

**OPA1 ANTISENSE OLIGOMERS FOR TREATMENT
OF CONDITIONS AND DISEASES**

CROSS-REFERENCE

[0001] This application claims benefit of United States Provisional Patent Application No. 63/505,333 filed May 31, 2023, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Alternative splicing events in genes can lead to nonproductive mRNA transcripts which in turn can lead to aberrant or reduced protein expression, and therapeutic agents which can target the alternative splicing events in genes can modulate the expression level of functional proteins in patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition or disease caused by the protein deficiency.

[0003] Autosomal dominant optic atrophy (ADOA) is one of the most commonly diagnosed optic neuropathies. This optic nerve disease is associated with structural and functional mitochondrial deficits that lead to degeneration of the retinal ganglion cells and progressive, irreversible loss of vision. A majority of ADOA patients carry mutations in OPA1 and most mutations lead to haploinsufficiency (Lenaers G. *et al.* Orphanet J Rare Dis 2012). OPA1 encodes a mitochondrial GTPase with a critical role in mitochondrial fusion, ATP synthesis and apoptosis. Currently, there is no approved disease-modifying treatment for ADOA patients and there is a need for such treatments.

SUMMARY

[0004] Described herein, in some aspects, is a method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,

wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, and

wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

[0005] Described herein, in some aspects, is a method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,

wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299,

wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by having:

- (i) a heterozygous *OPAI* gene variant;
- (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments;
- (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity;
- (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or
- (v) any combination of (i)-(iv).

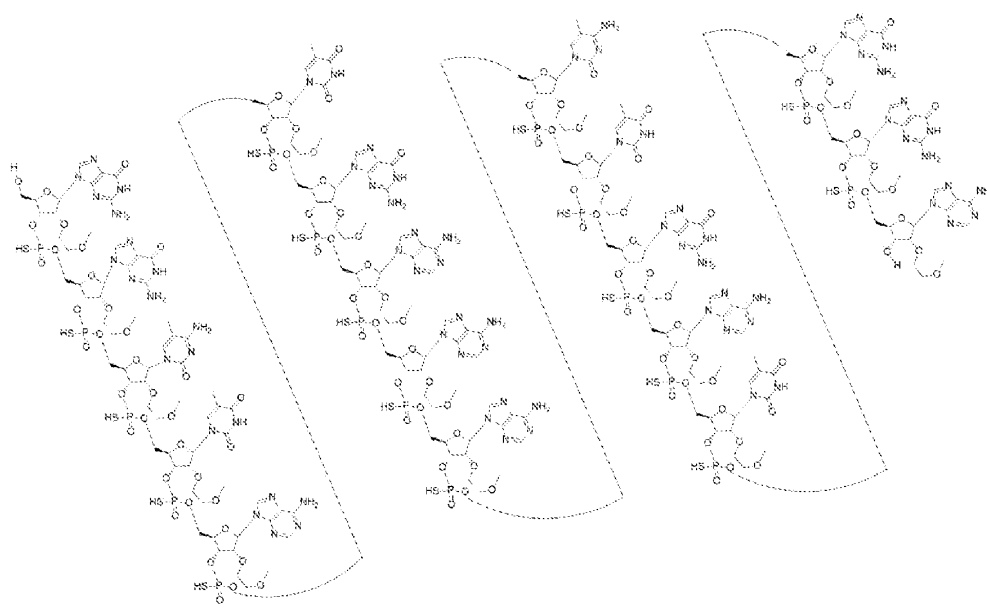
[0006] Described herein, in some aspects, is a method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,

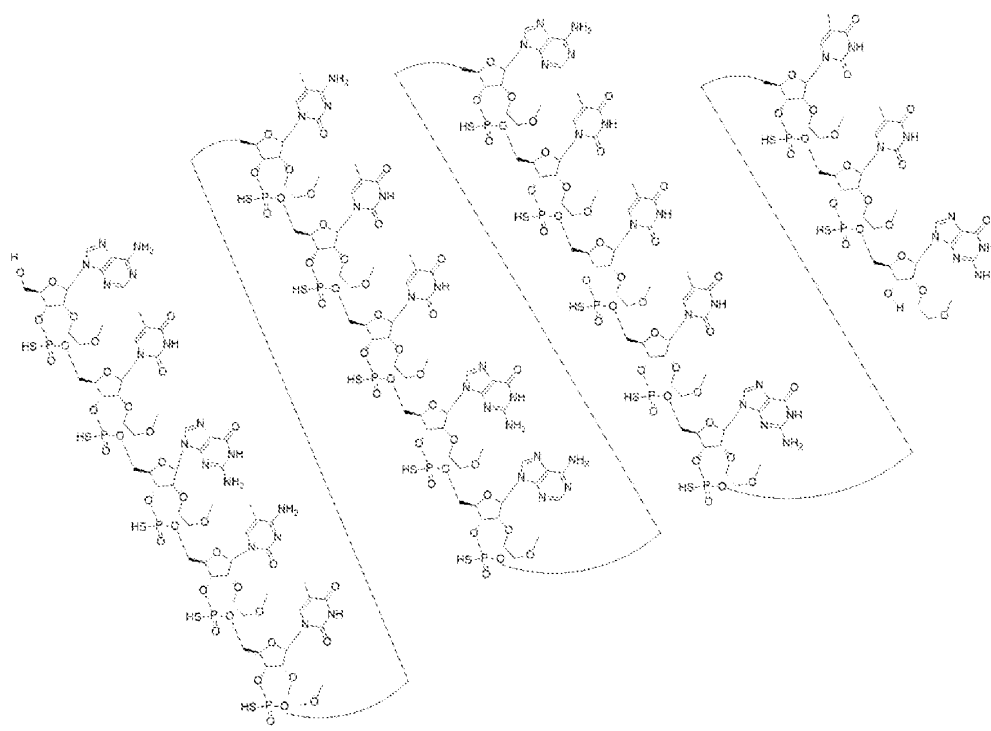
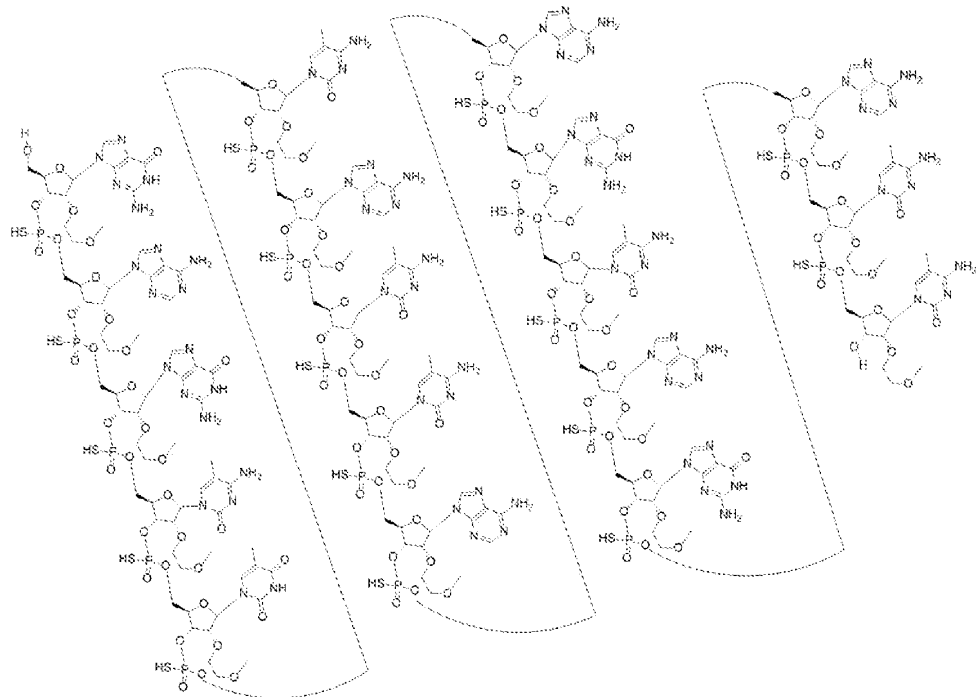
wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by:

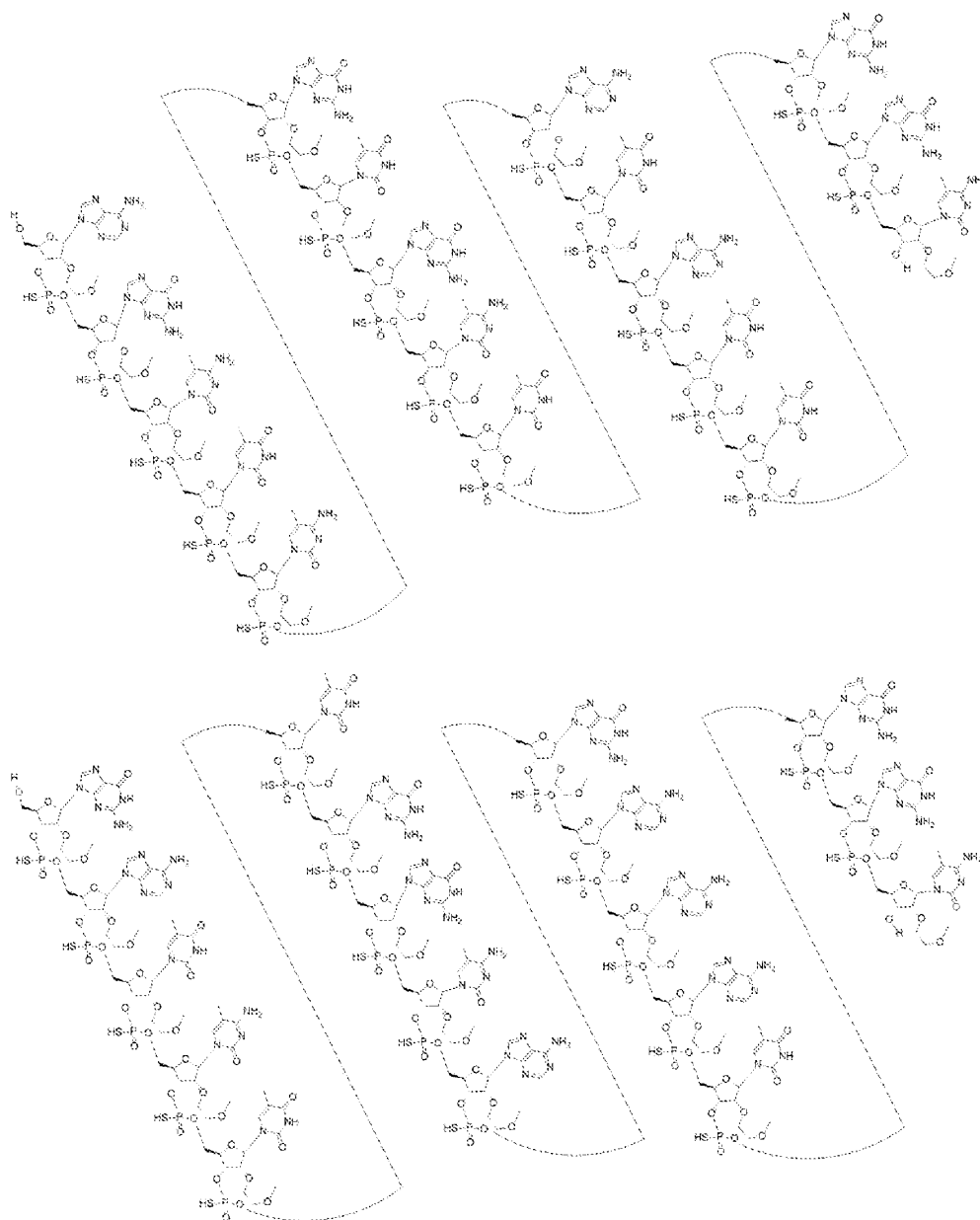
- (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPAI* gene;
- (2) not having only benign or likely benign variants in the *OPAI* gene;
- (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus);
- (4) not having been diagnosed with Behr syndrome;
- (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases;
- (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy;
- (7) not having or having a history of any ocular condition in either eye;
- (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering;
- (9) not having a history of retinal photocoagulation;
- (10) not having a history or presence of retinal vein occlusion;

- (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering;
- (12) not having dry age-related macular degeneration in either eye;
- (13) not having high myopia (>6 diopters);
- (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated);
- (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy;
- (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery; or
- (17) any combination of (1)-(16).

[0007] Described herein, in some aspects, is a method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer is a compound according to any one of the following chemical structures:







or a pharmaceutically acceptable salt thereof.

[0008] In some aspects, the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

[0009] In some aspects, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg to about 15 mg, about 0.01 mg to about 10 mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg,

about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

[0010] In some aspects, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

[0011] In some aspects, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

[0012] In some aspects, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer.

[0013] In some aspects, the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μ L to about 250 μ L, about 10 μ L to about 250 μ L, about 20 μ L to about 250 μ L, about 30 μ L to about 250 μ L, about 40 μ L to about 250 μ L,

[0014] In some aspects, the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μL , about 8 μL , about 10 μL , about 12 μL , about 15 μL , about 18 μL , about 20 μL , about 25 μL , about 28 μL , about 30 μL , about 35 μL , about 40 μL , about 45 μL , about 48 μL , about 50 μL , about 55 μL , about 60 μL , about 65 μL ,

about 70 μ L, about 75 μ L, about 80 μ L, about 90 μ L, about 100 μ L, about 120 μ L, about 150 μ L, about 160 μ L, about 180 μ L, about 200 μ L, about 220 μ L, or about 250 μ L.

[0015] In some aspects, the method comprises administering the pharmaceutical composition to both left eye and right eye of the subject.

[0016] In some aspects, the method comprises administering the pharmaceutical composition at the same dose to both the left eye and the right eye of the subject.

[0017] In some aspects, the method comprises administering the pharmaceutical composition at different doses to the left eye and the right eye of the subject.

[0018] In some aspects, the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to the sequence set forth in any one of SEQ ID NOs: 6-275 or 280-299.

[0019] In some aspects, the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to the sequence set forth in any one of SEQ ID NO: 36, 236, 242, 250, 280-283, 288, or 290-292.

[0020] In some aspects, the antisense oligomer modulates splicing of a non-sense mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA in a cell of the subject, wherein the pre-mRNA encodes the OPA1 protein and comprises the NMD exon, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating expression of the OPA1 protein in the cell.

[0021] In some aspects, the antisense oligomer:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the NMD exon; or
- (c) a combination of (a) and (b).

[0022] In some aspects, the targeted portion of the pre-mRNA is proximal to the NMD exon.

[0023] In some aspects, the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.

[0024] In some aspects, the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10

nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.

[0025] In some aspects, the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.

[0026] In some aspects, the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.

[0027] In some aspects, the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

[0028] In some aspects, the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

[0029] In some aspects, the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[0030] In some aspects, the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[0031] In some aspects, the targeted portion of the pre-mRNA is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon.

[0032] In some aspects, the targeted portion of the pre-mRNA at least partially overlaps with the NMD exon.

[0033] In some aspects, the targeted portion of the pre-mRNA at least partially overlaps with an intron upstream or downstream of the NMD exon.

[0034] In some aspects, the targeted portion of the pre-mRNA comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction.

[0035] In some aspects, the targeted portion of the pre-mRNA is within the NMD exon.

[0036] In some aspects, the targeted portion of the pre-mRNA comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[0037] In some aspects, the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.

[0038] In some aspects, the NMD exon comprises a sequence of SEQ ID NO: 279.

[0039] In some aspects, the targeted portion of the pre-mRNA is within the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.

[0040] In some aspects, the targeted portion of the pre-mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.

[0041] In some aspects, the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193628509 to 193628616.

[0042] In some aspects, the OPA1 protein expressed from the processed mRNA is a full-length OPA1 protein or a wild-type OPA1 protein.

[0043] In some aspects, the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.

[0044] In some aspects, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.

[0045] In some aspects, the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

[0046] In some aspects, the antisense oligomer promotes exclusion of the NMD exon from the pre-mRNA.

[0047] In some aspects, the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-

fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.

[0048] In some aspects, the method results in an increase in the level of the processed mRNA in the cell.

[0049] In some aspects, the level of the processed mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.

[0050] In some aspects, the method results in an increase in the expression of the OPA1 protein in the cell.

[0051] In some aspects, a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.

[0052] In some aspects, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[0053] In some aspects, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety.

[0054] In some aspects, the antisense oligomer comprises at least one modified sugar moiety.

[0055] In some aspects, each sugar moiety is a modified sugar moiety.

[0056] In some aspects, the antisense oligomer comprises a 5'-methylcytosine (5'-MeC).

[0057] In some aspects, each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC).

[0058] In some aspects, the antisense oligomer comprises a 5'-methyluracil (5'-MeU).

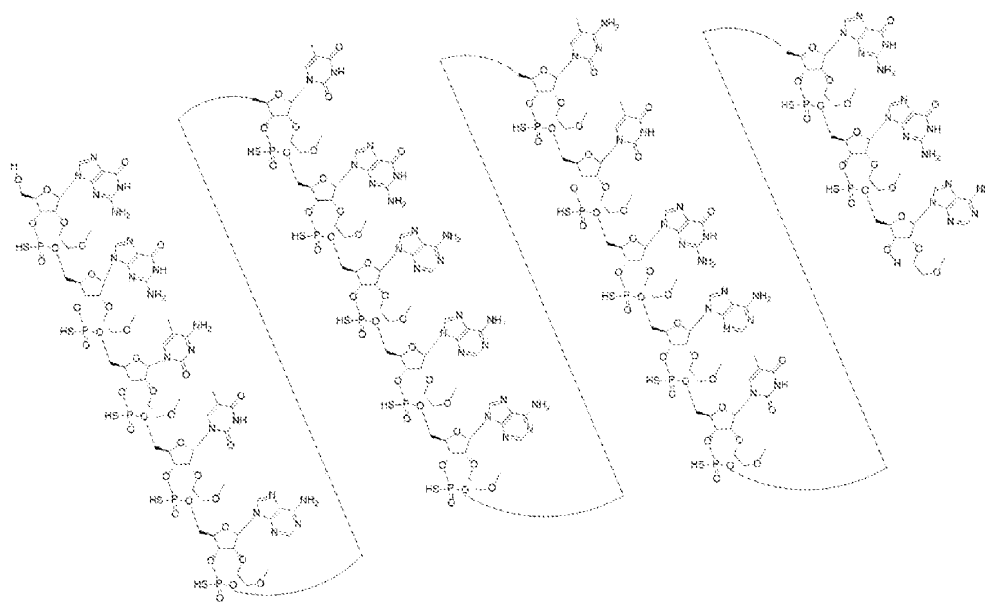
[0059] In some aspects, the antisense oligomer comprises a phosphorothioate linkage.

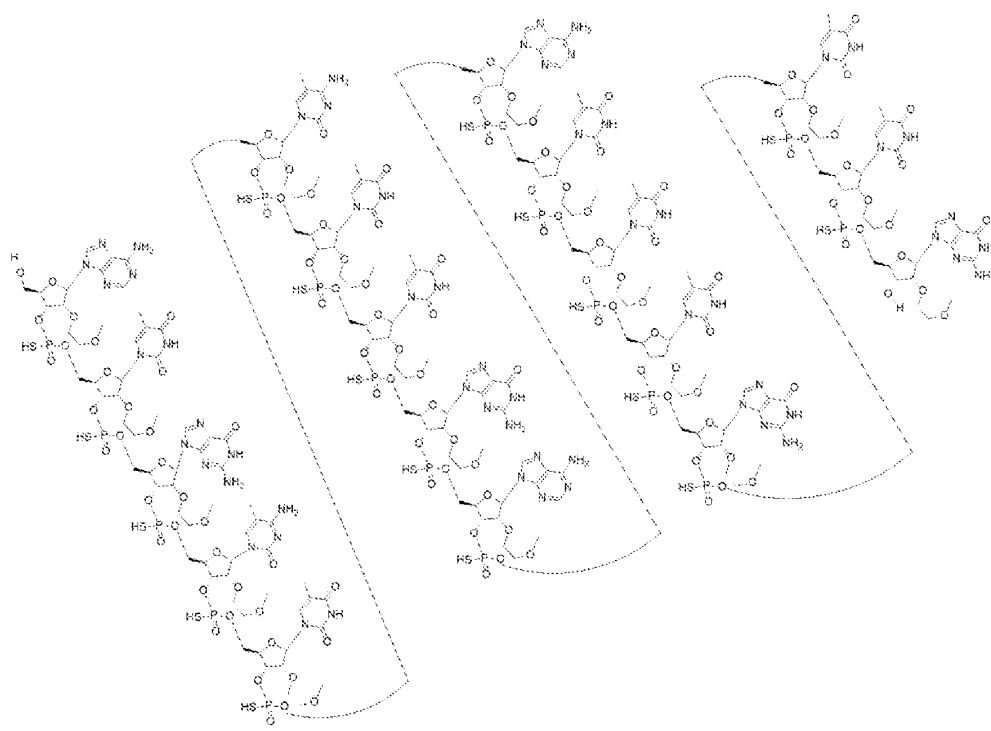
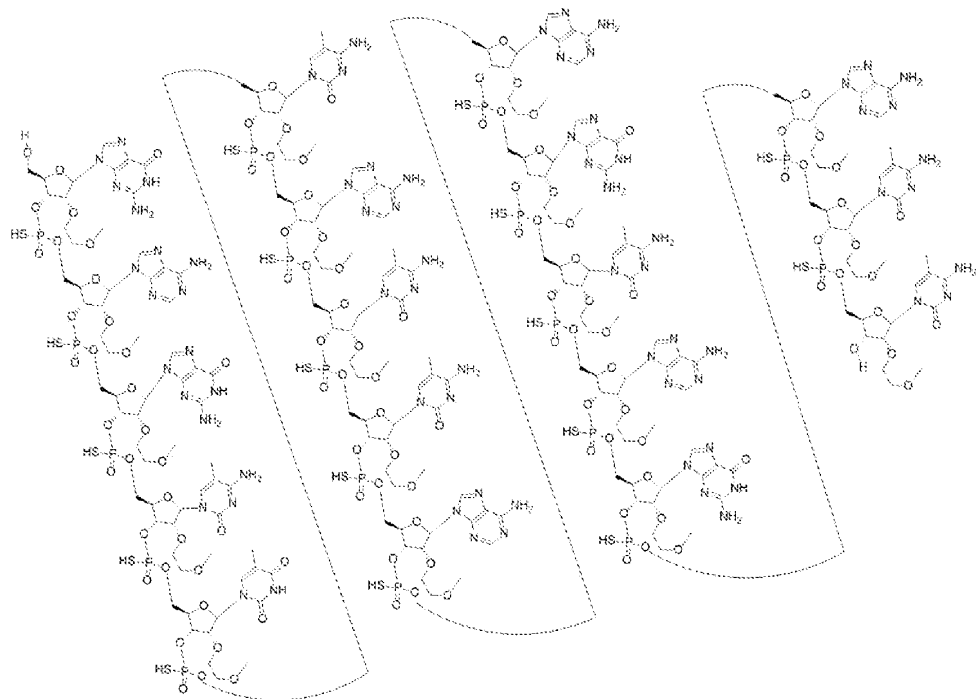
[0060] In some aspects, each internucleoside linkage of the ASO is a phosphorothioate linkage.

