for inhibiting a severe acute respiratory syndrome coronavirus (SARS-CoV)

5 wherein the siRNA targets a genomic site of a SARS-CoV virus, which optionally is SARS-CoV-1 or SARS-CoV-2.

2. The use of claim 1, wherein the genomic site is in a SARS-CoV gene selected from the group consisting of POL gene, Spike gene, Helicase gene, and Envelop gene.

10 3. The use of claim 1 or claim 2, wherein the genomic site comprises the nucleotide sequence selected from the group consisting of:

- (i) 5'- GAGGCACGUCAACAUCUUA-3' (SEQ ID NO: 2),
- (ii) 5'- CAGCAUUAAAUCACACUAA-3' (SEQ ID NO: 4),
- 15 (iii) 5'- CGGUGUUUAAACCGUGUUU-3' (SEQ ID NO: 6),
- (iv) 5'- GUGGUACAACUACACUAA-3' (SEQ ID NO: 8),
- (v) 5'- UGGCUUGAUGACGUAGUUU-3' (SEQ ID NO: 10),
- (vi) 5'- CUGUCAAAACCCGGUAAUUU-3' (SEQ ID NO: 12),
- (vii) 5'- GCGGUUCACUAUAUGUAA-3' (SEQ ID NO: 14),
- 20 (viii) 5'- GCCACUAGUCUCUAGUCAG-3' (SEQ ID NO: 16),
- (ix) 5'- CUCCUACUUGGCGUGUUUA-3' (SEQ ID NO: 18),
- (x) 5'- CGCACAUUGCUAACUAAGG-3' (SEQ ID NO: 20), and
- (xi) 5'- CAGGUACGUAAUAGUAA-3' (SEQ ID NO: 22).

25 4. The use of claim 1 or claim 2, wherein the siRNA targets a site in an RNA-dependent RNA polymerase (RdRP) gene of the SARS-CoV virus.

5. The use of claim 4, wherein the siRNA targets a site in the RdRP messenger RNA (mRNA).

6. The use of claim 5, wherein the siRNA targets a site in the mRNA, and wherein the target site is in the nucleotide sequence of 5'-UUGC UUUUCAAACUGUCAAAACCCGGUAAUUUUAAACAAAGA-3' (SEQ ID NO: 23).

5 7. The use of claim 6, wherein the target site is in the nucleotide sequence of 5'-UUUCAAAACUGUCAAAACCCGGUAAUUUU-3' (SEQ ID NO: 24).

8. The use of any one of claims 1-7, wherein the siRNA is a double-strand molecule comprising a sense chain and an antisense chain.

10 9. The use of claim 8, wherein the sense chain and the antisense chain comprises the following nucleotide sequences, respectively:

- (i) 5'- GAGGCACGUCAACAUCUUX₁-3' (SEQ ID NO: 25) and
5'- X₂AAGAUGUUGACGUGCCUCN₁N₂-3' (SEQ ID NO: 26);
- 15 (ii) 5'- CAGCAUUAAAUCACACUAX₁-3' (SEQ ID NO: 27), and
5'- X₂UAGUGUGAUUUAAUGCUGN₁N₂-3' (SEQ ID NO: 28);
- (iii) 5'- CGGUGUUUAAACCGUGUUX₁-3' (SEQ ID NO: 29), and
5'- X₂AACACGGUUUAAACACCGN₁N₂-3' (SEQ ID NO: 30);
- (iv) 5'- GUGGUACAACUACACUAX₁-3' (SEQ ID NO: 31), and
20 5'- X₂UAAGUGUAGUUGUACCACN₁N₂-3' (SEQ ID NO: 32),
- (v) 5'- UGGCUUGAUGACGUAGUUX₁-3' (SEQ ID NO: 33), and
5'- X₂AACUACGUCAUCAAGCCAN₁N₂-3' (SEQ ID NO: 34);
- (vi) 5'- CUGUCAAAACCCGGUAAUUX₁-3' (SEQ ID NO: 35), and
5'- X₂AAUUACCGGGUUUGACAGN₁N₂-3' (SEQ ID NO: 36);
- 25 (vii) 5'- GCGGUUCACUAUAUGUUAX₁-3' (SEQ ID NO: 37), and
5'- X₂UAACAUAUAGUGAACCGCN₁N₂-3' (SEQ ID NO: 38);
- (viii) 5'- GCCACUAGUCUCUAGUCAX₁-3' (SEQ ID NO: 39), and
5'- X₂UGACUAGAGACUAGUGGCN₁N₂-3' (SEQ ID NO: 40);
- (ix) 5'- CUCCUACUUGGCGUGUUUX₁-3' (SEQ ID NO: 41), and
30 5'- X₂AAACACGCCAAGUAGGAGN₁N₂-3' (SEQ ID NO: 42);
- (x) 5'- CGCACAUUGCUAACUAAGX₁-3' (SEQ ID NO: 43), and

5'-X₂CUUAGUUAGCAAUGUGCGN₁N₂-3' (SEQ ID NO: 44); or
 (xi) 5'-CAGGUACGUAAUAGUUAX₁-3' (SEQ ID NO: 45) and
 5'-X₂UAAACUAUUAACGUACCUGN₁N₂-3' (SEQ ID NO: 46);

wherein X₁ and X₂ in each of the sense chain and antisense chain of each of (i)-(xi),

5 independently, are A and U, respectively or vice versa, or G and C, respectively, or vice versa; and
 wherein each of N₁ and N₂ in each of the sense chain and antisense chain of each of (i)-(xi),
 independently, is A, U, G, or C; optionally wherein N₂ is U.

