

(nucleus). Thus, there is a need for AONs with improved characteristics for treating, preventing and/or delaying DMD.

SUMMARY

[0006a] In one aspect, the present disclosure provides a hydroxyalkoxylated AON consisting of one antisense oligonucleotide and one or two hydroxyalkoxy groups, wherein said hydroxyalkoxy group comprises or consists of an ethylene glycol monomer, ethylene glycol oligomer or ethylene glycol polymer, wherein the antisense oligonucleotide is represented by a nucleotide sequence comprising or consisting of:

- i) any one of SEQ ID NO: 9-404, or
- ii) a fragment of any one of SEQ ID NO: 9-404, or
- iii) any one of SEQ ID NO: 9-404 with 1, 2, 3, 4, or 5 additional nucleotides, or
- iv) any one of SEQ ID NO: 9-404 with 1, 2, 3, 4, or 5 nucleotides missing from said SEQ ID NO, or
- v) a nucleotide sequence which has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, or at least 95%, or at least 97%, identity with any one of SEQ ID NO: 9-404.

[0006b] In another aspect, the present disclosure provides a pharmaceutical composition, comprising the hydroxyalkoxylated AON as described herein and a pharmaceutically acceptable carrier. Other aspects of the present disclosure relate to the hydroxyalkoxylated AON or the pharmaceutical composition as described herein for use as a medicament; use of the hydroxyalkoxylated AON or the pharmaceutical composition as described herein in the manufacture of a medicament for preventing, treating, and/or delaying Duchenne Muscular Dystrophy (DMD) in a subject.

[0006c] Yet another aspect of the present disclosure relates to an *in vitro* method of inducing skipping of exon 51 of dystrophin pre-mRNA, comprising contacting the dystrophin pre-mRNA with the hydroxyalkoxylated AON as described herein.

[0007] In one example, provided herein is a hydroxyalkoxylated AON. In another example, provided is a hydroxyalkoxylated AON consisting of one antisense oligonucleotide (AON) and one or two hydroxyalkoxy groups. In certain examples, the hydroxyalkoxy group comprises or consists of an ethylene glycol monomer, ethylene glycol oligomer or ethylene glycol polymer. In other examples, the AONs for use in the hydroxyalkoxylated AONs provided herein are represented by a nucleotide sequence comprising or consisting of:

[0008] any one of SEQ ID NO: 9-404, or

- [0009]** a fragment of any one of SEQ ID NO: 9-404, or
- [0010]** any one of SEQ ID NO: 9-404 with 1, 2, 3, 4, or 5 additional nucleotides, or
- [0011]** any one of SEQ ID NO: 9-404 with 1, 2, 3, 4, or 5 nucleotides missing from said SEQ ID NO, or
- [0012]** a nucleotide sequence which has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, or at least 95%, or at least 97%, identity with any one of SEQ ID NO: 9-404.
- [0013]** In certain examples, the hydroxyalkoxylated AON provided herein consists of 2'-O-methyl RNA monomers linked by phosphorothioate backbone linkages. In another example, the hydroxyalkoxylated AON provided herein consists of 2'-O-methyl RNA monomers linked by phosphate backbone linkages. In another example, the hydroxyalkoxylated AON provided herein consists of 2'-O-methyl RNA monomers linked by a mixture of phosphorothioate and phosphate backbone linkages.
- [0014]** In some examples, the AONs for use in the hydroxyalkoxylated AONs provided herein are complementary or reverse complementary to a portion of an exon in human dystrophin pre-mRNA. In some examples, the AONs for use in the hydroxyalkoxylated AONs provided herein are complementary to a portion of an exon in human dystrophin pre-mRNA. In certain examples, the AONs are complementary to a portion of one of exons 51, 45, 53, 52 or 55 of human dystrophin pre-mRNA. In certain examples, the AONs are complementary to a portion of exon 51 of human dystrophin pre-mRNA.
- [0015]** In further examples, provided is a pharmaceutical composition comprising a hydroxyalkoxylated AON provided herein and a pharmaceutically acceptable carrier.