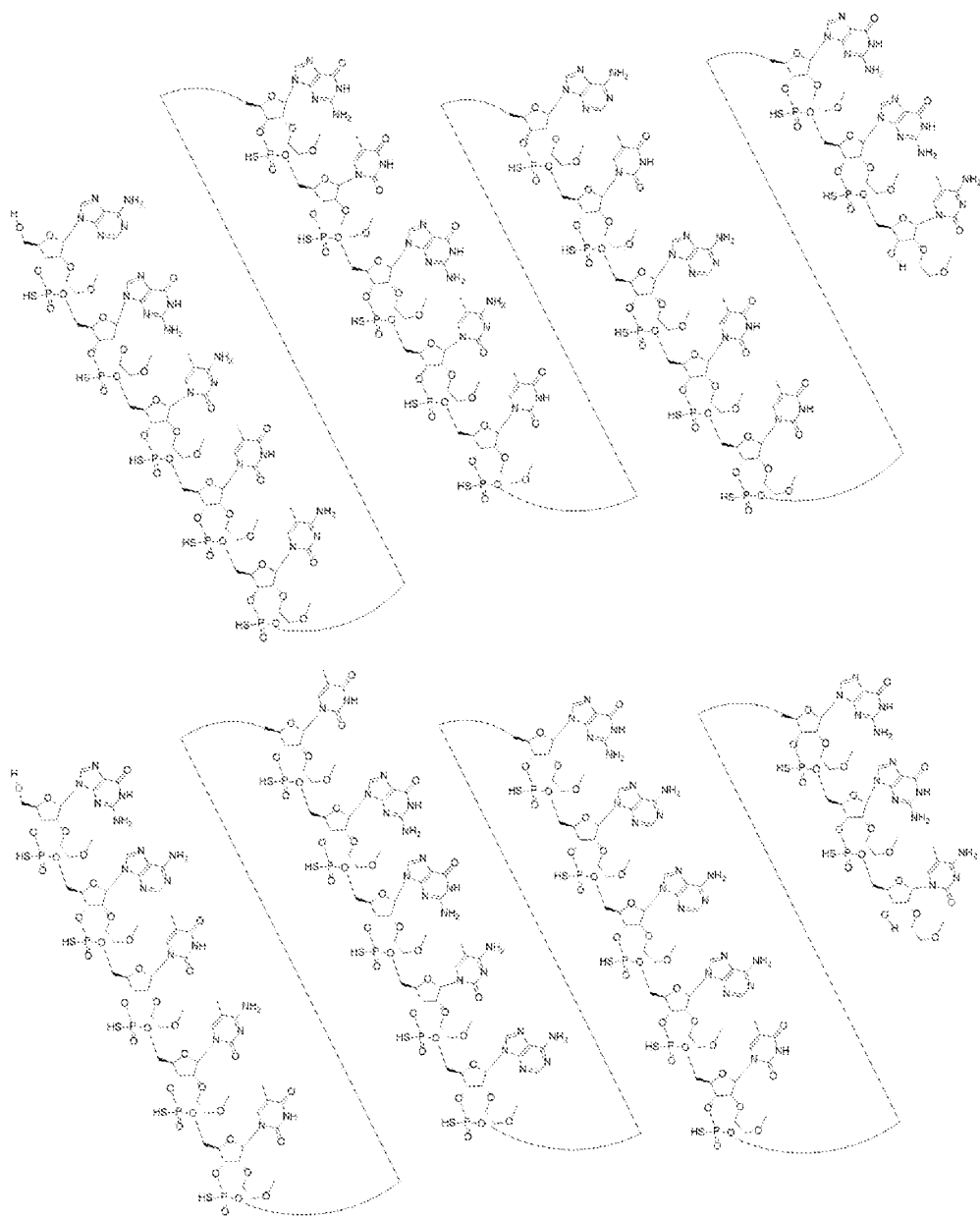
[0061] In some aspects, the antisense oligomer comprises a locked nucleic acid (LNA).

[0062] In some aspects, the antisense oligomer is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases in length.

[0063] In some aspects, the antisense oligomer is a compound according to any one of the following chemical structures:

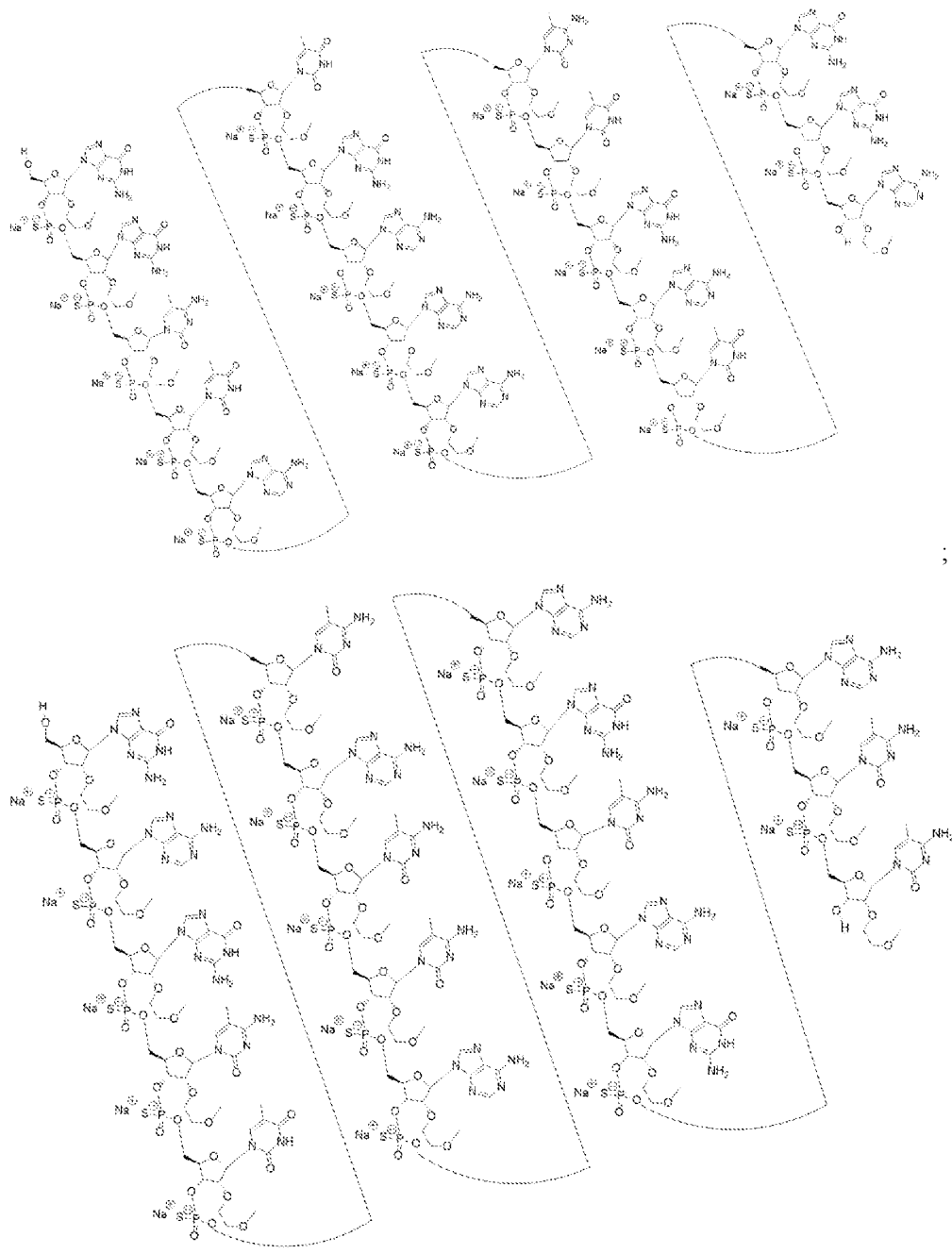


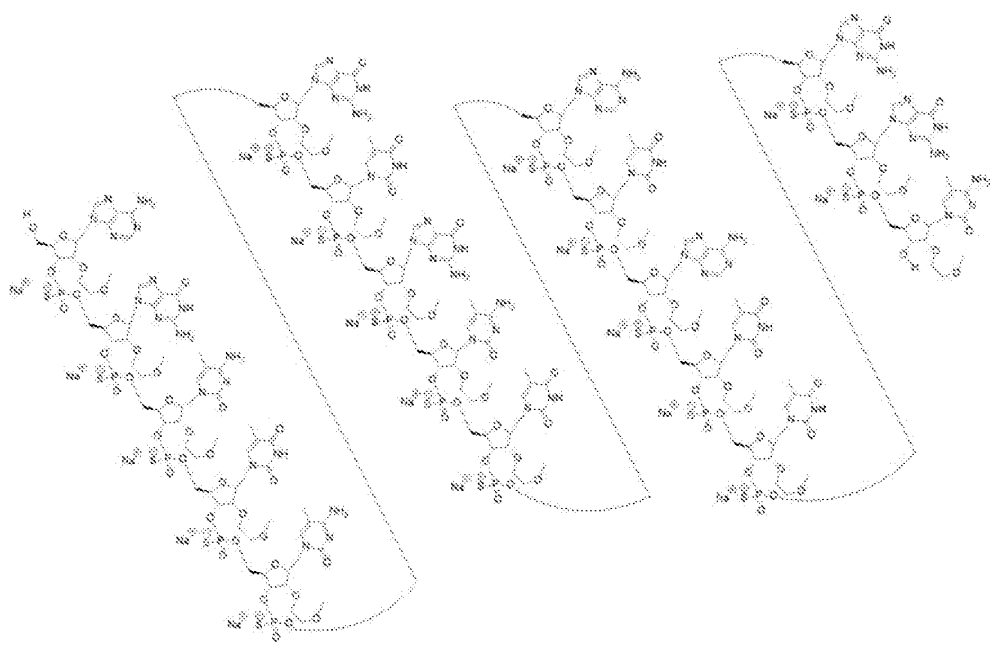
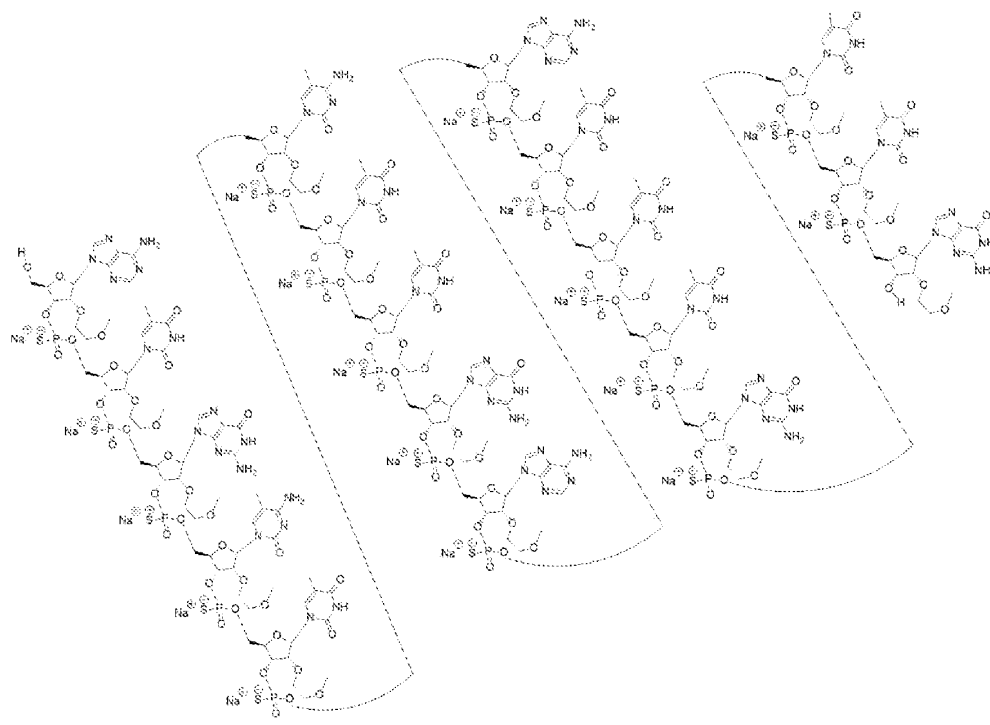




or a pharmaceutically acceptable salt thereof.

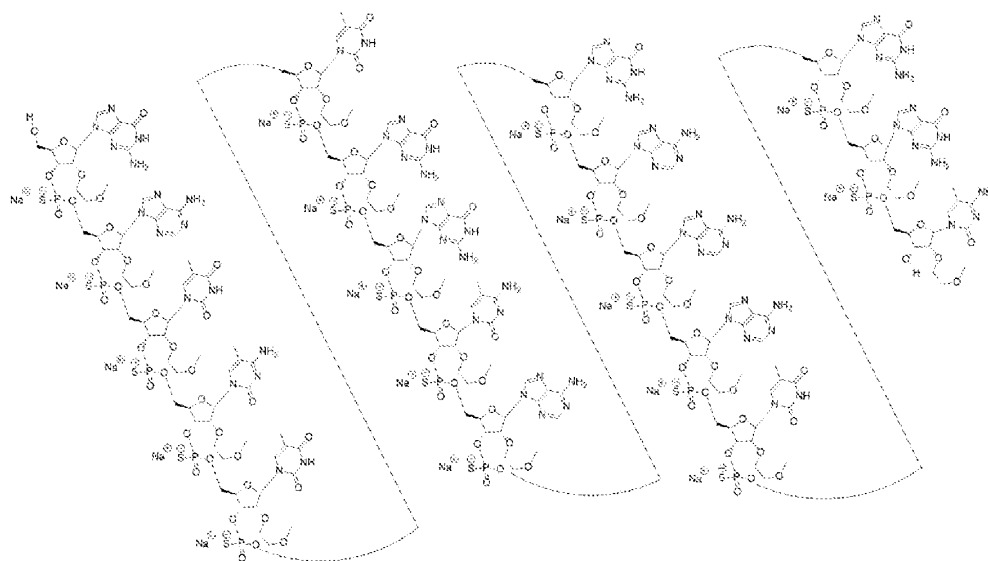
[0064] In some aspects, the antisense oligomer is a compound according to any of the following structures:





;

or



[0065] In some aspects, the pharmaceutical composition is a liquid composition.

[0066] In some aspects, the method comprises administering the pharmaceutical composition as a bolus injection.

[0067] In some aspects, the method comprises administering the pharmaceutical composition as a bolus injection over 1 to 60 minutes, 1 to 50 minutes, 1 to 40 minutes, 1 to 30 minutes, 1 to 20 minutes, 1 to 10 minutes, 1 to 5 minutes, or 1 to 3 minutes.

[0068] In some aspects, the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

[0069] In some aspects, the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

[0070] In some aspects, the antisense oligomer is solubilized or diluted in an isotonic solution.

[0071] In some aspects, the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8.

[0072] In some aspects, the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution.

[0073] In some aspects, the pharmaceutical formulation does not comprise a preservative.

[0074] In some aspects, the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL.

[0075] In some aspects, the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL, about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about

200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

[0076] In some aspects, the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[0077] In some aspects, the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer.

[0078] In some aspects, the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to

about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[0079] In some aspects, the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[0080] In some aspects, the concentrate is a phosphate-buffered solution.

[0081] In some aspects, the subject is a human subject.

[0082] In some aspects, the disease or condition is associated with a deficient amount or activity of the OPA1 protein.

[0083] In some aspects, the disease or condition comprises an eye disease or condition.

[0084] In some aspects, the disease or condition comprises a cardiovascular disease or condition.

[0085] In some aspects, the disease or condition comprises a neurological disease or condition.

[0086] In some aspects, the disease or condition comprises ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy,

lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[0087] In some aspects, the disease or condition comprises Optic atrophy type 1.

[0088] In some aspects, the disease or condition comprises autosomal dominant optic atrophy (ADOA).

[0089] In some aspects, the subject is characterized by having:

- (i) a heterozygous *OPA1* gene variant;
- (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments;
- (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity;
- (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or
- (v) any combination of (i)-(iv).

[0090] In some aspects, the subject is additionally characterized by:

- (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPA1* gene;
- (2) not having only benign or likely benign variants in the *OPA1* gene;
- (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus);
- (4) not having been diagnosed with Behr syndrome;
- (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases;
- (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy;
- (7) not having or having a history of any ocular condition in either eye;
- (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering;
- (9) not having a history of retinal photocoagulation;
- (10) not having a history or presence of retinal vein occlusion;
- (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering;
- (12) not having dry age-related macular degeneration in either eye;

- (13) not having high myopia (>6 diopters);
- (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated);
- (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy;
- (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery;
- (17) any combination of (1)-(16).

[0091] In some aspects, administering comprises administering multiple doses of the pharmaceutical composition to the human subject.

[0092] In some aspects, administering comprises administering a first dose of the pharmaceutical composition to the human subject and a subsequent dose of the pharmaceutical composition to the human subject.

[0093] In some aspects, the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is not tolerated.

[0094] In some aspects, the subsequent dose is the same as the previous dose following an indication that administration of the previous dose is tolerated.

[0095] In some aspects, the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is tolerated.

[0096] In some aspects, the subsequent dose is the same as the previous dose following an indication that administration of the previous dose is effective.

[0097] In some aspects, the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is effective.

[0098] In some aspects, the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is not effective.

[0099] In some aspects, the pharmaceutical composition is administered via intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

[00100] In some aspects, the pharmaceutical composition is administered via intravitreal injection.

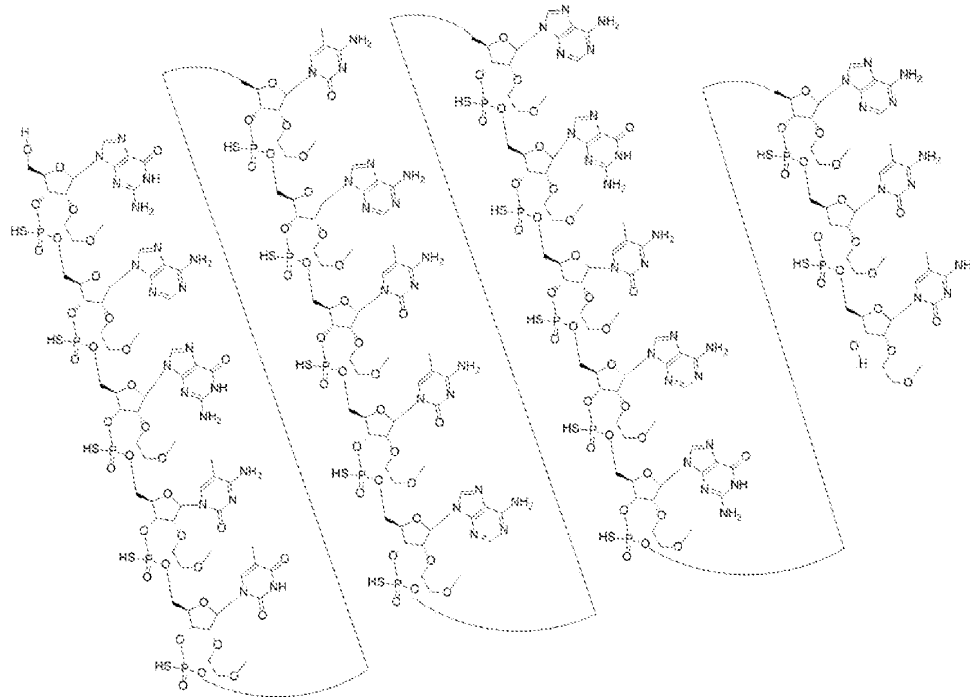
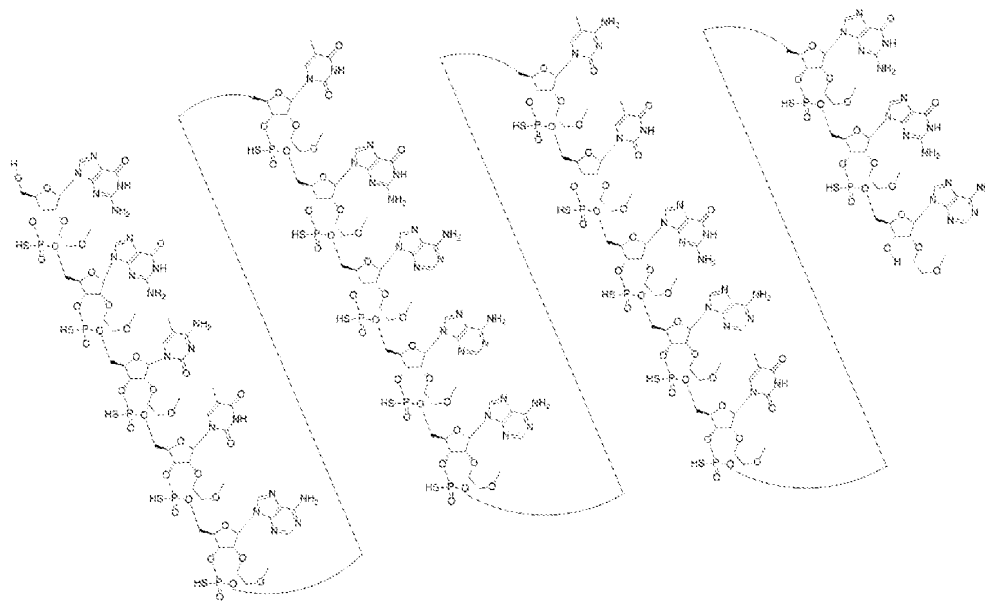
[00101] In some aspects, the method further comprises administering an additional therapeutic agent.

