[0007] In order to further reduce the 2′-F content and increase the 2′-OMe content concomitantly so that the stability and tolerability can be improved without compromising RNAi activity, fine-tuning of the positions of the 2′-OMe and 2′-F (modification patterns) is necessary in DsiRNA conjugates that have already shown good potency and duration. A recent report has attempted to optimize modification patterns of a 21/23mer siRNA GalNAc conjugate platform (Foster *et al.*, Mol. Ther. 26:708-717, 2018). That report, however, did not identify patterns of 2′-OMe and 2′-F that confer an oligonucleotide with high potency and duration as disclosed herein, including positions having poor tolerability to 2′-OMe substitution. Nor did that report identify advanced designs with minimal 2′-F content specifically for triloop and tetraloop GalXC platforms as disclosed herein.

SUMMARY ~~OF THE INVENTION~~

[0008] The present disclosure is based on the development of strategies for modifying an oligonucleotide (*e.g.*, RNA interference oligonucleotide) with 2′-deoxy-2′-fluoro (2′-F) and 2'-O-Methyl (2′-OMe) modifications to increase its potency and duration.

[0009] Accordingly, one aspect~~s~~ of the present disclosure provide 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 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 regions 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 nucleotides 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.

[0009a] In another aspect, the present disclosure provides the oligonucleotide as described herein for use in reducing RNA expression, wherein the sugar moiety of each nucleotide in the second duplex is modified with 2′-O-methyl (2′-OMe).

[0009b] In another aspect, the present disclosure provides 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).

[0009c] In another aspect, the present disclosure provides a pharmaceutical composition comprising the oligonucleotide as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure provides a use of an oligonucleotide as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for treating or preventing a disease or disorder in a subject by reducing or inhibiting the expression of a target gene causing the disease in the subject.

[0010] The details of one or more ~~embodiment~~examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the detailed description of several ~~embodiment~~examples and from the appended claims.

BRIEF DESCRIPTION OF FIGURES

[0011] **Figures 1A-1C** shows data from a sense strand structure activity relationship (SAR). HAO1 target mRNA knockdown was measured at 48 hours after transfection of different concentrations of a nicked tetraloop GalNAc conjugate in a HAO1 stable cell line. Potency was determined as half maximal inhibitory concentration (IC_{50}). **Figure 1A** is a graph showing potency of a sense strand in which positions 17 and 19 on the sense strand are modified with 2′-F. **Figure 1B** is a graph showing potency of a sense strand in which position 19 of the sense strand is modified with 2′-F and position 17 of the sense strand is modified with 2′-OMe. **Figure 1C** is a graph showing potency of a sense strand in which positions 17 and 19 on the sense strand are modified with 2′-OMe.

[0012] **Figures 2A-2D** shows data from an antisense strand structure activity relationship (SAR). HAO1 target mRNA knockdown was measured at 48 hours after transfection of different concentrations of a nicked tetraloop GalNAc conjugate in a HAO1 stable cell line. Potency was determined as half maximal inhibitory concentration (IC_{50}). **Figure 2A** is a graph showing potency of an antisense strand in which positions 15, 17 and 19 on the sense strand are modified with 2′-F. **Figure 2B** is a graph showing potency of an antisense strand in which positions 15 and 17 of the antisense strand are modified with 2′-F and position 19 of the antisense strand is modified with 2′-OMe. **Figure 2C** is a graph showing potency of an antisense strand in which position 15 of the antisense strand is modified with 2′-F and positions 17 and 19 of the antisense strand are modified with 2′-OMe. **Figure 2D** is a graph showing potency of an antisense strand in which positions 15, 17, and 19 of the antisense strand are modified with 2′-OMe.

[0013] **Figures 3A-3H** shows data from an antisense strand structure activity relationship (SAR). HAO1 target mRNA knockdown was measured at 48 hours after transfection of different concentrations of a nicked tetraloop GalNAc conjugate in a HAO1 stable cell line. Potency was determined as half maximal inhibitory concentration (IC_{50}). **Figure 3A** is a graph showing potency of an antisense strand in which positions 1-3 and 5-10 of the antisense strand are modified with 2′-F and position 4 of the antisense strand is modified with 2′-OMe. **Figure 3B** is a graph showing

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:

(i) 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); or

(ii) 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); or

(iii) 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); or

(iv) 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); or

(v) 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); or

(vi) 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); or

~~6.~~ (vii) 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).

~~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.~~7. The oligonucleotide of claim 1 or ~~12~~6, wherein the antisense strand has 3 nucleotides that are modified at the 2′-position of the sugar moiety with 2′-F.

~~14.~~8. The oligonucleotide of any of the preceding claims, wherein the second duplex has a length of 1-6 base pairs.

~~15.~~9. The oligonucleotide of any of the preceding claims, wherein the second duplex comprises at least one bicyclic nucleotide.

~~16.~~10. The oligonucleotide of claim ~~15~~9, wherein the second duplex has a length of 1-3 base pairs.

~~17.~~11. 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.~~12. The oligonucleotide of claim 1 for use in 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.~~13. The oligonucleotide of any of the preceding claims for use in 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.~~14. The oligonucleotide of claim 13 for use in 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; and wherein the ligand comprises *N*-acetylgalactosamine.

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

~~22.~~15. The oligonucleotide of the preceding claims for use in reducing RNA expression ~~of the preceding claims~~, wherein the nucleotide at position 1 of the antisense strand comprises a phosphate mimic.

~~23.~~16. The oligonucleotide of any of the preceding claims for use in reducing RNA expression ~~of any of the preceding claims~~, wherein the sense strand comprises 36 nucleotides and the antisense strand comprises 22 nucleotides.

~~24.~~17. 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.~~18. The single-stranded oligonucleotide of claim ~~24~~17, wherein the single-stranded oligonucleotide comprises 20 nucleotides; or 21 nucleotides; or from 20 to 23 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.~~19. A pharmaceutical composition comprising any one of the preceding claims and a pharmaceutically acceptable carrier.

~~29.~~20. ~~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~~ Use of an oligonucleotide of any one of claims 1-11 or 17-18, or a pharmaceutical composition of claim 19 in the manufacture of a medicament for treating or preventing a disease or disorder in a subject by reducing or inhibiting the expression of a target gene causing the disease 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.~~