[0016] In another example, provided is a method of preventing, treating, and/or delaying Duchenne Muscular Dystrophy (DMD), comprising administering to a subject a hydroxyalkoxylated AON provided herein or a pharmaceutical composition provided herein.

[0017] In another example, provided is a method of inducing skipping of exon 51 of the dystrophin pre-mRNA by contacting the dystrophin pre-mRNA with a hydroxyalkoxylated AON provided herein.

[0018] In some examples, linking an AON described herein to one or two hydroxyalkoxy moieties leads to a hydroxyalkoxylated AON that shows improved characteristics for treatment of genetic disorders, such as DMD, as compared to the AON lacking a hydroxyalkoxy group.

BRIEF DESCRIPTION OF THE FIGURES

[0019] **FIG. 1** shows dystrophin expression and exon skipping levels in quadriceps and heart of hDMD Δ 52/mdx mice treated with the AON with SEQ ID NO: 22333 (“Cmpd 1”, 18 mg/kg) or the PS-linked hydroxyalkoxylated AON with SEQ ID NO: 22345 (“Cmpd 2 PS”, 18.7 mg/kg) compared to mice treated with vehicle in a 13-week treatment (by weekly intravenous tail vein injections).

[0020] **FIG. 2** shows dystrophin expression and exon skipping levels in quadriceps of hDMD Δ 52/mdx mice treated with the PS-linked hydroxyalkoxylated AON with SEQ ID NO: 22345 (Cmpd 2 PS, 9.4, 18.7 or 37.5 mg/kg) at 14 or 28 days post-dosing QW, Q2W or Q4W.

[0021] **FIG. 3A** shows effects of the AON with SEQ ID NO: 22333 (Cmpd 1) or the PS-linked or PO-linked hydroxyalkoxylated AON with SEQ ID NO: 22345 (Cmpd 2 PS or Cmpd 2 PO, respectively) on liver function measured by alkaline phosphatase (“ALP”), alanine aminotransferase (“ALT”) and aspartate transaminase (“AST”) levels in healthy CD-1 mice. **FIG. 3B** shows effects of the AON with SEQ ID NO: 22333 (Cmpd 1) or the hydroxyalkoxylated AON with SEQ ID NO: 22345 (Cmpd 2 PS) on liver function measured by alanine aminotransferase (“ALT”) and aspartate transaminase (“AST”) levels in hDMD Δ 52/mdx mice. **FIG. 3C** shows effects of the hydroxyalkoxylated AON with SEQ ID NO: 22345 (Cmpd 2 PS) on kidney function in hDMD Δ 52/mdx mice.

[0022] **FIG. 4** shows effects of the AON with SEQ ID NO: 22333 (Cmpd 1) or the hydroxyalkoxylated AON with SEQ ID NO: 22345 (Cmpd 2 PO) on complement parameters Bb and C3a.

WHAT IS CLAIMED IS:

1. A hydroxyalkoxylated AON consisting of one antisense oligonucleotide and one or two hydroxyalkoxy groups, wherein said hydroxyalkoxy group comprises or consists of an ethylene glycol monomer, ethylene glycol oligomer or ethylene glycol polymer, wherein the antisense oligonucleotide is represented by a nucleotide sequence comprising or consisting of:
 - i) any one of SEQ ID NO: 9-404, or
 - ii) a fragment of any one of SEQ ID NO: 9-404, or
 - iii) any one of SEQ ID NO: 9-404 with 1, 2, 3, 4, or 5 additional nucleotides, or
 - iv) any one of SEQ ID NO: 9-404 with 1, 2, 3, 4, or 5 nucleotides missing from said SEQ ID NO, or
 - v) a nucleotide sequence which has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, or at least 95%, or at least 97%, identity with any one of SEQ ID NO: 9-404.
2. The hydroxyalkoxylated AON of claim 1, wherein the antisense oligonucleotide consists of 2'-O-methyl RNA monomers linked by phosphorothioate backbone linkages.
3. The hydroxyalkoxylated AON of claim 1 or 2, wherein the hydroxyalkoxy group is diethylene glycol, triethylene glycol (TEG), tetraethylene glycol, pentaethylene glycol or hexaethylene glycol (HEG), or wherein said hydroxyalkoxy group is TEG.
4. The hydroxyalkoxylated AON of any one of claims 1-3, wherein the 5' terminal monomer or the 3' terminal monomer of said antisense oligonucleotide is linked to the hydroxyalkoxy group.
5. The hydroxyalkoxylated AON of any one of claims 1-3, consisting of two hydroxyalkoxy groups, wherein the 5' terminal monomer of said antisense oligonucleotide is linked to a first hydroxyalkoxy group and wherein the 3' terminal monomer of said antisense oligonucleotide is linked to a second hydroxyalkoxy group, wherein the first and second hydroxyalkoxy groups comprise or consist of an ethylene glycol monomer, ethylene glycol oligomer or ethylene glycol polymer, or wherein the first and second hydroxyalkoxy group are TEG.
6. The hydroxyalkoxylated AON of any one of claims 1-5, wherein the hydroxyalkoxy group is attached to the AON through a phosphate linker (PO).
7. The hydroxyalkoxylated AON of any one of claims 1-5, wherein the hydroxyalkoxy group is attached to the AON through a phosphorothioate linker (PS).