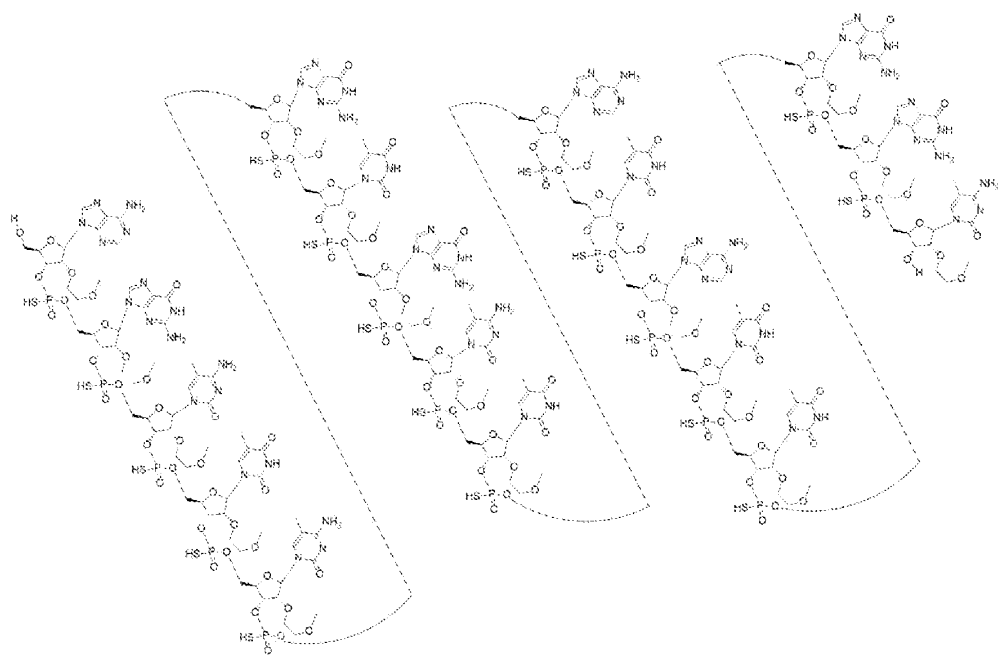
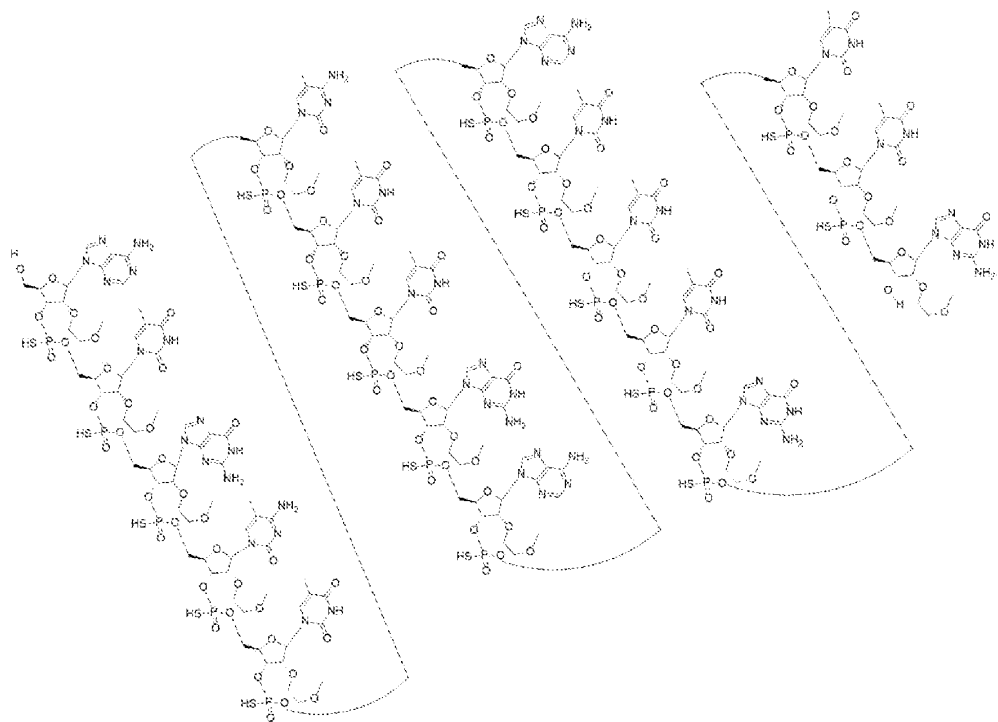
[00102] In some aspects, the additional therapeutic agent comprises a small molecule.

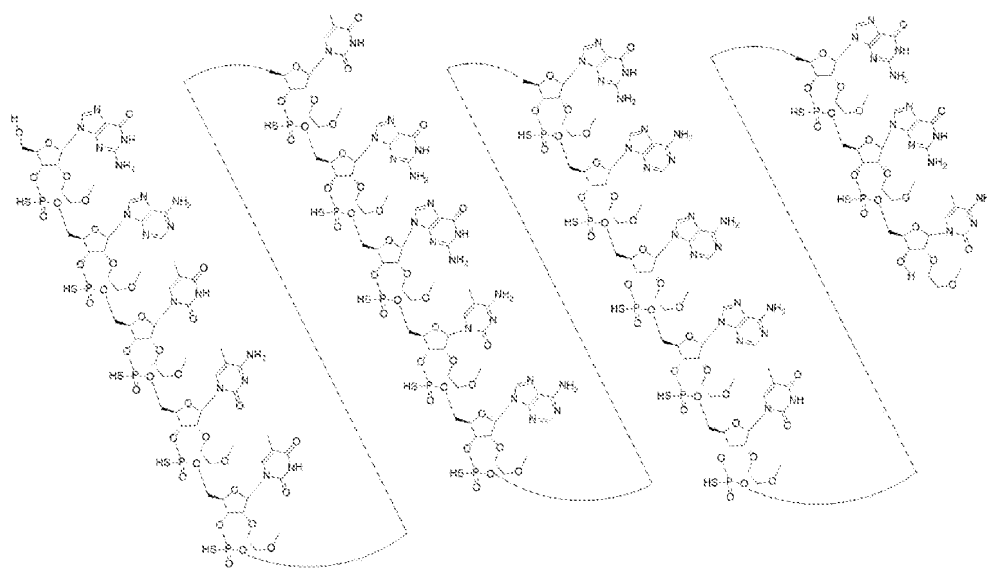
[00103] In some aspects, the additional therapeutic agent comprises an antisense oligomer.

[00104] In some aspects, the additional therapeutic agent comprises an ophthalmologic drug.

[00105] Described herein, in some aspects, is an antisense oligomer that is a compound according to any one of the following structures:

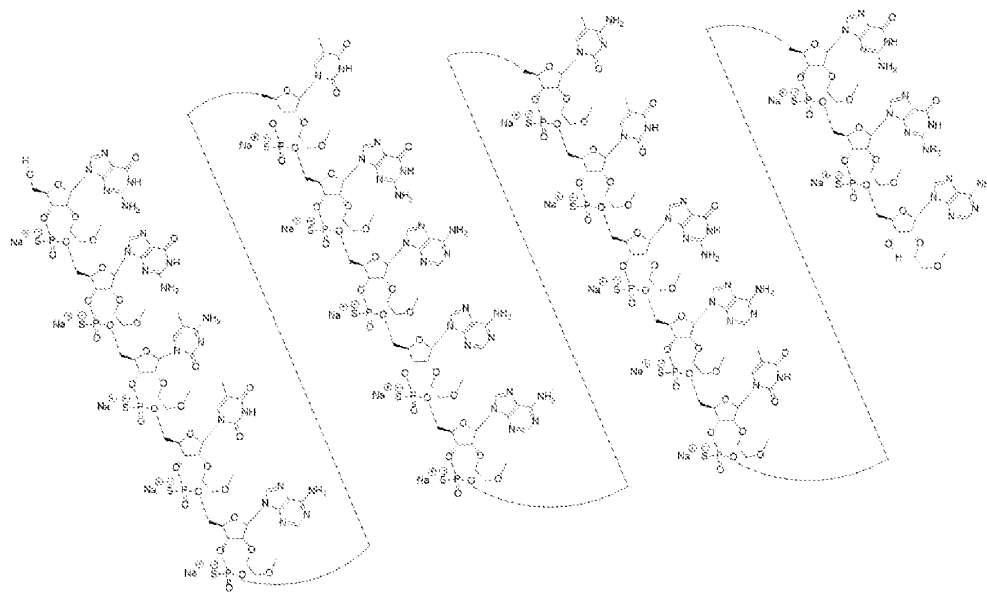


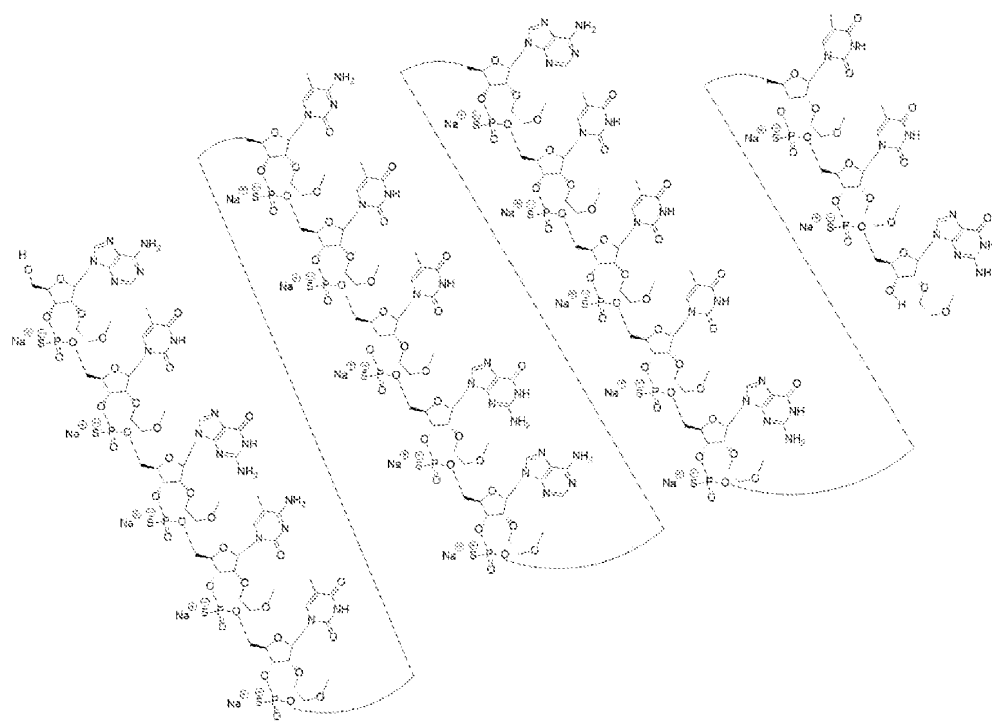
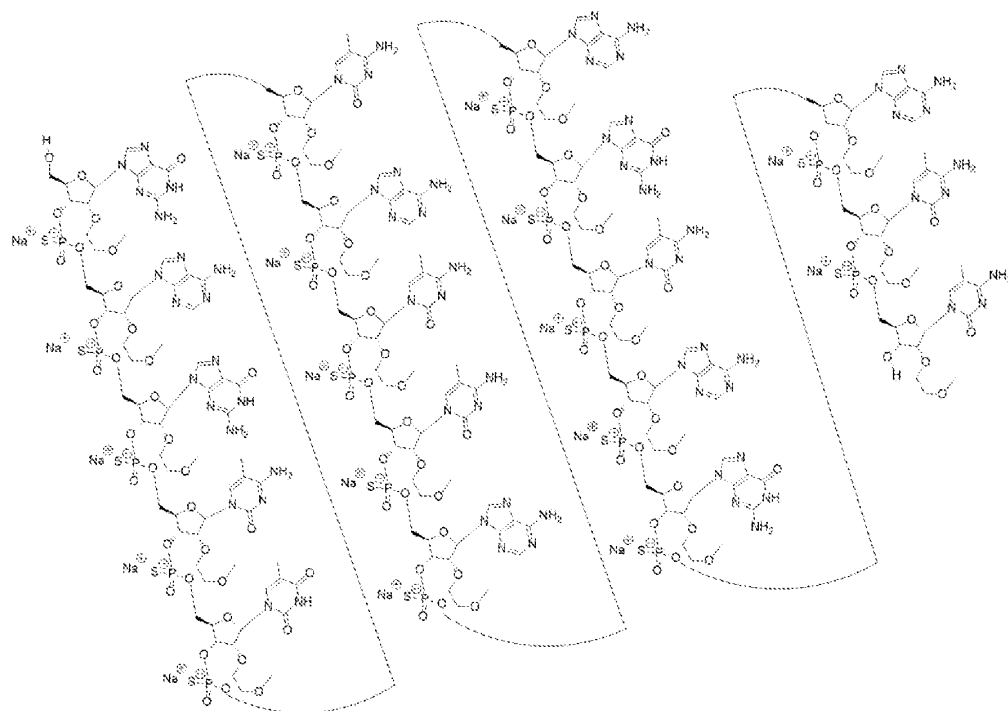


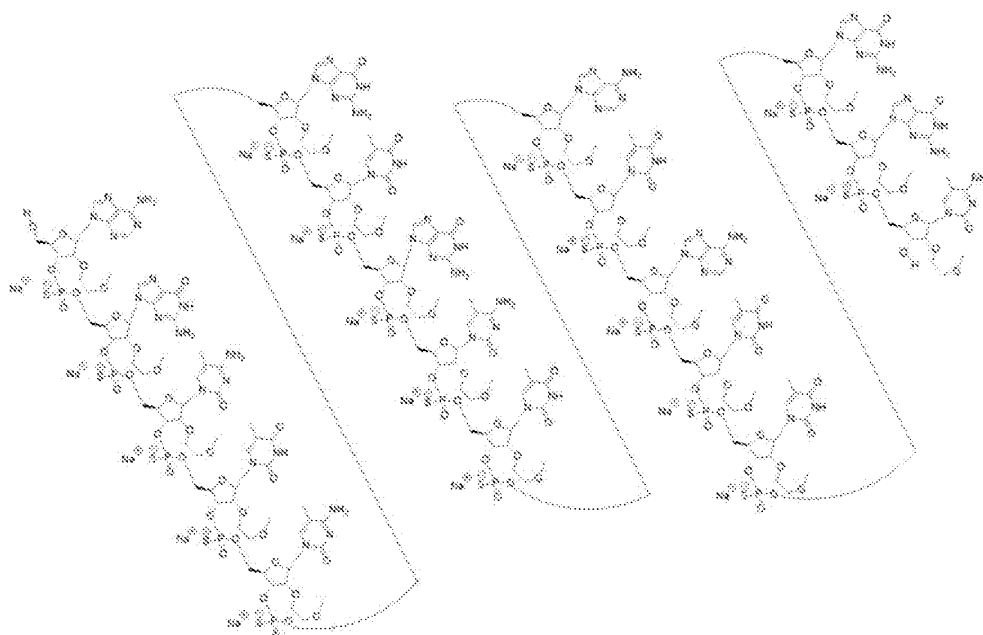


or a pharmaceutically acceptable salt thereof.

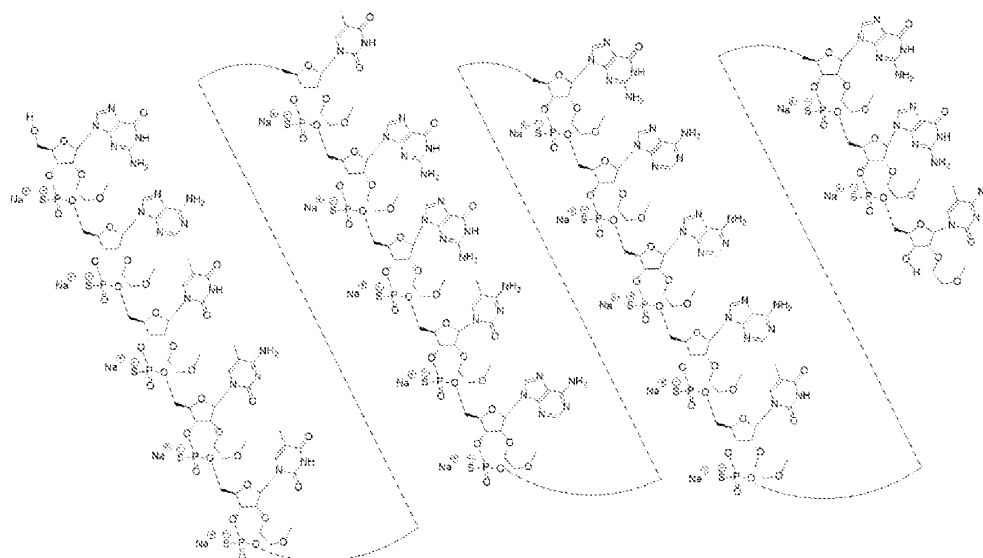
[00106] Described herein, in some aspects, is an antisense oligomer having any of the following structures:







; or



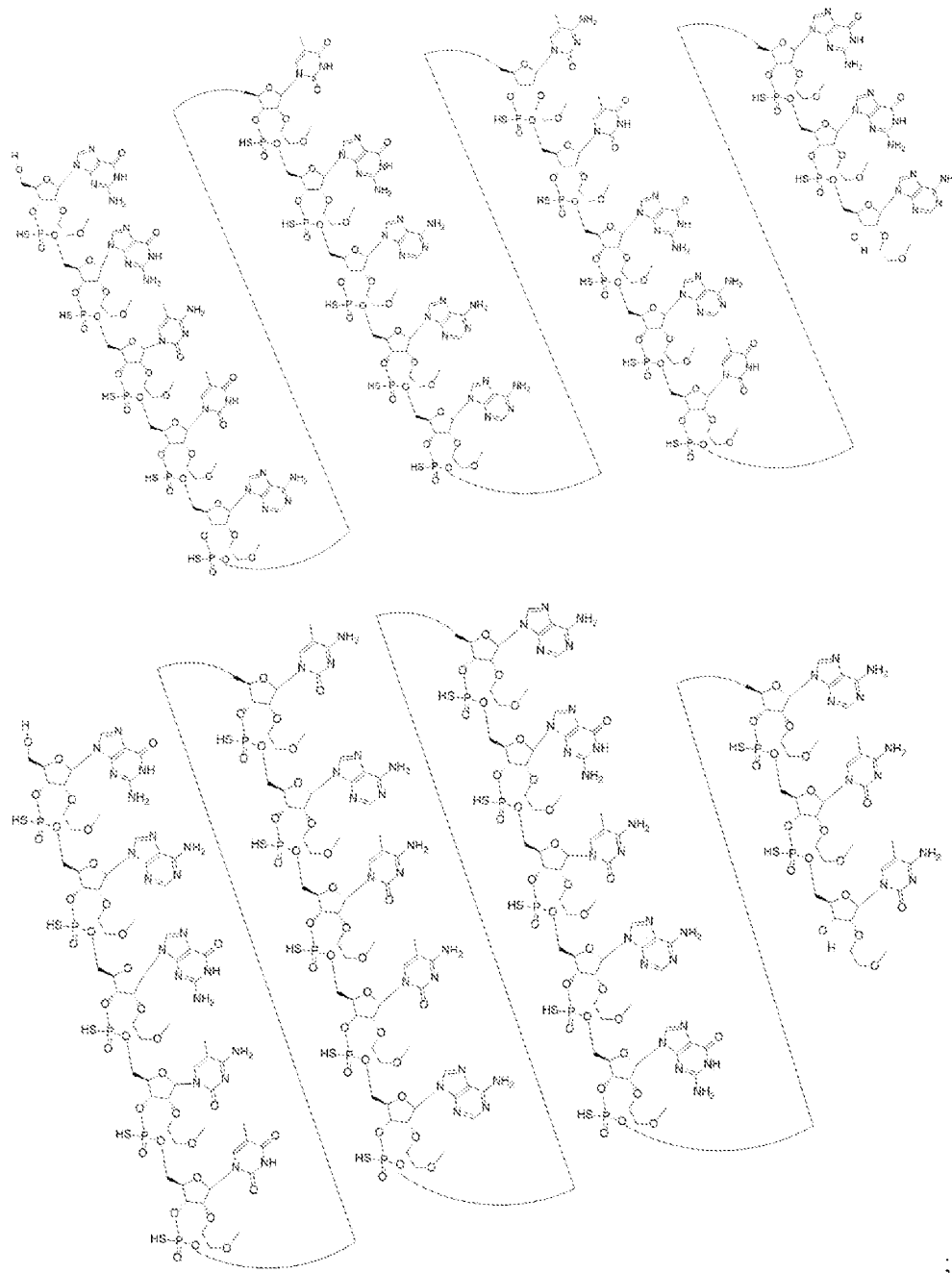
[00107] Described herein, in some aspects, is a pharmaceutical formulation comprising:

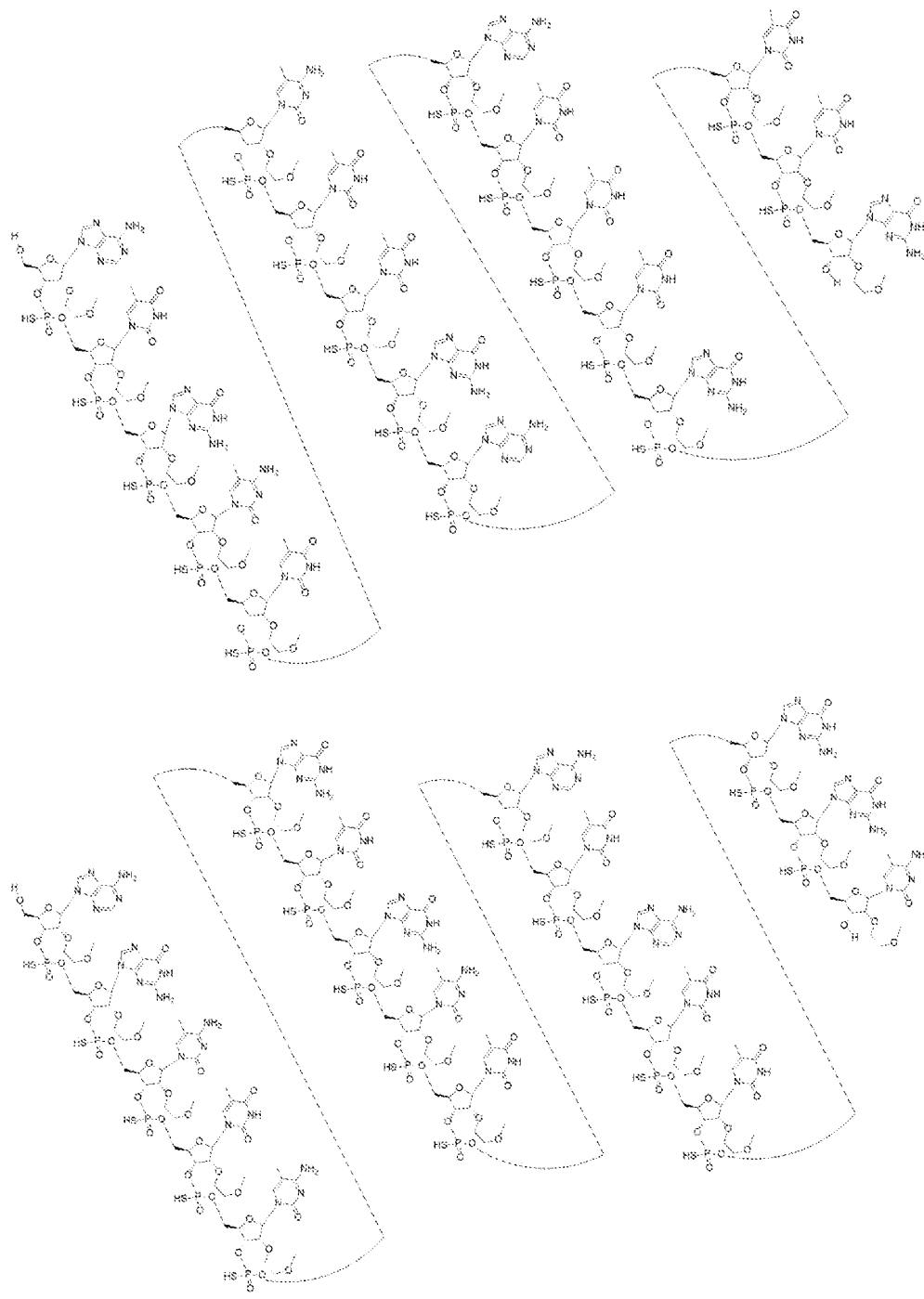
- (a) an antisense oligomer, wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and
 - (b) a pharmaceutically acceptable diluent;
- wherein the antisense oligomer is dissolved or suspended in a solution at a concentration of about 1 mg/mL to about 200 mg/mL.

