

CLAIMS

What is claimed is:

1. An oligonucleotide comprising:
 - a sense strand comprising 17-36 nucleotides, wherein the sense strand has a first region (R1) and a second region (R2), wherein the second region (R2) of the sense strand comprises a first subregion (S1), a second subregion (S2) and a tetraloop (L) or triloop (triL) that joins the first and second regions, wherein the first and second subregions form a second duplex (D2);
 - an antisense strand comprising 20-22 nucleotides, wherein the antisense strand includes at least 1 single-stranded nucleotide at its 3'-terminus, wherein the sugar moiety of the nucleotide at position 5 of the antisense strand is modified with a 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-fluoro (2'-F), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), 2'-O-[2-(methylamino)-2-oxoethyl] (2'-O-NMA), and 2'-deoxy-2'-fluoro- β -D-arabinonucleic acid (2'-FANA), and wherein the sense strand and antisense strand are separate strands; and
 - a first duplex (D1) formed by the first region of the sense strand and the antisense strand, wherein the first duplex has a length of 12-20 base pairs and has 7-10 nucleotides that are modified at the 2'-position of the sugar moiety with 2'-F.
2. The oligonucleotide of claim 1, wherein the sugar moiety at positions 2 and 14 of the antisense strand is modified with 2'-F.
3. The oligonucleotide of claim 2, wherein the sugar moiety at each of up to 3 nucleotides at positions 1, 3, 7, and 10 of the antisense strand is additionally modified with 2'-F.
4. The oligonucleotide of any one of claims 1-3, wherein the sugar moiety of each of the nucleotides at positions 8-11 of the sense strand is additionally modified with 2'-F.
5. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 1-7 and 12-17 or 12-20 of the sense strand are modified with a

modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), 2'-O-[2-(methylamino)-2-oxoethyl] (2'-O-NMA), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

6. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 2, 5, and 14 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

7. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 1, 2, 5, and 14 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

8. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 1, 2, 3, 5, 7, and 14 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

9. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 2, 3, 5, 7, and 14 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

10. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 1, 2, 3, 5, 10, and 14 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

11. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 2, 3, 5, 10, and 14 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

12. The oligonucleotide of claim 1, wherein the sugar moiety of each of the nucleotides at positions 2, 3, 5, 7, 10, and 14 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid (2'-FANA).

13. The oligonucleotide of claim 1 or 12, wherein the antisense strand has 3 nucleotides that are modified at the 2'-position of the sugar moiety with 2'-F.

14. The oligonucleotide of any of the preceding claims, wherein the second duplex has a length of 1-6 base pairs.

15. The oligonucleotide of any of the preceding claims, wherein the second duplex comprises at least one bicyclic nucleotide.

16. The oligonucleotide of claim 15, wherein the second duplex has a length of 1-3 base pairs.

17. The oligonucleotide of any of the preceding claims, wherein the triloop has a nucleotide sequence of GAA or AAA or wherein the tetraloop is an RNA tetraloop selected from the group consisting of GAAA, UNCG, GNRA, or CUUG or a DNA tetraloop selected from the group consisting of d(GNAB), d(CNNG), or d(TNCG), wherein N is any one of U, A, C, G and R is G or A.

18. The oligonucleotide for reducing RNA expression of claim 1, wherein the sugar moiety of each nucleotide in the second duplex is modified with 2'-O-methyl (2'-OMe).

19. The oligonucleotide for reducing RNA expression of any of the preceding claims, wherein at least one of the nucleotides in the tetraloop or the triloop is conjugated to a ligand.

20. The oligonucleotide for reducing RNA expression of claim 19, wherein 1-3 nucleotides in the triloop or 1-4 nucleotides in the tetraloop are conjugated to a ligand.

21. The oligonucleotide for reducing RNA expression of claim 19 or 20, wherein the ligand comprises *N*-acetylgalactosamine.

22. The oligonucleotide for reducing RNA expression of the preceding claims, wherein the nucleotide at position 1 of the antisense strand comprises a phosphate mimic.

23. The oligonucleotide for reducing RNA expression of any of the preceding claims, wherein the sense strand comprises 36 nucleotides and the antisense strand comprises 22 nucleotides.

24. A single-stranded oligonucleotide comprising 20-22 nucleotides, wherein the sugar moiety of each of the nucleotides at positions 2, 5, and 14 and optionally up to 3 of the nucleotides at positions 1, 3, 7, and 10 of the antisense strand is modified with 2'-F and the sugar moiety of each of the remaining nucleotides of the antisense strand is modified with a modification selected from the group consisting of 2'-O-propargyl, 2'-O-propylamin, 2'-amino, 2'-ethyl, 2'-aminoethyl (EA), 2'-O-methyl (2'-OMe), 2'-O-methoxyethyl (2'-MOE), 2'-O-[2-(methylamino)-2-oxoethyl] (2'-O-NMA), and 2'-deoxy-2'-fluoro- β -D-arabinonucleic acid (2'-FANA).

25. The single-stranded oligonucleotide of claim 24, wherein the single-stranded oligonucleotide comprises 20 nucleotides.

26. The single-stranded oligonucleotide of claim 24, wherein the single-stranded oligonucleotide comprises 21 nucleotides.

27. The single-stranded oligonucleotide of claim 24, wherein the single-stranded oligonucleotide comprises from 20 to 23 nucleotides.

28. A pharmaceutical composition comprising any one of the preceding claims and a pharmaceutically acceptable carrier.

29. A method for reducing expression of a target gene in a subject, comprising administering the oligonucleotide of any one of claims 1-23, the single stranded oligonucleotide of claims 24-27, or the composition of claim 28 to the subject in an amount sufficient to reduce expression of a target gene in the subject.

30. A method of treating or preventing a disease or disorder in a subject comprising administering to the subject the oligonucleotide of any one of claims 1-23, the single stranded oligonucleotide of claims 24-27, or the composition of claim 28 in an amount sufficient to inhibit expression of a gene causing disease in the subject.