8. The hydroxyalkoxylated AON of any one of claims 1-7, wherein the antisense oligonucleotide has a length from 8 to 33 nucleotides, from 12 to 24 nucleotides, or from 16 to 22 nucleotides.
9. The hydroxyalkoxylated AON of any one of claim 1-8, wherein all cytosine bases of the antisense oligonucleotide are 5-methylcytosine bases.
10. The hydroxyalkoxylated AON of any one of claim 1-9, wherein the antisense oligonucleotide comprises or consists of 1, 2, 3, 4, 5, 6, 7, 8 or 9 monomers that comprise a bicyclic nucleic acid (BNA) scaffold modification.
11. The hydroxyalkoxylated AON of claim 10, wherein the antisense oligonucleotide comprises or consists of BNA modifications as selected from the set consisting of:
- (i) a single BNA scaffold modification in the monomer at the 5'-terminus,
 - (ii) a single BNA scaffold modification in the monomer at the 3'-terminus,
 - (iii) two BNA scaffold modifications where one is in the monomer at the 5'-terminus and the other is in the monomer at the 3'-terminus,
 - (iv) two BNA scaffold modifications, one in the monomer at the 5'-terminus and the other in the adjacent monomer,
 - (v) two BNA scaffold modifications, one in the monomer at the 3'-terminus and the other in the adjacent monomer, and
 - (vi) four BNA scaffold modifications, one in the monomer at the 5'-terminus, one in the monomer adjacent to the 5'-terminus, one in the monomer at the 3'-terminus and one in the monomer adjacent to the 3'-terminus;
- optionally 1, 2, 3, 4 or 5 additional BNA scaffold modifications are present.
12. The hydroxyalkoxylated AON of claim 10 or 11, wherein the BNA modification is a bridged nucleic acid scaffold modification, or a locked nucleic acid (LNA) scaffold modification.
13. The hydroxyalkoxylated AON of any one of claims 1-12, wherein all uracil bases of the antisense oligonucleotide are 5-methyluracil bases.
14. The hydroxyalkoxylated AON of any one of claims 1-4 and 6-13 that has SEQ ID NO: 22345, 22357, 22369, 22488, 22489 or 22490.
15. The hydroxyalkoxylated AON of claim 14 that has SEQ ID NO: 22345.
16. A pharmaceutical composition, comprising the hydroxyalkoxylated AON of any one of claims 1-15 and a pharmaceutically acceptable carrier.
17. The hydroxyalkoxylated AON of any one of claims 1-15 or the pharmaceutical composition of claim 16 for use as a medicament.

18. Use of the hydroxyalkoxylated AON of any one of claims 1-15 or the pharmaceutical composition of claim 16 in the manufacture of a medicament for preventing, treating, and/or delaying Duchenne Muscular Dystrophy (DMD) in a subject.
19. An *in vitro* method of inducing skipping of exon 51 of dystrophin pre-mRNA, comprising contacting the dystrophin pre-mRNA with the hydroxyalkoxylated AON of any one of claims 1-15.