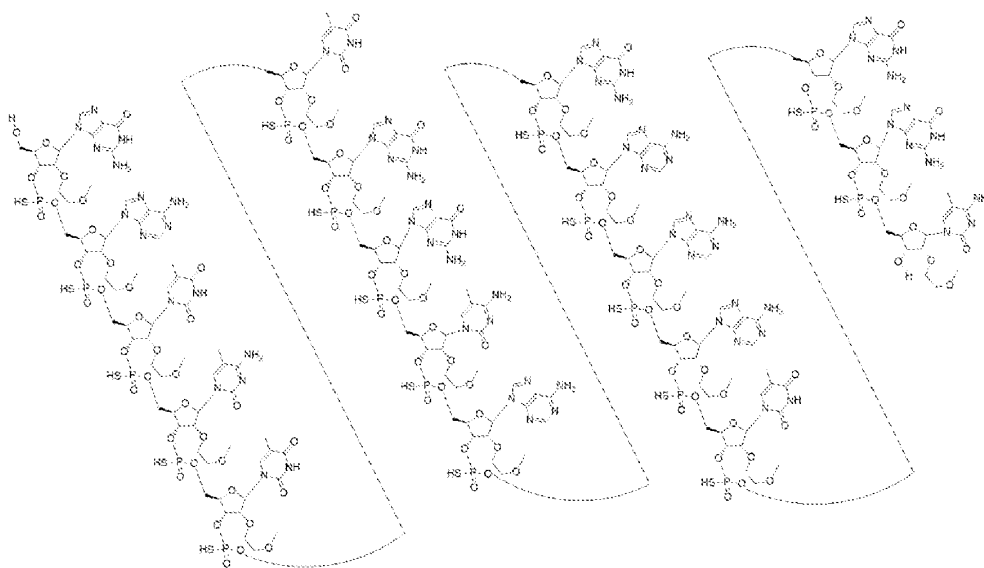
[00108] Described herein, in some aspects, is a pharmaceutical formulation comprising:

- (a) an antisense oligomer; and
 - (b) a pharmaceutically acceptable diluent;
- wherein the antisense oligomer is dissolved or suspended in a solution, and

wherein the antisense oligomer is a compound according to any one of the following chemical structures:







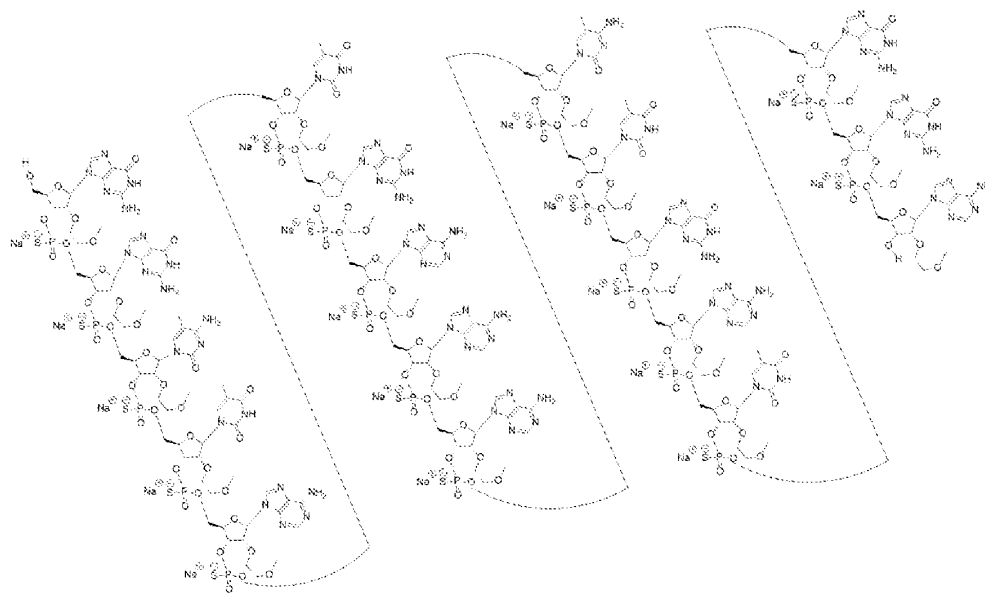
or a pharmaceutically acceptable salt thereof.

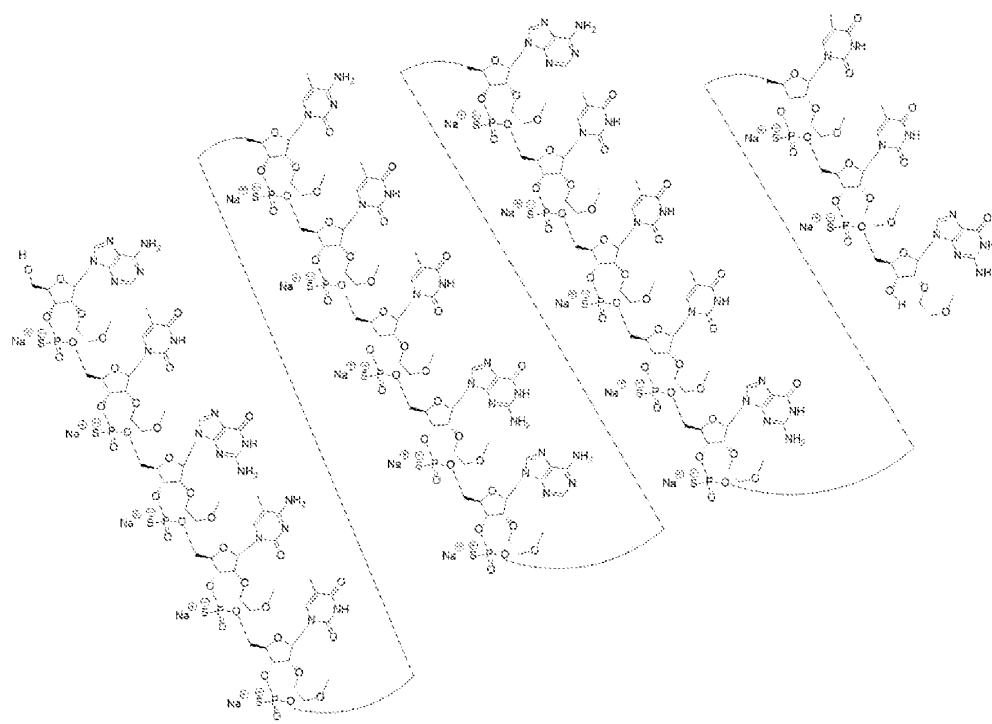
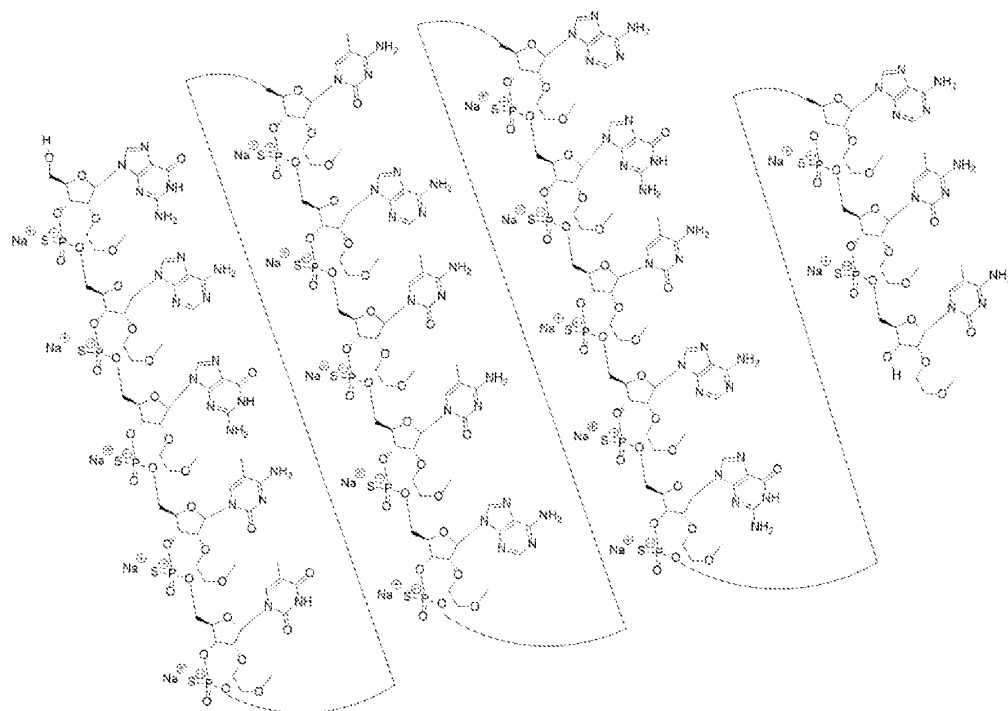
[00109] Described herein, in some aspects, is a pharmaceutical formulation comprising:

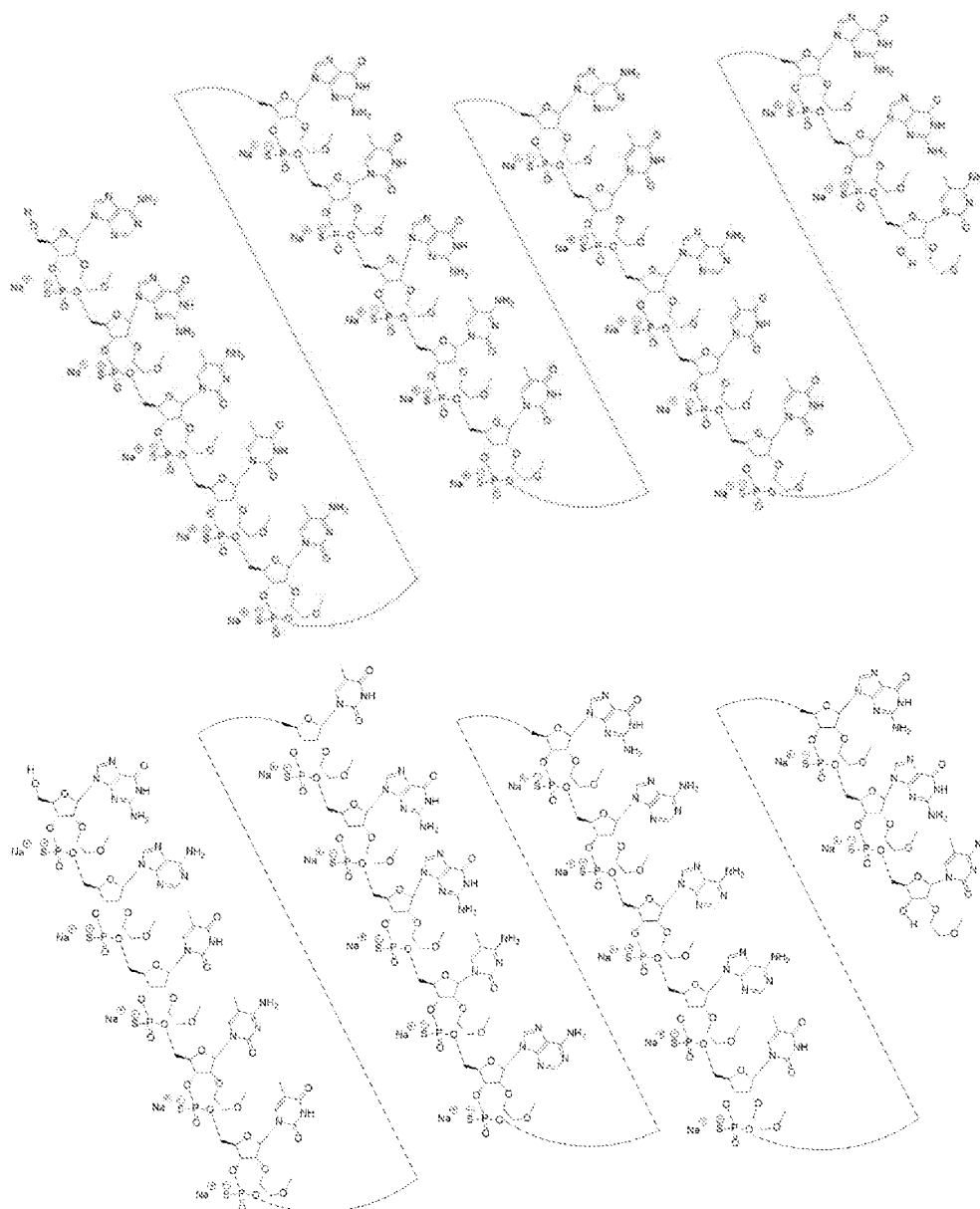
- (a) an antisense oligomer; and
- (b) a pharmaceutically acceptable diluent;

wherein the antisense oligomer is dissolved or suspended in a solution, and

wherein the antisense oligomer has any one of the following chemical structures:







; or

[00110] In some aspects, the antisense oligomer is present in the solution at a concentration of about 1 mg/mL to about 200 mg/mL.

[00111] In some aspects, the antisense oligomer is present in the solution at a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about

150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

[00112] In some aspects, the antisense oligomer is present in the solution at a concentration of about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00113] In some aspects, the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer.

[00114] In some aspects, the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL,

about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00115] In some aspects, the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00116] In some aspects, the concentrate is phosphate buffered.

[00117] In some aspects, the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

[00118] In some aspects, the antisense oligomer is solubilized or diluted in a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

[00119] In some aspects, the antisense oligomer is solubilized or diluted in an isotonic solution.

[00120] In some aspects, the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8.

[00121] In some aspects, the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution.

[00122] In some aspects, the pharmaceutical formulation does not comprise a preservative.

[00123] In some aspects, the pharmaceutical formulation is suitable for an intravitreal injection.

[00124] In some aspects, the pharmaceutical formulation is packaged in a single-use vial.

[00125] Described herein, in some aspects, is a kit comprising:

- (i) a concentrate comprising an antisense oligomer (ASO), wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and
- (ii) a diluent, wherein the concentrate is miscible with the diluent; and
- (iii) instructions for diluting the concentrate with the diluent.

[00126] In some aspects, the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to

about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00127] In some aspects, the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00128] In some aspects, the concentrate is phosphate buffered.

[00129] In some aspects, the diluent is a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

[00130] In some aspects, the diluent is a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

[00131] In some aspects, the diluent comprises an isotonic solution.

[00132] In some aspects, the diluent comprises a phosphate-buffered solution with at least pH 5.8.

[00133] In some aspects, the diluent comprises a phosphate-buffered (pH 6.6 - 7.6) solution.

[00134] In some aspects, the concentrate or the diluent does not comprise a preservative.

[00135] In some aspects, the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 2 mg/mL to 200 mg/mL in the diluent.

[00136] In some aspects, the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about

150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL in the diluent.

[00137] In some aspects, the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the antisense oligomer to a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL in the diluent.

[00138] In some aspects, the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 or 280-299.

[00139] In some aspects, the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

[00140] In some aspects, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00141] In some aspects, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety.

[00142] In some aspects, the antisense oligomer comprises at least one modified sugar moiety.

[00143] In some aspects, each sugar moiety is a modified sugar moiety.

[00144] In some aspects, the antisense oligomer comprises a 5'-methylcytosine (5'-MeC).

[00145] In some aspects, each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC).

[00146] In some aspects, the antisense oligomer comprises a 5'-methyluracil (5'-MeU).

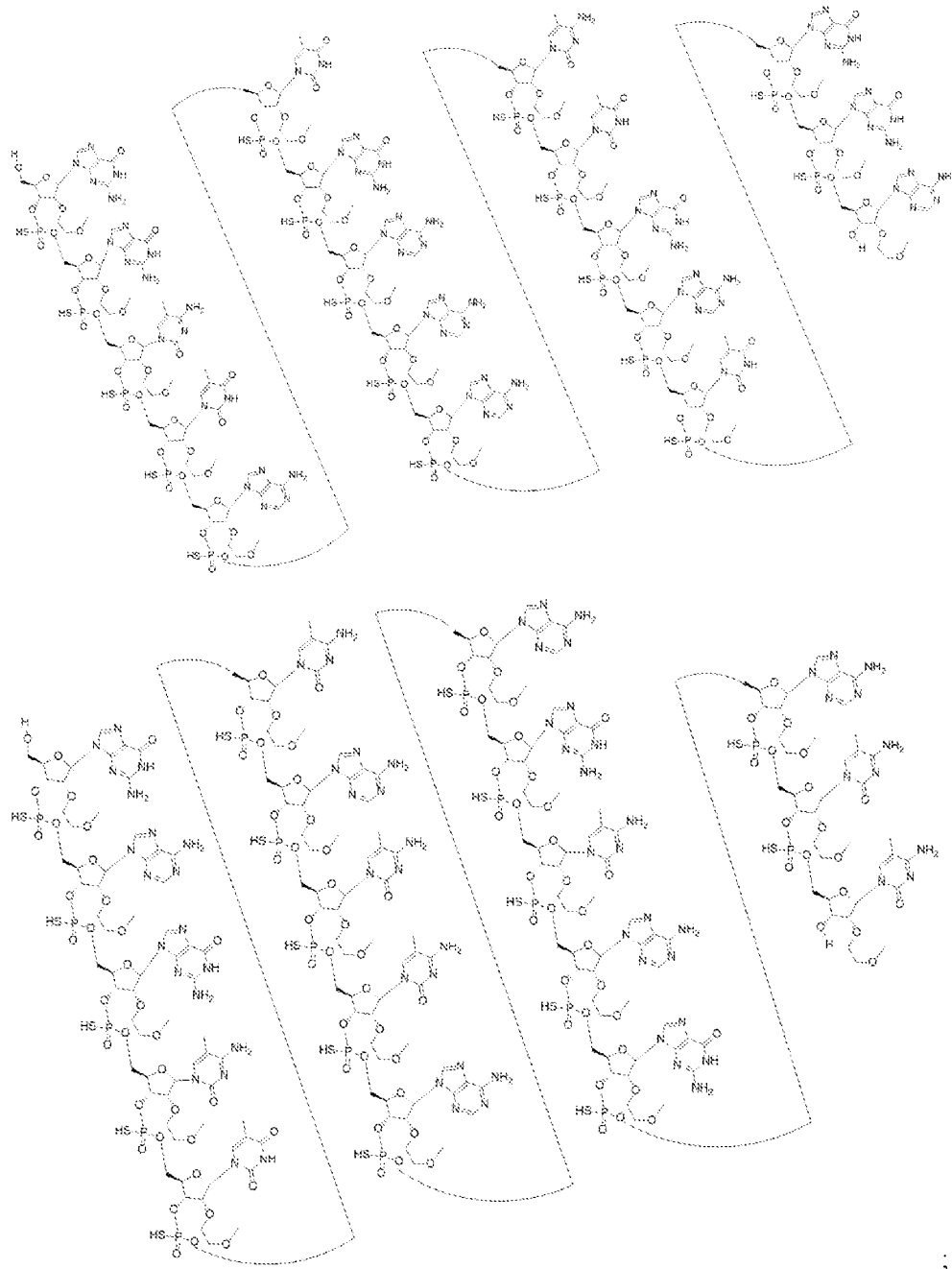
[00147] In some aspects, the antisense oligomer comprises a phosphorothioate linkage.

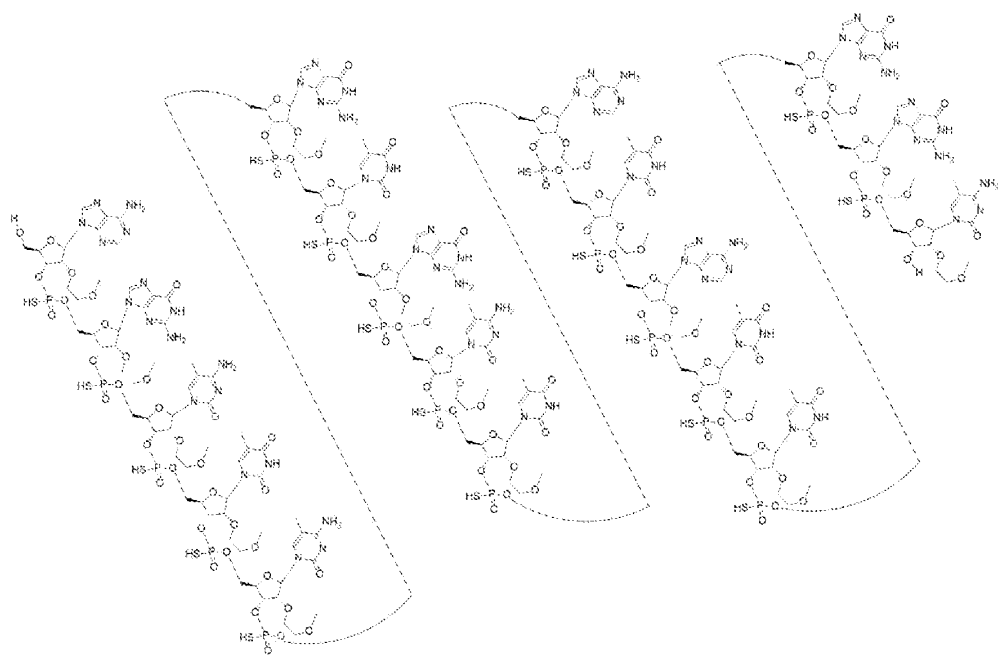
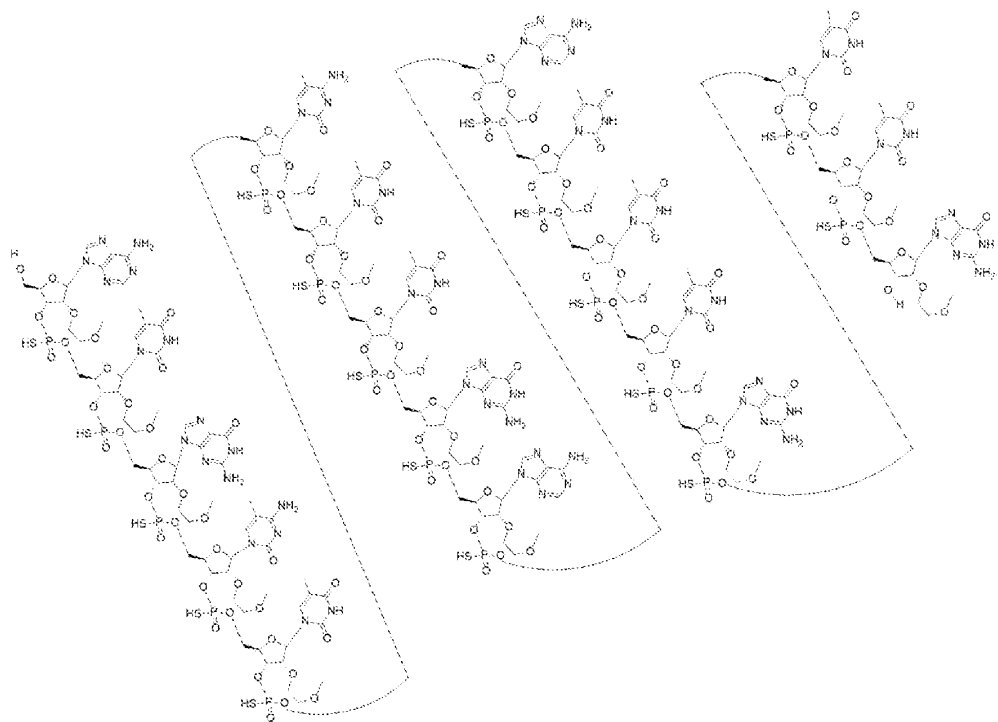
[00148] In some aspects, each internucleoside linkage of the ASO is a phosphorothioate linkage.