10. The use of claim 6, wherein the sense chain and the antisense chain comprises the
 10 following nucleotide sequences, respectively:

- (i) 5'-GAGGCACGUCAACAUCUUX₁-3' (SEQ ID NO: 25) and
 5'-X₂AAGAUGUUGACGUGCCUCUU-3' (SEQ ID NO: 47);
- (ii) 5'-CAGCAUUAUAUCACACUAX₁-3' (SEQ ID NO: 27), and
 5'-X₂UAGUGUGAUUUAUAGCUGUU-3' (SEQ ID NO: 48);
- 15 (iii) 5'-CGGUGUUUAAACCGUGUUX₁-3' (SEQ ID NO: 29), and
 5'-X₂AACACGGUUUAAACACCGUU-3' (SEQ ID NO: 49);
- (iv) 5'-GUGGUACAACUACACUAX₁-3' (SEQ ID NO: 31), and
 5'-X₂UAAGUGUAGUUGUACCACUU-3' (SEQ ID NO: 50);
- (v) 5'-UGGCUUGAUGACGUAGUUX₁-3' (SEQ ID NO: 33), and
 20 5'-X₂AACUACGUCAUCAAGCCAUU-3' (SEQ ID NO: 51);
- (vi) 5'-CUGUCAAAACCCGGUAAUUX₁-3' (SEQ ID NO: 35), and
 5'-X₂AAUUAACCGGUUUGACAGUU-3' (SEQ ID NO: 52);
- (vii) 5'-GCGGUUCACUAUAUGUUAX₁-3' (SEQ ID NO: 37), and
 5'-X₂UAACAUAUAGUGAACCGCUU-3' (SEQ ID NO: 53);
- 25 (viii) 5'-GCCACUAGUCUCUAGUCAX₁-3' (SEQ ID NO: 39), and
 5'-X₂UGACUAGAGACUAGUGGCUU-3' (SEQ ID NO: 54);
- (ix) 5'-CUCCUACUUGGCGUGUUX₁-3' (SEQ ID NO: 41), and
 5'-X₂AAACACGCCAAGUAGGAGUU-3' (SEQ ID NO: 55);
- (x) 5'-CGCACAUUGCUAACUAAGX₁-3' (SEQ ID NO: 43), and
 30 5'-X₂CUUAGUUAGCAAUGUGCGUU-3' (SEQ ID NO: 56); or
- (xi) 5'-CAGGUACGUAAUAGUUAX₁-3' (SEQ ID NO: 45) and

5'-X₂UAACUAUUAACGUACCUGUU-3' (SEQ ID NO: 57);

wherein X₁ and X₂ in each of the sense chain and antisense chain of each of (i)-(xi), independently, are A and U, respectively, or vice versa.

5 11. The use of claim 9 or claim 10, wherein the sense chain and the antisense chain comprise the nucleotide sequences set forth in (vi), (vii), (viii), (x), or (xi).

 12. The use of claim 1, wherein the siRNA is selected from the group consisting of C6, C7, C8, C10, and C11, optionally wherein the siRNA is C6.

10 13. The use of any one of claims 1-12, wherein the siRNA comprises one or more modified nucleotides.

 14. The use of claim 13, wherein the one or more modified nucleotides comprise 2'-
15 fluoro, 2'-O-methyl, or a combination thereof.

 15. The use of any one of claims 1-14, wherein the siRNA comprises one or more phosphorothioate bonds.

20 16. The use of claim 13, wherein the siRNA is C6G25S, C8G25S, or C10G31A, optionally wherein the siRNA is C6G25S.

 17. The use of any one of claims 1-16, wherein the siRNA is to be administered to a subject having infected by the SARS-CoV virus.

25 18. The use of claim 17, wherein the siRNA is formulated in a pharmaceutical composition, which further comprises a pharmaceutically acceptable carrier.

 19. The use of claim 17 or claim 18, wherein the subject is a human patient having
30 COVID-19.

20. The use of any one of claims 17-19, wherein the subject is to be further administered an agent for treatment of infection caused by the SARS-CoV, which optionally is SARS-CoV-1 or SARS-CoV-2, preferably wherein the infection is caused by SARS-CoV-2.

5 21. The use of claim 20, wherein the agent comprises an anti-SARS-CoV-2 antibody, remdesivir, a steroid, an anti-SARS-CoV vaccine, or a combination thereof.

22. The use of claim 17 or claim 18, wherein the subject is a human patient at risk for SARS-CoV infection.

10 23. The use of any one of claims 17-22, wherein the siRNA is to be administered to the subject by intranasal instillation, aerosol inhalation, or a combination thereof.

15 24. A small interfering RNA (siRNA) that targets a SARS-CoV virus, wherein the siRNA comprises a sequence complementary to a genomic site of the SARS-CoV virus, wherein the siRNA is set forth in any one of claims 1-16.

25. A pharmaceutical composition comprising a small interfering RNA of claim 22 and a pharmaceutically acceptable carrier.