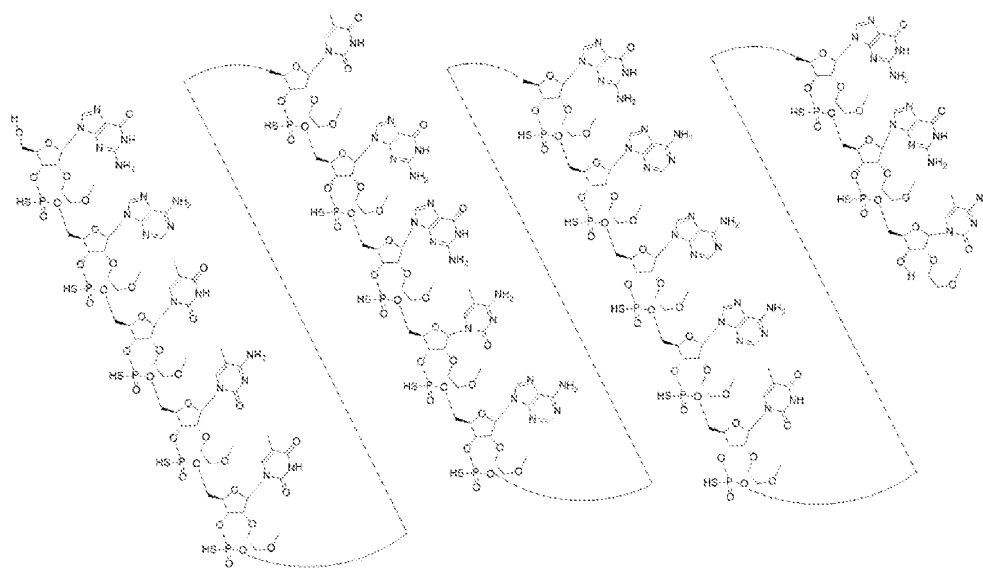
[00149] In some aspects, the antisense oligomer comprises a locked nucleic acid (LNA).

[00150] In some aspects, the antisense oligomer is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases in length.

[00151] In some aspects, the antisense oligomer is a compound according to any one of the following chemical structures:

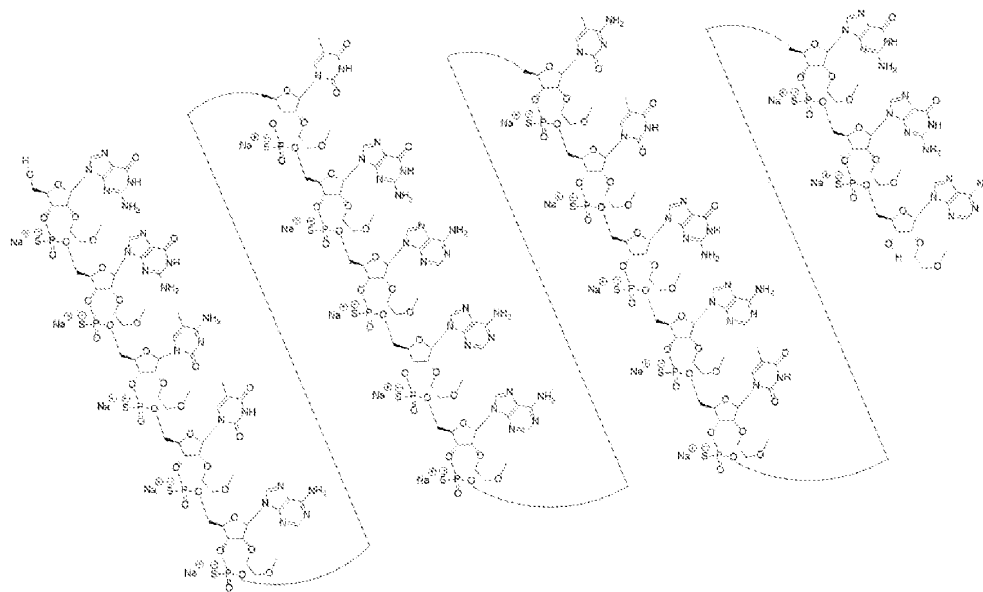


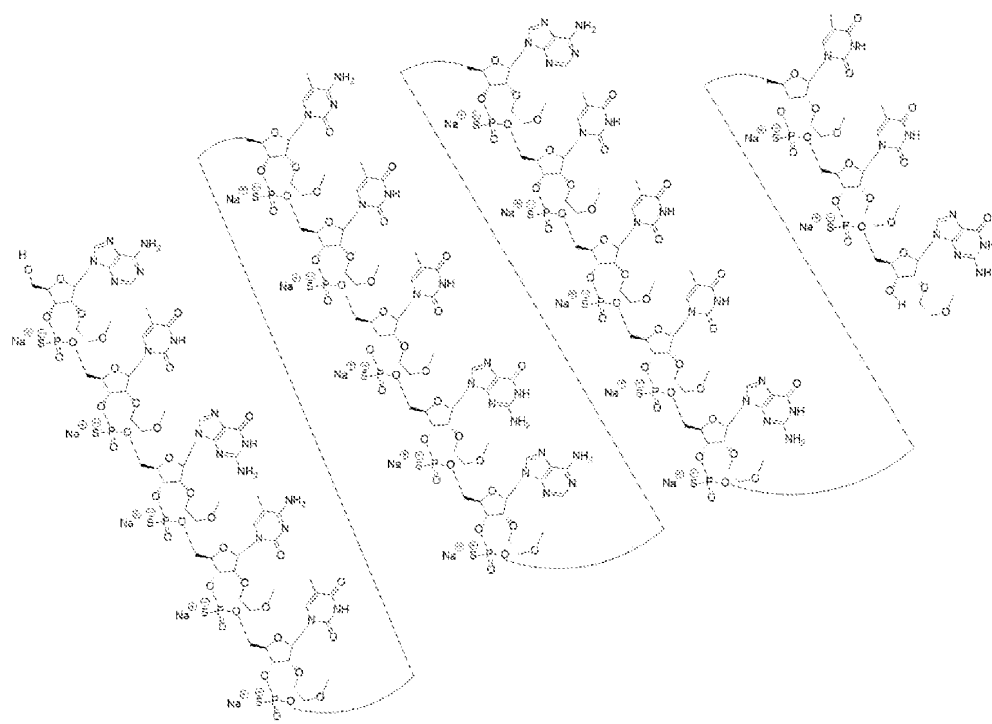
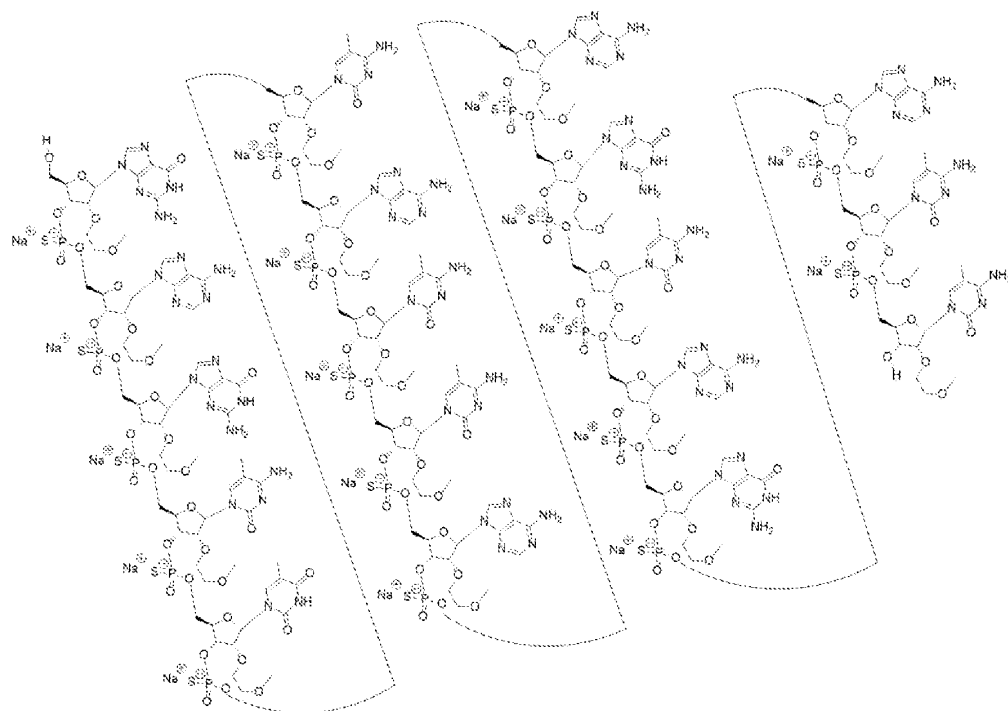


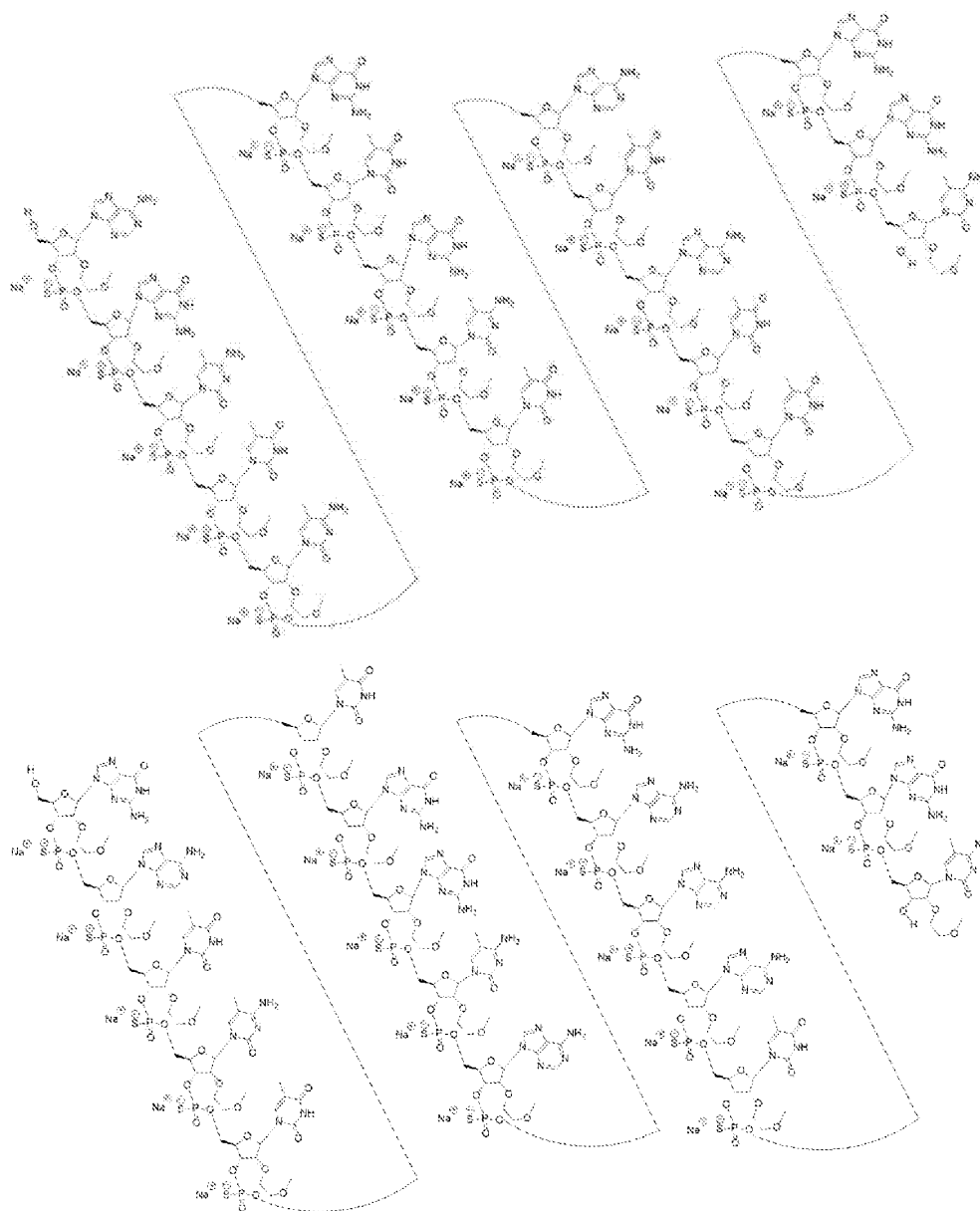


or a pharmaceutically acceptable salt thereof.

[00152] In some aspects, the antisense oligomer has any one of the following chemical structures:







[00153] Described herein, in some aspects, are uses of an antisense oligomer for the manufacture of a medicament for treating a disease or condition characterized by a reduced expression or function of OPA1 protein in a human subject in need thereof, or reducing likelihood of the subject developing the disease or condition, wherein the medicament is administered to one eye of the subject at a dose of about 0.005 mg to about 20 mg, and wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299.

[00154] In some aspects, the medicament is administered to the one eye of the subject at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg to about 15 mg, about 0.01 mg to about 10

mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg, about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

[00155] In some aspects, the medicament is administered to the one eye of the subject at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

[00156] In some aspects, the medicament is administered to the one eye of the subject at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

[00157] In some aspects, the medicament is administered to the one eye of the subject at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer.

INCORPORATION BY REFERENCE

[00158] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[00159] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[00160] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[00161] **FIG. 1A** depicts a schematic representation of a target mRNA that contains a nonsense-mediated mRNA decay-inducing exon (NMD exon mRNA) and therapeutic agent-mediated exclusion of the nonsense-mediated mRNA decay-inducing exon to increase expression of the full-length target protein or functional RNA. In particular, **FIG. 1A** shows an example schematic of the inclusion of an alternative exon (designated as Exon X) identified in the *OPAI* gene which leads to the introduction of a premature termination codon (PTC) resulting in a nonproductive mRNA transcript degraded by nonsense-mediated decay (NMD). The schematic also shows the location of the two primers used to detect both the productive and nonproductive mRNA transcripts by RT-PCR.

[00162] **FIG. 1B** illustrates expression of *OPAI* transcripts containing the NMD exon in HEK293 cells treated with increasing amounts of cycloheximide. Treatment of HEK293 cells with cycloheximide for 3 hours increases the abundance of nonproductive mRNA transcripts. Nonproductive transcript abundance was calculated as the intensity of the (nonproductive transcript / [the nonproductive + productive transcript]) and expressed as a percentage. A representative gel image is shown, and the percentage abundance of the nonproductive transcript indicated below is an average of three biological replicates.

[00163] **FIG. 1C** shows RT-PCR data from nonproductive transcripts in RNA isolated from retinal tissue punches from macular and peripheral regions of both eyes of a human donor. The relative abundance of the nonproductive transcript in each sample is included at the bottom of each lane. OD: oculus dexter (right eye), OS: oculus sinister (left eye).

[00164] **FIG. 2A** shows RT-PCR data for nonproductive *OPAI* mRNAs in HEK293 cells after treatment with ASO-1 and cycloheximide *in vitro*.

[00165] **FIG. 2B** illustrates quantification of productive *OPAI* mRNAs in HEK293 cells after treatment with ASO-1 in the absence of cycloheximide.

[00166] **FIG. 2C** illustrates protein expression of OPA1 in HEK293 cells after treatment with ASO-1 in the absence of cycloheximide.

[00167] **FIG. 2D** is an EC50 evaluation of ASO-1 in HEK293 cells and illustrates a representative dose-response curve fit based on levels of nonproductive transcript measured when various doses of ASO-1 were applied to HEK293 cells.

[00168] **FIG. 2E** are Western Blot gel images showing OPA1 protein expression after the transfection of HEK293 cells with vehicle (first three lanes), a non-targeting ASO (NT ASO) at various concentrations (1 nM, 5 nM, and 20 nM), and ASO-1 at various concentrations (1 nM, 5 nM, and 20 nM). Each condition comprised six biological replicates. Cells were lysed with RIPA buffer after transfection and Western Blots were probed with antibodies targeting OPA1 and β -tubulin. Multiple bands at the top of each gel correspond to different isoforms of OPA1.

[00169] **FIG. 2F** is a line chart showing relative cell viability over the course of 72 hours after treatment of HEK293 cells with various doses (1 μ M, 3 μ M, 10 μ M, and 20 μ M) of either ASO-1 or non-targeting ASO (NT ASO).

[00170] **FIG. 2G** is a line chart showing relative cell viability 72 hours after treatment of HEK293 cells with various concentrations of cycloheximide (8 ng/ml, 40 ng/ml, 200 ng/ml, 1000 ng/ml, 5000 ng/ml).

[00171] **FIGs. 3A-3E** represent data from Western Blot analyses showing reduced OPA1 protein levels in *OPAI*^{+/-} HEK293 cells compared to isogenic *OPAI*^{+/+} HEK293 cells. Western Blot gel images are shown in **FIG. 3A**. Histograms of the quantified Western Blots are shown in **FIG. 3B** and **FIG. 3E**, with the latter showing relevant data points. **FIG. 3C** is a histogram showing relative OPA1 protein expression when isogenic *OPAI*^{+/+} HEK293 and *OPAI*^{+/-} HEK293 cells were treated with Vehicle (no ASO) and ASO-1, and corresponds to quantification of Western Blot data from **FIG. 3D**. **FIG. 3D** shows an exemplary Western Blot gel image of *OPAI*^{+/+} HEK293 and *OPAI*^{+/-} HEK293 cells treated with either Vehicle or ASO-1 and probed with antibodies targeting either OPA1 or β -actin. **FIG. 3F** is a gel image of RT-PCR data from *OPAI*^{+/+} HEK293 and *OPAI*^{+/-} HEK293 cells treated with either DMSO or cycloheximide (CHX) and showing levels of nonproductive (upper bands) and productive (lower bands) *OPAI* transcripts. **FIG. 3G** is a histogram of quantified data from the Western Blot of **FIG. 3F** showing the percentage of nonproductive transcripts in the tested conditions comprising *OPAI*^{+/+} HEK293 and *OPAI*^{+/-} HEK293 cells treated with either DMSO or CHX. **FIG. 3H** is a histogram of qPCR data showing relative levels of nonproductive transcript in the tested conditions comprising *OPAI*^{+/+} HEK293 and *OPAI*^{+/-} HEK293 cells treated with either DMSO or CHX.

[00172] **FIGs. 4A-4E** show that ASO-1 increases *OPA1* mRNA and OPA1 protein expression in *OPA1*^{+/-} HEK293 cells. **FIGs. 4A-4D** are histograms of data from *OPA1*^{+/+} or *OPA1*^{+/-} HEK293 cells treated with Vehicle or 10 μM ASO-1 by gymnotic delivery for 72 hours and assessed for effect on *OPA1* mRNA expression (**FIGs. 4A-4B**) and OPA1 protein (**FIGs. 4C-4D**) 72 hours post treatment. **FIG. 4A** is histogram showing data for *OPA1* mRNA expression and **FIG. 4C** is histogram showing data for OPA1 protein levels when *OPA1*^{+/+} HEK293 cells are treated with Vehicle or 10 μM ASO-1 by gymnotic delivery. **FIG. 4B** is histogram showing data for *OPA1* mRNA expression and **FIG. 4D** is histogram showing data for OPA1 protein levels when *OPA1*^{+/-} HEK293 cells are treated with Vehicle or 10 μM ASO-1 by gymnotic delivery. **FIG. 4E** is a representative Western Blot image for data in **FIGs. 4C-4D**.

[00173] **FIGs. 5A-5D** show that ASO-1 reduces nonproductive exon inclusion and increases OPA1 expression in ADOA patient fibroblast cells. The upper panel of **FIG. 5A** shows source and identifying information of the wild-type *OPA1*^{+/+} fibroblasts tested (WT (ATCC), GM02036, and GM03234), and the lower panel of **FIG. 5A** contains *OPA1* variant information for the three ADOA patients designated as F34, F35 and F36. **FIGs. 5B-5C** are histograms representing data for WT (ATCC) and all three patient fibroblast cells treated with ASO-1 and harvested for analysis of nonproductive splicing (**FIG. 5B**) and *OPA1* mRNA levels (**FIG. 5C**). **FIG. 5D** shows a histogram of OPA1 protein levels determined by Western Blot after transfection and normalized to β-actin. **FIGs. 5E-5F** are histograms representing data for the three different types of wild-type cells from the upper panel of **FIG. 5A** (WT (ATCC), GM02036, and GM03234) and the three patient fibroblast cells with different *OPA1* pathogenic variants from the lower panel of **FIG. 5A** that were harvested for analysis of their *OPA1* mRNA levels (**FIG. 5E**) and relative OPA1 protein levels (**FIG. 5F**) prior to treatment with ASO-1. **FIG. 5G** is an exemplary Western Blot showing OPA1 protein levels of the three different wild-type fibroblasts and three ADOA patient dermal fibroblasts with different *OPA1* pathogenic variants prior to treatment with ASO-1, and cells were probed with antibodies targeting either OPA1 or β-actin. Multiple bands at the upper part of the gel image of **FIG. 5G** correspond to different isoforms of OPA1 and the lower bands correspond to β-actin. **FIG. 5H** shows a histogram of relative nonproductive *OPA1* transcript levels when the three wild-type cells (WT (ATCC), GM02036, and GM03234) and three ADOA patient dermal fibroblasts with different *OPA1* pathogenic variants (F34, F35, and F36) are treated with either DMSO or CHX. **FIG. 5I** are exemplary Western Blot gel images showing OPA1 protein levels when wild-type and ADOA patient fibroblasts are treated with either Vehicle (no ASO) or ASO-1, and cells were probed with antibodies targeting either OPA1 or β-actin. Each gel image within **FIG. 5I** represents a separate experiment, and each lane of each gel represents an individual biological replicate.

[00174] **FIGS. 6A-6D** are exemplary histograms of oxygen consumption rate that demonstrate ADOA patient-derived fibroblast cells have reduced mitochondrial function. Basal respiration (**FIG. 6A**), ATP-linked respiration (**FIG. 6B**), maximal respiration (**FIG. 6C**) and spare respiratory capacity (**FIG. 6D**) are shown. **FIGS. 6E-6H** are exemplary histograms of additional experiments assessing oxygen consumption rate that demonstrate ADOA patient-derived fibroblast cells have reduced mitochondrial function compared to three wild-type cells (WT (ATCC), GM02036, and GM03234). Basal respiration (**FIG. 6E**), ATP-linked respiration (**FIG. 6F**), maximal respiration (**FIG. 6G**) and spare respiratory capacity (**FIG. 6H**) are shown.

[00175] **FIGS. 7A-7D** are histograms that show ASO-1 increases mitochondrial function in all three ADOA patients' fibroblast cells. Oxygen consumption rate measurements were taken from patient fibroblast cells treated with 20, 40 or 60 nM ASO-1 or Vehicle. Basal respiration (**FIG. 7A**), ATP-linked respiration (**FIG. 7B**), maximal respiration (**FIG. 7C**) and spare respiratory capacity (**FIG. 7D**) are shown. **FIGS. 7E-7H** are histograms that show the effects of a nontargeting ASO control on mitochondrial function in ADOA patient F35's fibroblast cells. Oxygen consumption rate measurements were taken from patient fibroblast cells treated with 20, 40 or 60 nM ASO-1 or Vehicle (no ASO). Basal respiration (**FIG. 7E**), ATP-linked respiration (**FIG. 7F**), maximal respiration (**FIG. 7G**) and spare respiratory capacity (**FIG. 7H**) are shown.

[00176] **FIGS. 8A-8D** show that surrogate antisense oligonucleotide (ASO) ST-1102 increases OPA1 protein in Wild-Type (WT) rabbit retinal tissue following single intravitreal (IVT) injection. **FIG. 8A** illustrates the study design for the *in vivo* rabbit experiment of Example 9. **FIG. 8B** are histograms representing RT-PCR data measuring nonproductive splicing using rabbit retinal RNA. **FIG. 8C** shows histograms of quantitated Western Blot data for OPA1 protein normalized to actin. **FIG. 8D** shows a histogram of ST-1102 detected in retinal tissue on Day 29 by HELISA at all three dose levels.

[00177] **FIG. 9** shows RT-PCR gel images from ST-1102-injected rabbit retinas.

[00178] **FIG. 10** shows Western Blot images from ST-1102-injected rabbit retinas.

[00179] **FIGS. 11A-11C** show histograms demonstrating that intravitreal administration of ASO-1 induces dose-related reduction in nonsense-mediated decay exon inclusion and increased OPA1 protein with sustained tissue exposure in the retina of cynomolgus monkeys. **FIG. 11A** is a histogram of qPCR data measuring the presence of nonproductive exon-containing RNA transcripts in retinal tissue relative to total *OPA1* transcripts. **FIG. 11B** is a histogram representing OPA1 protein quantitated in retinal lysates by ELISA and normalized to total protein in the sample. **FIG. 11C** is a histogram representing ASO-1 levels in retinal tissue quantitated by HELISA. **FIG. 11D** is a gel image showing RT-PCR data of the right (oculus dexter, OD) and left (oculus sinister, OS) eyes of three cynomolgus monkeys. Upper bands represent nonproductive transcripts and

lower bands represent productive transcripts. Numbers at the bottom of the gel refer to the amount of A-X-B relative to the total (A-X-B plus A-B).

[00180] **FIG. 12A-12D** are images representative of ASO-1 and OPA1 protein in retinal ganglion cells (RGCs) after intravitreal (IVT) administration to cynomolgus monkeys. **FIG. 12A** shows miRNAscope™ staining with a specific probe to ASO-1 was performed on MDF-fixed, paraffin-embedded cynomolgus monkey eyes to demonstrate cellular location of ASO-1 (red) in RGCs. Increased ASO-1 signal is correlated with increased dose. Nuclei were counterstained with hematoxylin (blue). **FIG. 12B** shows immunofluorescence staining with OPA1 antibody indicating apparent dose-related protein increase (red) in the RGCs in/near the fovea. Nuclei are stained with DAPI (blue), and magnification was 40X. **FIG. 12C** shows immunohistochemically (IHC) stained image of RGCs after intravitreal administration of PBS to cynomolgus monkeys. **FIG. 12D** shows an *in situ* hybridization (ISH) of RGCs after intravitreal administration of ASO-1 to cynomolgus monkeys. **FIG. 12E** is a schematic depicting the experimental procedure entailing the IVT administration of either PBS or ASO-1 to cynomolgus monkeys and evaluation after 4 weeks. The right eyes of cynomolgus monkeys were fixed for *in situ* hybridization (ISH) and immunohistochemistry (IHC).

[00181] **FIG. 13A-13D** show that there is dose-related increase in ASO-1 in retinal ganglion cells (RGCs) after intravitreal (IVT) administration to cynomolgus monkeys. **FIGS. 13A-13B** are histograms representing quantitated copies of mRNA per cell and percent positive RGCs in or near foveal RGCs. **FIGS. 13B-13C** are histograms representing quantitated copies of mRNA per cell and percent positive RGCs in peripheral retina.

[00182] **FIG. 14A-14B** show quantitation of OPA1 protein immunofluorescence in cynomolgus monkey retinal ganglion cells (RGCs) after intravitreal ASO-1 administration. Fluorescence (FL) intensity was quantitated in the RGC layer in foveal/near foveal (**FIG. 14A**) and peripheral (**FIG. 14B**) retina.

[00183] **FIG. 15A** is a schematic depicting the experimental procedure entailing the IVT administration of 50- μ l aliquots of either PBS or ASO-1 to cynomolgus monkeys, followed by tissue collection and retina dissection 8 weeks. **FIG. 15B** shows the treatment conditions of each group in the cynomolgus monkey study. **FIG. 15C** is a schematic of retina dissection showing a map of the quadrants for downstream evaluation of OPA1 protein expression (Q1), degree of ASO-1 exposure (Q2 and Q4), and *OPA1* mRNA expression (Q3). **FIG. 15D** is a histogram of ASO-1 exposure in the retina for the five dose groups in **FIG. 15B** as assessed by the measured concentration of ASO-1 (units/g) resulting from hybridization ELISA (hELISA). **FIG. 15E** is a histogram of NMD exon inclusion in the retina for the five dose groups in **FIG. 15B** as assessed by the *OPA1* NMD exon inclusion relative to the control from qPCR data. **FIG. 15F** is a histogram

of OPA1 protein levels in the retina for the five dose groups in **FIG. 15B** as assessed by OPA1 measured in units/mg of total protein relative to the control resulting from ELISA.

DETAILED DESCRIPTION

[00184] Alternative splicing events in the *OPA1* gene can lead to nonproductive mRNA transcripts which in turn can lead to aberrant protein expression, and therapeutic agents which can target the alternative splicing events in the *OPA1* gene can modulate the expression level of functional proteins in DS patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition caused by OPA1 protein deficiency.

[00185] One of the alternative splicing events that can lead to nonproductive mRNA transcripts is the inclusion of an extra exon in the mRNA transcript that can induce nonsense-mediated mRNA decay. The present disclosure provides compositions and methods for modulating alternative splicing of *OPA1* to increase the production of protein-coding mature mRNA, and thus, translated functional OPA1 protein. These compositions and methods include antisense oligomers (ASOs) that can cause exon skipping, *e.g.*, pseudoexon skipping, and promote constitutive splicing of *OPA1* pre-mRNA. In various embodiments, functional OPA1 protein can be increased using the methods of the disclosure to treat a condition caused by OPA1 protein deficiency.

mRNA Splicing

[00186] Intervening sequences in RNA sequences or introns are removed by a large and highly dynamic RNA-protein complex termed the spliceosome, which orchestrates complex interactions between primary transcripts, small nuclear RNAs (snRNAs) and a large number of proteins. Spliceosomes assemble ad hoc on each intron in an ordered manner, starting with recognition of the 5' splice site (5'ss) by U1 snRNA or the 3' splice site (3'ss) by the U2 pathway, which involves binding of the U2 auxiliary factor (U2AF) to the 3'ss region to facilitate U2 binding to the branch point sequence (BPS). U2AF is a stable heterodimer composed of a U2AF2-encoded 65-kD subunit (U2AF65), which binds the polypyrimidine tract (PPT), and a U2AF1-encoded 35-kD subunit (U2AF35), which interacts with highly conserved AG dinucleotides at 3'ss and stabilizes U2AF65 binding. In addition to the BPS/PPT unit and 3'ss/5'ss, accurate splicing requires auxiliary sequences or structures that activate or repress splice site recognition, known as intronic or exonic splicing enhancers or silencers. These elements allow genuine splice sites to be recognized among a vast excess of cryptic or pseudo-sites in the genome of higher eukaryotes, which have the same sequences but outnumber authentic sites by an order of magnitude. Although they often have a regulatory function, the exact mechanisms of their activation or repression are poorly understood.

[00187] The decision of whether to splice or not to splice can be typically modeled as a stochastic rather than deterministic process, such that even the most defined splicing signals can sometimes splice incorrectly. However, under normal conditions, pre-mRNA splicing proceeds at surprisingly high fidelity. This is attributed in part to the activity of adjacent cis-acting auxiliary exonic and intronic splicing regulatory elements (ESRs or ISRs). Typically, these functional elements are classified as either exonic or intronic splicing enhancers (ESEs or ISEs) or silencers (ESSs or ISSs) based on their ability to stimulate or inhibit splicing, respectively. Although there is now evidence that some auxiliary cis-acting elements may act by influencing the kinetics of spliceosome assembly, such as the arrangement of the complex between U1 snRNP and the 5'ss, it seems very likely that many elements function in concert with trans-acting RNA-binding proteins (RBPs). For example, the serine- and arginine-rich family of RBPs (SR proteins) is a conserved family of proteins that have a key role in defining exons. SR proteins promote exon recognition by recruiting components of the pre-spliceosome to adjacent splice sites or by antagonizing the effects of ESSs in the vicinity. The repressive effects of ESSs can be mediated by members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family and can alter recruitment of core splicing factors to adjacent splice sites. In addition to their roles in splicing regulation, silencer elements are suggested to have a role in repression of pseudo-exons, sets of decoy intronic splice sites with the typical spacing of an exon but without a functional open reading frame. ESEs and ESSs, in cooperation with their cognate trans-acting RBPs, represent important components in a set of splicing controls that specify how, where and when mRNAs are assembled from their precursors.

[00188] Alternative splicing is a regulated process during gene expression that can result in multiple isoforms of mature mRNA transcripts that are processed from a single primary mRNA transcript that is transcribed from a single gene, and the resultant multiple proteins that are translated from at least some of the multiple mature mRNA isoforms. In this process, particular exons of a gene may be included within or excluded from the final, processed mRNA produced from that gene. Consequently, the proteins translated from alternatively spliced mRNAs will contain differences in their amino acid sequence and, in some cases, in their biological functions.

[00189] As described herein, an “alternatively spliced exon” can refer to an exon of a gene that can be either included or excluded naturally from a mature mRNA transcript, thus resulting in different protein products that are translated from the different mature mRNA transcripts. The inclusion or skipping of an alternatively spliced exon can take place naturally in a cell, either randomly, or in a regulated manner, *e.g.*, subject to regulation by external physiological or pathological stimuli, or intracellular signaling. In some cases, the production of alternatively spliced mRNAs, *e.g.*, the splicing of the alternatively spliced exon, is regulated by a system of

trans-acting proteins that bind to cis-acting sites on the primary transcript itself. In some cases, an alternatively spliced exon is a coding exon, *e.g.*, an exon that, when included in the mature mRNA transcript, is translated into an amino acid sequence as part of the protein product translated from the mature mRNA transcript. In some cases, the inclusion of an alternatively spliced exon in the mature mRNA transcript would maintain the canonical open reading frame as compared to a mature mRNA transcript without the alternatively spliced exon, *e.g.*, the number of nucleotides in the alternatively spliced exon is divisible by 3.

[00190] The sequences marking the exon-intron boundaries are degenerate signals of varying strengths that can occur at high frequency within human genes. In multi-exon genes, different pairs of splice sites can be linked together in many different combinations, creating a diverse array of transcripts from a single gene. This is commonly referred to as alternative pre-mRNA splicing. Although most mRNA isoforms produced by alternative splicing can be exported from the nucleus and translated into functional polypeptides, different mRNA isoforms from a single gene can vary greatly in their translation efficiency. Those mRNA isoforms with premature termination codons (PTCs) at least 50 bp upstream of an exon junction complex are likely to be targeted for degradation by the nonsense-mediated mRNA decay (NMD) pathway. Mutations in traditional (BPS/PPT/3'ss/5'ss) and auxiliary splicing motifs can cause aberrant splicing, such as exon skipping or cryptic (or pseudo-) exon inclusion or splice-site activation, and contribute significantly to human morbidity and mortality. Both aberrant and alternative splicing patterns can be influenced by natural DNA variants in exons and introns.

[00191] Given that exon-intron boundaries can occur at any of the three positions of a codon, it is clear that only a subset of alternative splicing events can maintain the canonical open reading frame. For example, only exons that are evenly divisible by 3 can be skipped or included in the mRNA without any alteration of reading frame. Splicing events that do not have compatible phases will induce a frame-shift. Unless reversed by downstream events, frame-shifts can certainly lead to one or more PTCs, probably resulting in subsequent degradation by NMD. NMD is a translation-coupled mechanism that eliminates mRNAs containing PTCs. NMD can function as a surveillance pathway that exists in all eukaryotes. NMD can reduce errors in gene expression by eliminating mRNA transcripts that contain premature stop codons. Translation of these aberrant mRNAs could, in some cases, lead to deleterious gain-of-function or dominant-negative activity of the resulting proteins. NMD targets not only transcripts with PTCs but also a broad array of mRNA isoforms expressed from many endogenous genes, suggesting that NMD is a master regulator that drives both fine and coarse adjustments in steady-state RNA levels in the cell.

[00192] An NMD-inducing exon ("NIE" or "NMD exon") is an exon or a pseudo-exon that is a region within an intron and can activate the NMD pathway if included in a mature RNA

transcript. In constitutive splicing events, the intron containing an NMD exon is usually spliced out, but the intron or a portion thereof (e.g., NMD exon) may be retained during alternative or aberrant splicing events. Mature mRNA transcripts containing such an NMD exon may be nonproductive due to frame shifts which induce the NMD pathway. Inclusion of a NMD exon in mature RNA transcripts may downregulate gene expression. mRNA transcripts containing an NMD exon may be referred to as “NIE-containing mRNA” or “NMD exon mRNA” in the current disclosure.

[00193] Cryptic (or pseudo- splice sites) have the same splicing recognition sequences as genuine splice sites but are not used in splicing reactions. They outnumber genuine splice sites in the human genome by an order of a magnitude and are normally repressed by thus far poorly understood molecular mechanisms. Cryptic 5' splice sites have the consensus NNN/GUNNNN or NNN/GCNNNN where N is any nucleotide and / is the exon-intron boundary. Cryptic 3' splice sites have the consensus NAG/N. Their activation is positively influenced by surrounding nucleotides that make them more similar to the optimal consensus of authentic splice sites, namely MAG/GURAGU and YAG/G, respectively, where M is C or A, R is G or A, and Y is C or U.

[00194] Splice sites and their regulatory sequences can be readily identified by a skilled person using suitable algorithms publicly available, listed for example, in Kralovicova, J. and Vorechovsky, I. (2007) Global control of aberrant splice site activation by auxiliary splicing sequences: evidence for a gradient in exon and intron definition. *Nucleic Acids Res.*, 35, 6399-6413 (www.ncbi.nlm.nih.gov/pmc/articles/PMC2095810/pdf/gkm680.pdf).

[00195] The cryptic splice sites or splicing regulatory sequences may compete for RNA-binding proteins, such as U2AF, with a splice site of the NMD exon. In some embodiments, an agent may bind to a cryptic splice site or splicing regulatory sequence to prevent binding of RNA-binding proteins and thereby favor binding of RNA-binding proteins to the NMD exon splice sites.

[00196] In some embodiments, the cryptic splice site may not comprise the 5' or 3' splice site of the NMD exon. In some embodiments, the cryptic splice site may be at least 10 nucleotides, at least 20 nucleotides, at least 50 nucleotides, at least 100 nucleotides or at least 200 nucleotides upstream of the NMD exon 5' splice site. In some embodiments, the cryptic splice site may be at least 10 nucleotides, at least 20 nucleotides, at least 50 nucleotides, at least 100 nucleotides, at least 200 nucleotides downstream of the NMD exon 3' splice site.

Target Transcripts

[00197] In some embodiments, the methods and compositions of the present disclosure exploit the presence of NMD exon in the pre-mRNA transcribed from the *OPAI* gene. Splicing of the identified *OPAI* NMD exon pre-mRNA species to produce functional mature *OPAI* mRNA may be induced using an agent such as an ASO that stimulates exon skipping of an NMD exon.

Induction of exon skipping may result in inhibition of an NMD pathway. The resulting mature *OPA1* mRNA can be translated normally without activating NMD pathway, thereby increasing the amount of OPA1 protein in the patient's cells and alleviating symptoms of a condition or disease associated with OPA1 deficiency, such as an eye disease or condition, Optic atrophy type 1, autosomal dominant optic atrophy (ADOA), ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[00198] In some embodiments, the methods and compositions of the present disclosure exploit the alternative splicing of the pre-mRNA transcribed from the *OPA1* gene. In some cases, splicing of a coding exon, *e.g.*, an alternatively spliced exon, *e.g.*, *OPA1* exon 7 (or an exon encoded by genomic region spanning from GRCh38/ hg38: chr3 193626092 to 193626202), can modulate the level of OPA1 protein expressed from the *OPA1* gene. As described herein, the term "*OPA1* exon 7" or grammatically equivalents thereof, is used interchangeably with the term "exon (GRCh38/ hg38: chr3 193626092 to 193626202)" or "an exon encoded by genomic region spanning from GRCh38/ hg38: chr3 193626092 to 193626202." Without wishing to be bound by a certain theory, the presence or absence of an amino acid sequence encoded by exon 7 or exon (GRCh38/ hg38: chr3 193626092 to 193626202) can modulate the stability of the OPA1 protein. For instance, in some cases, the OPA1 protein encoded by a mature mRNA transcript that lacks exon 7 can have fewer proteolytic cleavage sites as compared to an OPA1 protein encoded by a corresponding mature mRNA transcript that has contains exon 7. In some cases, the OPA1 protein an OPA1 protein encoded by a corresponding mature mRNA transcript that has contains encoded by a mature mRNA transcript that lacks exon 7 is a functional protein. The OPA1 protein encoded

by a mature mRNA transcript that lacks exon 7 can be at least partially functional as compared to an OPA1 protein encoded by a corresponding mature mRNA transcript that has contains exon 7. In some cases, the OPA1 protein encoded by a mature mRNA transcript that lacks exon 7 is at least partially functional as compared to a full-length wild-type OPA1 protein. In some cases, increase of OPA1 protein encoded by a mature mRNA transcript that lacks exon 7 in a cell can result in more functional OPA1 protein in the cell, due to the higher stability of the OPA1 protein lacking exon 7 and its at least partial functional equivalence.

[00199] In other embodiments, a coding exon of *OPA1* pre-mRNA other than exon 7 is targeted by an agent disclosed herein, which promotes exclusion of the coding exon other than exon 7. In these other embodiments, the agent that promotes exclusion of the coding exon other than exon 7 increases expression of OPA1 protein encoded by a mature mRNA transcript that lacks the excluded exon.

[00200] Alternative splicing of the *OPA1* pre-mRNA species, *e.g.*, skipping of a coding exon, *e.g.*, an alternatively spliced exon, *e.g.*, exon 7, to produce functional mature OPA1 protein may be induced using an agent such as an ASO that stimulates the exon skipping. Induction of exon skipping may result in modulation of levels of different alternatively spliced mRNA transcripts. The resulting mature *OPA1* mRNA can be translated into different OPA1 proteins, thereby modulating the amount of OPA1 protein in the patient's cells and alleviating symptoms of a condition or disease associated with OPA1 deficiency, such as an eye disease or condition, Optic atrophy type 1, autosomal dominant optic atrophy (ADOA), ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic

neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[00201] In some embodiments, the diseases or conditions that can be treated or ameliorated using the method or composition disclosed herein are not directly associated with the target protein (gene) that the therapeutic agent targets. In some embodiments, a therapeutic agent provided herein can target a protein (gene) that is not directly associated with a disease or condition, but the modulation of expression of the target protein (gene) can treat or ameliorate the disease or condition.

[00202] In some embodiments, the antisense oligomer modulates splicing of a nonsense-mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA in a cell of the subject, wherein the pre-mRNA encodes the OPA1 protein and comprises the NMD exon, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating expression of the OPA1 protein in the cell.

[00203] In some embodiments, the antisense oligomer: (a) binds to a targeted portion of the pre-mRNA; (b) modulates binding of a factor involved in splicing of the NMD exon; or (c) a combination of (a) and (b).

[00204] In some embodiments, the targeted portion of the pre-mRNA is proximal to the NMD exon.

[00205] In various embodiments, the present disclosure provides a therapeutic agent which can target *OPA1* mRNA transcripts to modulate splicing or protein expression level. The therapeutic agent can be a small molecule, polynucleotide, or polypeptide. In some embodiments, the therapeutic agent is an ASO. Various regions or sequences on the *OPA1* pre-mRNA can be targeted by a therapeutic agent, such as an ASO. In some embodiments, the ASO targets an *OPA1* pre-mRNA transcript containing an NMD exon. In some embodiments, the ASO targets a sequence within an NMD exon of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence upstream (or 5') from the 5' end of an NMD exon (3'ss) of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence downstream (or 3') from the 3' end of an NMD exon (5'ss) of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking on the 5' end of the NMD exon of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking the 3' end of the NMD exon of an *OPA1* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising an NMD exon-intron boundary of an *OPA1* pre-mRNA transcript. An NMD exon-intron boundary can refer to the junction of an intron sequence and an NMD exon region. The intron sequence can flank the 5' end of the NMD exon, or the 3' end of the NMD exon. In some embodiments, the ASO targets a sequence within an exon of an *OPA1* pre-mRNA

transcript. In some embodiments, the ASO targets a sequence within an intron of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising both a portion of an intron and a portion of an exon of an *OPAI* pre-mRNA transcript.

[00206] In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the NMD exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides upstream (or 5') from the 5' end of the NMD exon region. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from the 5' end of the NMD exon. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the NMD exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides downstream from the 3' end of the NMD exon. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the NMD exon.

[00207] In some embodiments, the *OPAI* NMD exon-containing pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 1. In some embodiments, the *OPAI* NMD exon pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 2-5.

[00208] In some embodiments, the *OPAI* NMD exon-containing pre-mRNA transcript (or NMD exon mRNA) comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, *OPAI* NMD exon-containing pre-mRNA transcript (or NMD exon mRNA) is encoded by a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, the targeted portion of the NMD exon mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5.

[00209] In some embodiments, the ASO targets exon 6x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 6, exon 7x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 7, or exon 28x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 28. In some embodiments, the ASO targets exon (GRCh38/ hg38: chr3 193628509 193628616) of *OPAI* pre-mRNA; or exon (GRCh38/ hg38: chr3 193603500 193603557) of

OPAI. In some embodiments, the ASO targets an NMD exon of *OPAI* pre-mRNA other than NMD exon (GRCh38/hg38: chr3 193628509 193628616).

[00210] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from GRCh38/ hg38: chr3 193628509 of *OPAI*; or GRCh38/ hg38: chr3 193603500 of *OPAI*.

[00211] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from GRCh38/ hg38: chr3 193628509 of *OPAI*; or GRCh38/ hg38: chr3 193603500 of *OPAI*.

[00212] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193628616 of *OPAI*; or GRCh38/ hg38: chr3 193603557 of *OPAI*.

[00213] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193628616 of *OPAI*; or GRCh38/ hg38: chr3 193603557 of *OPAI*.

[00214] In some embodiments, the ASO has a sequence complementary to the targeted portion of the NMD exon mRNA according to any one of SEQ ID NOs: 2-5, or 279.

[00215] In some embodiments, the ASO targets a sequence upstream from the 5' end of an NMD exon. For example, ASOs targeting a sequence upstream from the 5' end of an NMD exon (exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*) comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence upstream from the 5' end of an NMD exon (e.g., exon (GRCh38/ hg38: chr3 193628509 to 193628616) of *OPAI*; or exon (GRCh38/ hg38: chr3 193603500 193603557) of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5.

[00216] In some embodiments, the ASOs target a sequence containing an exon-intron boundary (or junction). For example, ASOs targeting a sequence containing an exon-intron boundary can comprise a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complimentary to at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5. In some embodiments, the ASOs target a sequence downstream from the 3' end of an NMD exon. For example, ASOs targeting a sequence downstream from the 3' end of an NMD exon (e.g., exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3, or at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence downstream from the 3' end of an NMD exon (e.g., exon (GRCh38/ hg38: chr3 193628509 to 193628616) of *OPAI*; or exon (GRCh38/ hg38: chr3 193603500 to 193603557) of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5, or at least 8 contiguous nucleic acids of SEQ ID NO: 4 or 5. In some embodiments, ASOs target a sequence within an NMD exon.

[00217] In some embodiments, the ASO targets exon 6x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 6, exon 7x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 7, or exon 28x of an *OPAI* NMD exon-containing pre-mRNA comprising NIE exon 28. In some embodiments, the ASO targets a sequence downstream (or 3') from the 5' end of exon 6x, exon 7x, or exon 28x of an *OPAI* pre-mRNA. In some embodiments, the ASO targets a sequence upstream (or 5') from the 3' end of exon 6x, exon 7x, or exon 28x of an *OPAI* pre-mRNA.

[00218] In some embodiments, the targeted portion of the *OPAI* NMD exon-containing pre-mRNA is in intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50. In some embodiments, hybridization of an ASO to the targeted portion of the NMD exon pre-mRNA results in exon skipping of at least one of NMD exon within intron 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50, and subsequently increases *OPAI* protein production. In some embodiments, the targeted portion of the *OPAI* NMD exon-containing pre-mRNA is in intron 6 of *OPAI*, or intron 28 of *OPAI*. In some embodiments, the targeted portion of the *OPAI* NMD exon-containing pre-mRNA is intron (GRCh38/ hg38: chr3 193626203 to 193631611) of *OPAI*; or intron (GRCh38/ hg38: chr3 193593374 to 193614710) of *OPAI*.

[00219] In some embodiments, the methods and compositions of the present disclosure are used to increase the expression of *OPA1* by inducing exon skipping of a pseudo-exon of an *OPAI* NMD exon-containing pre-mRNA. In some embodiments, the pseudo-exon is a sequence within any of introns 1-50. In some embodiments, the pseudo-exon is a sequence within any of introns 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50. In some embodiments, the pseudo-exon can be an *OPAI* intron or a portion thereof. In some embodiments, the pseudo-exon is within intron 6 of *OPAI*, or intron 28 of *OPAI*. In some embodiments, the pseudo-exon is within intron (GRCh38/ hg38: chr3 193626203 to 193631611) of *OPAI*; or intron (GRCh38/ hg38: chr3 193593374 to 193614710) of *OPA1*.

[00220] In some embodiments, the ASO targets an *OPAI* pre-mRNA transcript to induce exon skipping of a coding exon, *e.g.*, an alternatively spliced exon. In some embodiments, the ASO targets a sequence within a coding exon, *e.g.*, an alternatively spliced exon, of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence upstream (or 5') from the 5' end of a coding exon (3'ss) of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence downstream (or 3') from the 3' end of a coding exon (5'ss) of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron

flanking on the 5' end of the coding exon of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence that is within an intron flanking the 3' end of the coding exon of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising an exon-intron boundary of an *OPAI* pre-mRNA transcript. An exon-intron boundary can refer to the junction of an intron sequence and an exon sequence. The intron sequence can flank the 5' end of the coding exon, or the 3' end of the coding exon. In some embodiments, the ASO targets a sequence within an exon of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence within an intron of an *OPAI* pre-mRNA transcript. In some embodiments, the ASO targets a sequence comprising both a portion of an intron and a portion of an exon of an *OPAI* pre-mRNA transcript.

[00221] In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides upstream (or 5') from the 5' end of the coding exon, *e.g.*, alternatively spliced exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides upstream (or 5') from the 5' end of the coding exon region. In some embodiments, the ASO may target a sequence more than 300 nucleotides upstream from the 5' end of the coding exon. In some embodiments, the ASO targets a sequence about 4 to about 300 nucleotides downstream (or 3') from the 3' end of the coding exon. In some embodiments, the ASO targets a sequence about 1 to about 20 nucleotides, about 20 to about 50 nucleotides, about 50 to about 100 nucleotides, about 100 to about 150 nucleotides, about 150 to about 200 nucleotides, about 200 to about 250 nucleotides, or about 250 to about 300 nucleotides downstream from the 3' end of the coding exon. In some embodiments, the ASO targets a sequence more than 300 nucleotides downstream from the 3' end of the coding exon.

[00222] In some embodiments, the *OPAI* pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 1. In some embodiments, the *OPAI* pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 2-5.

[00223] In some embodiments, the *OPAI* pre-mRNA transcript (or NMD exon mRNA) comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, *OPAI* pre-mRNA transcript (or NMD exon mRNA) is encoded by a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to any one of SEQ ID NOs: 2-5. In some embodiments, the targeted portion of the *OPAI* pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100%

sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5.

[00224] In some embodiments, the ASO targets exon 7 of an *OPAI* pre-mRNA, *i.e.*, the ASO targets exon (GRCh38/ hg38: chr3 193626092 to 193626202) of *OPAI* pre-mRNA.

[00225] In some embodiments, the ASO targets a coding exon of an *OPAI* pre-mRNA other than exon 7, *i.e.*, the ASO targets an exon of *OPAI* pre-mRNA other than exon defined by (GRCh38/ hg38: chr3 193626092 to 193626202).

[00226] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from GRCh38/ hg38: chr3 193626092 of *OPAI*.

[00227] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from the 5' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream (or 5') from GRCh38/ hg38: 193626092 of *OPAI*.

[00228] In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70

nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193626202 of *OPAI*.

[00229] In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from the 3' end of exon 7 of *OPAI*. In some embodiments, the ASO targets a sequence at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream (or 3') from GRCh38/ hg38: chr3 193626202 of *OPAI*.

[00230] In some embodiments, the ASO has a sequence complementary to the targeted portion of the NMD exon mRNA according to any one of SEQ ID NOs: 2-5, or 277.

[00231] In some embodiments, the ASO targets a sequence upstream from the 5' end of a coding exon, *e.g.*, an alternatively spliced exon. For example, ASOs targeting a sequence upstream from the 5' end of a coding exon (*e.g.*, exon 7 of *OPAI*) comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence upstream from the 5' end of a coding exon (*e.g.*, exon (GRCh38/ hg38: 193626092 to 193626202) of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5.

[00232] In some embodiments, the ASOs target a sequence containing an exon-intron boundary (or junction). For example, ASOs targeting a sequence containing an exon-intron boundary can comprise a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5. In some embodiments, the ASOs target a sequence downstream from the 3' end of a coding exon, *e.g.*, an alternatively spliced exon. For example, ASOs targeting a sequence downstream from the 3' end of a coding exon (*e.g.*, exon 7 of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3, or at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3. For example, ASOs targeting a sequence downstream from the 3' end of a coding exon (*e.g.*, exon 7 of *OPAI*) can comprise a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5, or at least 8 contiguous nucleic acids of SEQ ID NO: 4 or 5. In some embodiments, ASOs target a sequence within a coding exon, *e.g.*, an alternatively spliced exon.

Protein Expression

[00233] In some embodiments, the methods described herein are used to increase the production of a functional OPA1 protein or RNA. As used herein, the term “functional” refers to the amount of activity or function of an OPA1 protein or RNA that is necessary to eliminate any one or more symptoms of a treated condition or disease, *e.g.*, Optic atrophy type 1. In some embodiments, the methods are used to increase the production of a partially functional OPA1 protein or RNA. As used herein, the term “partially functional” refers to any amount of activity or function of the OPA1 protein or RNA that is less than the amount of activity or function that is necessary to eliminate or prevent any one or more symptoms of a disease or condition. In some embodiments, a partially functional protein or RNA will have at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% less activity relative to the fully functional protein or RNA.

[00234] In some embodiments, the method is a method of increasing the expression of the OPA1 protein by cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a disease or condition, *e.g.*, Optic atrophy type 1, caused by a deficient amount of activity of OPA1 protein, and wherein the deficient amount of the OPA1 protein is caused by haploinsufficiency of the OPA1 protein. In such an embodiment, the subject has a first allele encoding a functional OPA1 protein, and a second allele from which the OPA1 protein is not produced. In another such embodiment, the subject has a first allele encoding a functional OPA1 protein, and a second allele encoding a nonfunctional OPA1 protein. In another such embodiment, the subject has a first allele encoding a functional OPA1 protein, and a second allele encoding a partially functional OPA1 protein. In any of these embodiments, the antisense oligomer binds to a targeted portion of the *OPA1* pre-mRNA transcribed from the second allele, thereby inducing exon skipping of the pseudo-exon from the pre-mRNA, and causing an increase in the level of mature mRNA encoding functional OPA1 protein, and an increase in the expression of the OPA1 protein in the cells of the subject.

[00235] In some embodiments, the method is a method of increasing the expression of the OPA1 protein by cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a disease or condition caused by a deficient amount of activity of OPA1 protein, and wherein the deficient amount of the OPA1 protein is caused by autosomal recessive inheritance.

[00236] In some embodiments, the method is a method of increasing the expression of the OPA1 protein by cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a disease or condition, *e.g.*, Optic atrophy type 1, caused by a deficient amount of activity of OPA1 protein, and wherein the deficient amount of the OPA1 protein is caused by autosomal dominant inheritance.

[00237] In related embodiments, the method is a method of using an ASO to increase the expression of a protein or functional RNA. In some embodiments, an ASO may be used to increase the expression of OPA1 protein in cells of a subject having an *OPA1* pre-mRNA, wherein the subject has a deficiency, *e.g.*, Optic atrophy type 1; in the amount or function of an OPA1 protein.

[00238] In some embodiments, the pre-mRNA transcript that encodes the protein that is causative of the disease or condition is targeted by the agent, *e.g.*, the oligonucleotides, described herein. In some cases, it is the NMD exon-containing pre-mRNA transcript targeted by the agent, *e.g.*, the oligonucleotides, described herein. In some cases, the agent, *e.g.*, the oligonucleotides, described herein, are designed to target a coding exon of the pre-mRNA. In some cases, the agent, *e.g.*, the oligonucleotides, described herein can induce skipping of the NMD exon, a coding exon, or both. In some embodiments, a NMD exon-containing pre-mRNA transcript that encodes a protein that is not causative of the disease is targeted by the ASOs. For example, a disease that is the result of a mutation or deficiency of a first protein in a particular pathway may be ameliorated by targeting a pre-mRNA that encodes a second protein, thereby increasing production of the second protein. In some embodiments, the function of the second protein is able to compensate for the mutation or deficiency of the first protein (which is causative of the disease or condition).

[00239] In some embodiments, the subject has:

- (a) a first mutant allele from which
 - (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the OPA1 protein or functional RNA is not produced; and
- (b) a second mutant allele from which
 - (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the OPA1 protein is not produced, and

wherein the NMD exon-containing pre-mRNA is transcribed from the first allele and/or the second allele. In these embodiments, the ASO binds to a targeted portion of the NMD exon-containing pre-mRNA transcribed from the first allele or the second allele, thereby inducing exon skipping of the pseudo-exon from the NMD exon-containing pre-mRNA, and causing an increase in the level of mRNA encoding OPA1 protein and an increase in the expression of the target protein or functional RNA in the cells of the subject. In these

embodiments, the target protein or functional RNA having an increase in expression level resulting from the exon skipping of the pseudo-exon from the NMD exon-containing pre-mRNA may be either in a form having reduced function compared to the equivalent wild-type protein (partially-functional), or having full function compared to the equivalent wild-type protein (fully-functional).

[00240] In some embodiments, the subject has:

- (a) a first mutant allele from which
 - (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the OPA1 protein or functional RNA is not produced; and
- (b) a second mutant allele from which
 - (i) the OPA1 protein is produced at a reduced level compared to production from a wild-type allele,
 - (ii) the OPA1 protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
 - (iii) the OPA1 protein is not produced, and

wherein the *OPA1* pre-mRNA is transcribed from the first allele and/or the second allele. In these embodiments, the ASO binds to a targeted portion of the *OPA1* pre-mRNA transcribed from the first allele or the second allele, thereby inducing exon skipping of a coding exon from the *OPA1* pre-mRNA, and causing an increase in the expression of the target OPA1 protein in the cells of the subject. In these embodiments, the target OPA1 protein having an increase in expression level resulting from the exon skipping of the coding exon from the *OPA1* pre-mRNA may be either in a form having reduced function compared to the equivalent full-length wild-type protein (partially-functional), or having full function compared to the equivalent full-length wild-type protein (fully-functional).

[00241] In some embodiments, the level of mRNA encoding OPA1 protein is increased 1.1 to 10-fold, when compared to the amount of mRNA encoding OPA1 protein that is produced in a control cell, *e.g.*, one that is not treated with the antisense oligomer or one that is treated with an antisense oligomer that does not bind to the targeted portion of the *OPA1* pre-mRNA.

[00242] In some embodiments, a subject treated using the methods of the present disclosure expresses a partially functional OPA1 protein from one allele, wherein the partially functional OPA1 protein may be caused by a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion. In some embodiments, a subject treated using the methods of the

disclosure expresses a nonfunctional OPA1 protein from one allele, wherein the nonfunctional OPA1 protein may be caused by a frameshift mutation, a nonsense mutation, a missense mutation, a partial gene deletion, in one allele. In some embodiments, a subject treated using the methods of the disclosure has an OPA1 whole gene deletion, in one allele.

Exon Inclusion

[00243] As used herein, a “NMD exon-containing pre-mRNA” is a pre-mRNA transcript that contains at least one pseudo-exon. Alternative or aberrant splicing can result in inclusion of the at least one pseudo-exon in the mature mRNA transcripts. The terms “mature mRNA,” and “fully-spliced mRNA,” are used interchangeably herein to describe a fully processed mRNA. Inclusion of the at least one pseudo-exon can be nonproductive mRNA and lead to NMD of the mature mRNA. NMD exon-containing mature mRNA may sometimes lead to aberrant protein expression.

[00244] In some embodiments, the included pseudo-exon is the most abundant pseudo-exon in a population of NMD exon-containing pre-mRNAs transcribed from the gene encoding the target protein in a cell. In some embodiments, the included pseudo-exon is the most abundant pseudo-exon in a population of NMD exon-containing pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of NMD exon-containing pre-mRNAs comprises two or more included pseudo-exons. In some embodiments, an antisense oligomer targeted to the most abundant pseudo-exon in the population of NMD exon-containing pre-mRNAs encoding the target protein induces exon skipping of one or two or more pseudo-exons in the population, including the pseudo-exon to which the antisense oligomer is targeted or binds. In some embodiments, the targeted region is in a pseudo-exon that is the most abundant pseudo-exon in a NMD exon-containing pre-mRNA encoding the OPA1 protein.

[00245] The degree of exon inclusion can be expressed as percent exon inclusion, *e.g.*, the percentage of transcripts in which a given pseudo-exon is included. In brief, percent exon inclusion can be calculated as the percentage of the amount of RNA transcripts with the exon inclusion, over the sum of the average of the amount of RNA transcripts with exon inclusion plus the average of the amount of RNA transcripts with exon exclusion.

[00246] In some embodiments, an included pseudo-exon is an exon that is identified as an included pseudo-exon based on a determination of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%, inclusion. In embodiments, a included pseudo-exon is an exon that is identified as a included pseudo-exon based on a determination of about 5% to about 100%, about 5% to about 95%, about 5% to about 90%, about 5% to about 85%, about 5% to about 80%, about 5% to about 75%, about 5% to about 70%, about 5% to about

65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 10% to about 100%, about 10% to about 95%, about 10% to about 90%, about 10% to about 85%, about 10% to about 80%, about 10% to about 75%, about 10% to about 70%, about 10% to about 65%, about 10% to about 60%, about 10% to about 55%, about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 10% to about 20%, about 15% to about 100%, about 15% to about 95%, about 15% to about 90%, about 15% to about 85%, about 15% to about 80%, about 15% to about 75%, about 15% to about 70%, about 15% to about 65%, about 15% to about 60%, about 15% to about 55%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 20% to about 100%, about 20% to about 95%, about 20% to about 90%, about 20% to about 85%, about 20% to about 80%, about 20% to about 75%, about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 25% to about 100%, about 25% to about 95%, about 25% to about 90%, about 25% to about 85%, about 25% to about 80%, about 25% to about 75%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, or about 25% to about 35%, inclusion. ENCODE data (described by, *e.g.*, Tilgner, *et al.*, 2012, “Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs,” *Genome Research* 22(9):1616-25) can be used to aid in identifying exon inclusion.

[00247] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of an *OPAI* pre-mRNA transcript results in an increase in the amount of OPA1 protein produced by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of OPA1 protein produced by the cell to which the antisense oligomer is contacted is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least

about 150%, at least about 200%, at least about 250%, or at least about 300%, compared to the amount of target protein produced by a control compound. In some embodiments, the total amount of OPA1 protein produced by the cell to which the antisense oligomer is contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the amount of target protein produced by a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to a targeted portion of the pre-mRNA.

[00248] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of an *OPA1* pre-mRNA transcript results in an increase in the amount of mRNA encoding OPA1, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding OPA1 protein, or the mature mRNA encoding the OPA1 protein, is increased by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In some embodiments, the total amount of the mRNA encoding OPA1 protein, or the mature mRNA encoding OPA1 protein produced in the cell to which the antisense oligomer is contacted is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300%, compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. In some embodiments, the total amount of the mRNA encoding OPA1 protein, or the mature mRNA encoding OPA1 protein produced in the cell to which the antisense oligomer is contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-

fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to a targeted portion of the OPA1 NMD exon-containing pre-mRNA.

[00249] The NMD exon can be in any length. In some embodiments, the NMD exon comprises a full sequence of an intron, in which case, it can be referred to as intron retention. In some embodiments, the NMD exon can be a portion of the intron. In some embodiments, the NMD exon can be a 5' end portion of an intron including a 5'ss sequence. In some embodiments, the NMD exon can be a 3' end portion of an intron including a 3'ss sequence. In some embodiments, the NMD exon can be a portion within an intron without inclusion of a 5'ss sequence. In some embodiments, the NMD exon can be a portion within an intron without inclusion of a 3'ss sequence. In some embodiments, the NMD exon can be a portion within an intron without inclusion of either a 5'ss or a 3'ss sequence. In some embodiments, the NMD exon can be from 5 nucleotides to 10 nucleotides in length, from 10 nucleotides to 15 nucleotides in length, from 15 nucleotides to 20 nucleotides in length, from 20 nucleotides to 25 nucleotides in length, from 25 nucleotides to 30 nucleotides in length, from 30 nucleotides to 35 nucleotides in length, from 35 nucleotides to 40 nucleotides in length, from 40 nucleotides to 45 nucleotides in length, from 45 nucleotides to 50 nucleotides in length, from 50 nucleotides to 55 nucleotides in length, from 55 nucleotides to 60 nucleotides in length, from 60 nucleotides to 65 nucleotides in length, from 65 nucleotides to 70 nucleotides in length, from 70 nucleotides to 75 nucleotides in length, from 75 nucleotides to 80 nucleotides in length, from 80 nucleotides to 85 nucleotides in length, from 85 nucleotides to 90 nucleotides in length, from 90 nucleotides to 95 nucleotides in length, or from 95 nucleotides to 100 nucleotides in length. In some embodiments, the NMD exon can be at least 10 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides in length, at least 90 nucleotides, or at least 100 nucleotides in length. In some embodiments, the NMD exon can be from 100 to 200 nucleotides in length, from 200 to 300 nucleotides in length, from 300 to 400 nucleotides in length, from 400 to 500 nucleotides in length, from 500 to 600 nucleotides in length, from 600 to 700 nucleotides in length, from 700 to 800 nucleotides in length, from 800 to 900 nucleotides in length, from 900 to 1,000 nucleotides in length. In some embodiments, the NMD exon may be longer than 1,000 nucleotides in length.

[00250] Inclusion of a pseudo-exon can lead to a frameshift and the introduction of a premature termination codon (PTC) in the mature mRNA transcript rendering the transcript a target of NMD. Mature mRNA transcript containing NMD exon can be nonproductive mRNA transcript which does not lead to protein expression. The PTC can be present in any position downstream of an NMD exon. In some embodiments, the PTC can be present in any exon downstream of an NMD exon. In some embodiments, the PTC can be present within the NMD exon. For example, inclusion of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*, in an mRNA transcript encoded by the *OPAI* gene can induce a PTC in the mRNA transcript. For example, inclusion of exon (GRCh38/ hg38: chr3 193628509 193628616) of *OPAI*; or exon (GRCh38/ hg38: chr3 193603500 193603557) of *OPAI* in an mRNA transcript encoded by the *OPAI*.

[00251] In some aspects, provided herein is a method of modulating expression of an *OPAI* protein by promoting inclusion of a coding exon. The method can comprise contacting an agent to a cell having an *OPAI* pre-mRNA, wherein the agent comprises an oligonucleotide that binds to: (a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or (b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA; whereby the agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell. In some cases, the coding exon to be included is an alternatively spliced exon. In some cases, the method promotes inclusion of the coding exon in the processed mRNA during splicing of the pre-mRNA in the cell.

[00252] In some of these embodiments for inclusion of coding exon, the target portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of a 5' end of the coding exon. In some cases, the target portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of a 3' end of the coding exon. In some cases, the coding exon is exon 7 of *OPAI*. In some cases, the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277. In some cases, the coding exon comprises SEQ ID NO: 277. The targeted portion of the pre-mRNA can be within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.

[00253] In some cases, the inclusion of the coding exon in the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

Exclusion of Both NMD Exon and Coding Exon

[00254] In some embodiments, provided herein is a method of modulating expression of a target protein by targeting a pre-mRNA and modulating exclusion of both a coding exon and a nonsense-mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA. In some cases, the method comprises contacting an agent to the cell, and the agent promotes exclusion of both the coding exon and the NMD exon from the pre-mRNA, thereby increasing level of a processed mRNA that is processed from the pre-mRNA and lacks both the coding exon and the NMD exon. In some cases, the agent binds to a targeted portion of the pre-mRNA, or modulates binding of a factor involved in splicing of the coding exon, the NMD exon, or both. In some cases, the agent interferes with binding of the factor involved in splicing of the coding exon, the NMD exon, or both, to a region of the targeted portion. In some cases, the NMD exon is within an intronic region adjacent to the coding exon. In some cases, the NMD exon is within an intronic region immediately upstream of the coding exon. In some cases, the NMD exon is within an intronic region immediately downstream of the coding exon. In some cases, the coding exon is an alternatively spliced exon.

[00255] In some cases, the targeted portion of the pre-mRNA is proximal to the coding exon. The targeted portion of the pre-mRNA can be located in an intronic region immediately upstream of the coding exon. The targeted portion of the pre-mRNA can be located in an intronic region immediately downstream of the coding exon. In some cases, the targeted portion of the pre-mRNA can be located within the coding exon. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the coding exon to 100 nucleotides downstream of the coding exon. In some cases, the targeted portion comprises about

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.

[00256] In some cases, the targeted portion of the pre-mRNA is proximal to the NMD exon. In some cases, the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the NMD exon. In some cases, the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the NMD exon. In some cases, the targeted portion of the pre-mRNA is located within the NMD exon. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the NMD exon to 100 nucleotides downstream of the NMD exon.

[00257] In some embodiments, the method described herein is applicable to modulation of expression of OPA1 protein by modulating exclusion of both exon 7 and an NMD exon (*e.g.*, exon 7x) of *OPA1* pre-mRNA that contains both exon 7 and exon 7x. In some cases, the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277. In some cases, the coding exon comprises SEQ ID NO: 277. In some cases, the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of GRCh38/ hg38: chr3 193626092. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. In some cases, the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the targeted portion of the pre-mRNA comprises an exon-intron junction of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202. In some cases, the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279. In some cases, the NMD exon comprises SEQ ID NO: 279. In some cases, the targeted portion of the pre-mRNA is immediately upstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some cases, the targeted portion of the pre-mRNA is immediately downstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some cases, the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[00258] In some cases, the targeted portion of the pre-mRNA is within the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616. In some cases, the targeted portion of the pre-mRNA comprises an exon-intron junction of the NMD exon GRCh38/ hg38: chr3 193628509 to

193628616. In some cases, the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[00259] In some cases, the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent. In some cases, the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent. In some cases, the method results in an increase in the level of the processed mRNA in the cell. The level of the processed mRNA in the cell contacted with the agent can be increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent.

[00260] In some cases, the method results in an increase in expression of the OPA1 protein in the cell. A level of the OPA1 protein expressed from the processed mRNA in the cell contacted

with the agent can be increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of contacting with the agent.

[00261] In some cases, a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of contacting with the agent.

[00262] In some cases, the OPA1 protein expressed from the processed mRNA that lacks exon 7 and exon 7x is a functional OPA1 protein. The OPA1 protein expressed from the processed mRNA that lacks exon 7 and exon 7x can be at least partially functional as compared to a wild-type OPA1 protein. The OPA1 protein expressed from the processed mRNA that lacks exon 7 and exon 7x can be at least partially functional as compared to a full-length wild-type OPA1 protein.

Therapeutic Agents

[00263] In various embodiments of the present disclosure, compositions and methods comprising a therapeutic agent are provided to modulate protein expression level of OPA1. In some embodiments, provided herein are compositions and methods to modulate alternative splicing of *OPA1* pre-mRNA. In some embodiments, provided herein are compositions and methods to induce exon skipping in the splicing of *OPA1* pre-mRNA, *e.g.*, to induce skipping of a pseudo-exon during splicing of *OPA1* pre-mRNA. In other embodiments, therapeutic agents may be used to induce the inclusion of an exon in order to decrease the protein expression level.

[00264] A therapeutic agent disclosed herein can be a NIE repressor agent. A therapeutic agent may comprise a polynucleic acid polymer.

[00265] According to one aspect of the present disclosure, provided herein is a method of treatment or prevention of a condition or disease associated with a functional OPA1 protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of the pre-mRNA transcript to decrease inclusion of the NMD exon in the mature transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of an intron containing an NMD

exon (*e.g.*, exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*) of the pre-mRNA transcript or to a NMD exon-activating regulatory sequence in the same intron. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of an intron containing an NMD exon (*e.g.*, exon (GRCh38/ hg38: chr3 193628509 193628616) of *OPAI*; or exon (GRCh38/ hg38: chr3 193603500 193603557) of *OPAI*) of the pre-mRNA transcript or to a NMD exon-activating regulatory sequence in the same intron. In some embodiments, the method comprises administering a NIE repressor agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of an intron containing an NMD exon (*e.g.*, exon of *OPAI* other than exon 7x defined by (GRCh38/ hg38: chr3 193628509 193628616) or exon defined by (GRCh38/ hg38: chr3 193603500 193603557)) of the pre-mRNA transcript or to a NMD exon-activating regulatory sequence in the same intron. In some embodiments, the therapeutic agent promotes exclusion of an NMD exon of *OPAI* pre-mRNA other than exon 7x defined by (GRCh38/ hg38: chr3 193628509 193628616) or exon defined by (GRCh38/ hg38: chr3 193603500 193603557). In some embodiments, the composition disclosed herein includes an agent that promotes exclusion of an NMD exon of *OPAI* pre-mRNA other than exon 7x defined by (GRCh38/ hg38: chr3 193628509 193628616) or exon defined by (GRCh38/ hg38: chr3 193603500 193603557).

[00266] Where reference is made to reducing NMD exon inclusion in the mature mRNA, the reduction may be complete, *e.g.*, 100%, or may be partial. The reduction may be clinically significant. The reduction/correction may be relative to the level of NMD exon inclusion in the subject without treatment, or relative to the amount of NMD exon inclusion in a population of similar subjects. The reduction/correction may be at least 10% less NMD exon inclusion relative to the average subject, or the subject prior to treatment. The reduction may be at least 20% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 40% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 50% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 60% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 80% less NMD exon inclusion relative to an average subject, or the subject prior to treatment. The reduction may be at least 90% less NMD exon inclusion relative to an average subject, or the subject prior to treatment.

[00267] According to one aspect of the present disclosure, provided herein is a method of treatment or prevention of a condition or disease associated with a functional OPA1 protein

deficiency, comprising administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region of the pre-mRNA transcript to decrease inclusion of a coding exon (*e.g.*, exon 7) in the mature transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region containing a coding exon (*e.g.*, exon 7 of *OPA1*) of the pre-mRNA transcript. For example, provided herein is a method of treatment or prevention of a condition associated with a functional OPA1 protein deficiency, comprising administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region containing a coding exon (*e.g.*, exon (GRCh38/ hg38: chr3 193626092 to 193626202) of *OPA1*) of the pre-mRNA transcript. In some embodiments, the method comprises administering an agent to a subject to increase levels of functional OPA1 protein, wherein the agent binds to a region containing a coding exon (*e.g.*, exon of *OPA1* other than exon 7 defined by (GRCh38/ hg38: chr3 193626092 to 193626202)) of the pre-mRNA transcript. In some embodiments, the therapeutic agent promotes exclusion of a coding exon of *OPA1* pre-mRNA other than exon 7 defined by (GRCh38/ hg38: chr3 193626092 to 193626202). In some embodiments, the composition disclosed herein includes an agent that promotes exclusion of a coding exon of *OPA1* pre-mRNA other than exon 7 defined by (GRCh38/ hg38: chr3 193626092 to 193626202).

[00268] Where reference is made to increasing active OPA1 protein levels, the increase may be clinically significant. The increase may be relative to the level of active OPA1 protein in the subject without treatment, or relative to the amount of active OPA1 protein in a population of similar subjects. The increase may be at least 10% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 20% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 40% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 50% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 80% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 100% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 200% more active OPA1 protein relative to the average subject, or the subject prior to treatment. The increase may be at least 500% more active OPA1 protein relative to the average subject, or the subject prior to treatment.

[00269] In embodiments wherein the NIE repressor agent comprises a polynucleic acid polymer, the polynucleic acid polymer may be about 50 nucleotides in length. The polynucleic acid polymer may be about 45 nucleotides in length. The polynucleic acid polymer may be about

40 nucleotides in length. The polynucleic acid polymer may be about 35 nucleotides in length. The polynucleic acid polymer may be about 30 nucleotides in length. The polynucleic acid polymer may be about 24 nucleotides in length. The polynucleic acid polymer may be about 25 nucleotides in length. The polynucleic acid polymer may be about 20 nucleotides in length. The polynucleic acid polymer may be about 19 nucleotides in length. The polynucleic acid polymer may be about 18 nucleotides in length. The polynucleic acid polymer may be about 17 nucleotides in length. The polynucleic acid polymer may be about 16 nucleotides in length. The polynucleic acid polymer may be about 15 nucleotides in length. The polynucleic acid polymer may be about 14 nucleotides in length. The polynucleic acid polymer may be about 13 nucleotides in length. The polynucleic acid polymer may be about 12 nucleotides in length. The polynucleic acid polymer may be about 11 nucleotides in length. The polynucleic acid polymer may be about 10 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 50 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 45 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 40 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 35 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 30 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 25 nucleotides in length. The polynucleic acid polymer may be between about 10 and about 20 nucleotides in length. The polynucleic acid polymer may be between about 15 and about 25 nucleotides in length. The polynucleic acid polymer may be between about 15 and about 30 nucleotides in length. The polynucleic acid polymer may be between about 12 and about 30 nucleotides in length.

[00270] The sequence of the polynucleic acid polymer may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% complementary to a target sequence of an mRNA transcript, *e.g.*, a partially processed mRNA transcript. The sequence of the polynucleic acid polymer may be 100% complementary to a target sequence of a pre-mRNA transcript.

[00271] The sequence of the polynucleic acid polymer may have 4 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 3 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 2 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have 1 or fewer mismatches to a target sequence of the pre-mRNA transcript. The sequence of the polynucleic acid polymer may have no mismatches to a target sequence of the pre-mRNA transcript.

[00272] The polynucleic acid polymer may specifically hybridize to a target sequence of the pre-mRNA transcript. For example, the polynucleic acid polymer may have 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 100% sequence complementarity to a target sequence of the pre-mRNA transcript. The hybridization may be under high stringent hybridization conditions.

[00273] The polynucleic acid polymer comprising a sequence with at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2-5. The polynucleic acid polymer may comprise a sequence with 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2-5.

[00274] Where reference is made to a polynucleic acid polymer sequence, the skilled person will understand that one or more substitutions may be tolerated, optionally two substitutions may be tolerated in the sequence, such that it maintains the ability to hybridize to the target sequence; or where the substitution is in a target sequence, the ability to be recognized as the target sequence. References to sequence identity may be determined by BLAST sequence alignment using standard/default parameters. For example, the sequence may have 99% identity and still function according to the present disclosure. In other embodiments, the sequence may have 98% identity and still function according to the present disclosure. In another embodiment, the sequence may have 95% identity and still function according to the present disclosure. In another embodiment, the sequence may have 90% identity and still function according to the present disclosure.

Antisense Oligomers

[00275] Provided herein is a composition comprising an antisense oligomer that induces exon skipping by binding to a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA. As used herein, the terms “ASO” and “antisense oligomer” are used interchangeably and refer to an oligomer such as a polynucleotide, comprising nucleobases that hybridizes to a target nucleic acid (*e.g.*, an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA) sequence by Watson-Crick base pairing or wobble base pairing (G-U). The ASO may have exact sequence complementary to the target sequence or near complementarity (*e.g.*, sufficient complementarity to bind the target sequence and enhancing splicing at a splice site). ASOs are designed so that they bind (hybridize) to a target nucleic acid (*e.g.*, a targeted portion of a pre-mRNA transcript) and remain hybridized under physiological conditions. Typically, if they hybridize to a site other than the intended (targeted) nucleic acid sequence, they hybridize to a limited number of sequences that are not a target nucleic acid (to a few sites other than a target nucleic acid). Design of an ASO can take into consideration the occurrence of the nucleic acid sequence of the targeted portion of the pre-mRNA transcript or a sufficiently similar nucleic acid sequence in other locations in the genome or cellular pre-mRNA or transcriptome, such that the likelihood the ASO will bind other sites and cause “off-target” effects is limited. Any antisense

oligomers known in the art (for example, in PCT Application No. PCT/US2014/054151, published as WO 2015/035091, titled “Reducing Nonsense-Mediated mRNA Decay,” incorporated by reference herein), can be used to practice the methods described herein.

[00276] In some embodiments, ASOs “specifically hybridize” to or are “specific” to a target nucleic acid or a targeted portion of an *OPAI* pre-mRNA, *e.g.*, a NMD exon-containing pre-mRNA. Typically, such hybridization occurs with a T_m substantially greater than 37 °C, preferably at least 50 °C, and typically between 60 °C to approximately 90 °C. Such hybridization preferably corresponds to stringent hybridization conditions. At a given ionic strength and pH, the T_m is the temperature at which 50% of a target sequence hybridizes to a complementary oligonucleotide.

[00277] Oligomers, such as oligonucleotides, are “complementary” to one another when hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides. A double-stranded polynucleotide can be “complementary” to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. Complementarity (the degree to which one polynucleotide is complementary with another) is quantifiable in terms of the proportion (*e.g.*, the percentage) of bases in opposing strands that are expected to form hydrogen bonds with each other, according to generally accepted base-pairing rules. The sequence of an antisense oligomer (ASO) need not be 100% complementary to that of its target nucleic acid to hybridize. In certain embodiments, ASOs can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an ASO in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleobases may be clustered together or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. Percent complementarity of an ASO with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul, *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656).

[00278] An ASO need not hybridize to all nucleobases in a target sequence and the nucleobases to which it does hybridize may be contiguous or noncontiguous. ASOs may hybridize over one or more segments of a pre-mRNA transcript, such that intervening or adjacent segments are not involved in the hybridization event (*e.g.*, a loop structure or hairpin structure may be formed). In certain embodiments, an ASO hybridizes to noncontiguous nucleobases in a target pre-

mRNA transcript. For example, an ASO can hybridize to nucleobases in a pre-mRNA transcript that are separated by one or more nucleobase(s) to which the ASO does not hybridize.

[00279] The ASOs described herein comprise nucleobases that are complementary to nucleobases present in a target portion of an *OPAI* pre-mRNA, *e.g.*, a NMD exon-containing pre-mRNA. The term ASO embodies oligonucleotides and any other oligomeric molecule that comprises nucleobases capable of hybridizing to a complementary nucleobase on a target mRNA but does not comprise a sugar moiety, such as a peptide nucleic acid (PNA). The ASOs may comprise naturally-occurring nucleotides, nucleotide analogs, modified nucleotides, or any combination of two or three of the preceding. The term “naturally occurring nucleotides” includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” includes nucleotides with modified or substituted sugar groups and/or having a modified backbone. In some embodiments, all of the nucleotides of the ASO are modified nucleotides. Chemical modifications of ASOs or components of ASOs that are compatible with the methods and compositions described herein will be evident to one of skill in the art and can be found, for example, in U.S. Patent No. 8,258,109 B2, U.S. Patent No. 5,656,612, U.S. Patent Publication No. 2012/0190728, and Dias and Stein, *Mol. Cancer Ther.* 2002, 347-355, herein incorporated by reference in their entirety.

[00280] One or more nucleobases of an ASO may be any naturally occurring, unmodified nucleobase such as adenine, guanine, cytosine, thymine and uracil, or any synthetic or modified nucleobase that is sufficiently similar to an unmodified nucleobase such that it is capable of hydrogen bonding with a nucleobase present on a target pre-mRNA. Examples of modified nucleobases include, without limitation, hypoxanthine, xanthine, 7-methylguanine, 5, 6-dihydrouracil, 5-methylcytosine, and 5-hydroxymethylcytosine.

[00281] The ASOs described herein also comprise a backbone structure that connects the components of an oligomer. The term “backbone structure” and “oligomer linkages” may be used interchangeably and refer to the connection between monomers of the ASO. In naturally occurring oligonucleotides, the backbone comprises a 3'-5' phosphodiester linkage connecting sugar moieties of the oligomer. The backbone structure or oligomer linkages of the ASOs described herein may include, but are not limited to, phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoramidate, and the like. See, *e.g.*, LaPlanche, *et al.*, *Nucleic Acids Res.* 14:9081 (1986); Stec, *et al.*, *J. Am. Chem. Soc.* 106:6077 (1984), Stein, *et al.*, *Nucleic Acids Res.* 16:3209 (1988), Zon, *et al.*, *Anti-Cancer Drug Design* 6:539 (1991); Zon, *et al.*, *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec, *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews* 90:543 (1990). In some embodiments, the backbone structure of the ASO does not contain phosphorous

but rather contains peptide bonds, for example, in a peptide nucleic acid (PNA), or linking groups including carbamate, amides, and linear and cyclic hydrocarbon groups. In some embodiments, the backbone modification is a phosphorothioate linkage. In some embodiments, the backbone modification is a phosphoramidate linkage.

[00282] In some embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is random. In some embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is controlled and is not random. For example, U.S. Pat. App. Pub. No. 2014/0194610, “Methods for the Synthesis of Functionalized Nucleic Acids,” incorporated herein by reference, describes methods for independently selecting the handedness of chirality at each phosphorous atom in a nucleic acid oligomer. In some embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein in Tables 5 and 6, comprises an ASO having phosphorus internucleotide linkages that are not random. In some embodiments, a composition used in the methods of the disclosure comprises a pure diastereomeric ASO. In some embodiments, a composition used in the methods of the disclosure comprises an ASO that has diastereomeric purity of at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, about 90% to about 100%, about 91% to about 100%, about 92% to about 100%, about 93% to about 100%, about 94% to about 100%, about 95% to about 100%, about 96% to about 100%, about 97% to about 100%, about 98% to about 100%, or about 99% to about 100%.

[00283] In some embodiments, the ASO has a nonrandom mixture of Rp and Sp configurations at its phosphorus internucleotide linkages. For example, it has been suggested that a mix of Rp and Sp is required in antisense oligonucleotides to achieve a balance between good activity and nuclease stability (Wan, *et al.*, 2014, “Synthesis, biophysical properties and biological activity of second-generation antisense oligonucleotides containing chiral phosphorothioate linkages,” *Nucleic Acids Research* 42(22): 13456-13468, incorporated herein by reference). In some embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein in SEQ ID NOs: 2-5, comprises about 5-100% Rp, at least about 5% Rp, at least about 10% Rp, at least about 15% Rp, at least about 20% Rp, at least about 25% Rp, at least about 30% Rp, at least about 35% Rp, at least about 40% Rp, at least about 45% Rp, at least about 50% Rp, at least about 55% Rp, at least about 60% Rp, at least about 65% Rp, at least about 70% Rp, at least about 75% Rp, at least about 80% Rp, at least about 85% Rp, at least about 90% Rp, or at least about 95% Rp, with the remainder Sp, or about 100% Rp. In some embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of

the ASOs set forth herein comprise a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5, comprises about 10% to about 100% Rp, about 15% to about 100% Rp, about 20% to about 100% Rp, about 25% to about 100% Rp, about 30% to about 100% Rp, about 35% to about 100% Rp, about 40% to about 100% Rp, about 45% to about 100% Rp, about 50% to about 100% Rp, about 55% to about 100% Rp, about 60% to about 100% Rp, about 65% to about 100% Rp, about 70% to about 100% Rp, about 75% to about 100% Rp, about 80% to about 100% Rp, about 85% to about 100% Rp, about 90% to about 100% Rp, or about 95% to about 100% Rp, about 20% to about 80% Rp, about 25% to about 75% Rp, about 30% to about 70% Rp, about 40% to about 60% Rp, or about 45% to about 55% Rp, with the remainder Sp.

[00284] In some embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein comprise a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5, comprises about 5-100% Sp, at least about 5% Sp, at least about 10% Sp, at least about 15% Sp, at least about 20% Sp, at least about 25% Sp, at least about 30% Sp, at least about 35% Sp, at least about 40% Sp, at least about 45% Sp, at least about 50% Sp, at least about 55% Sp, at least about 60% Sp, at least about 65% Sp, at least about 70% Sp, at least about 75% Sp, at least about 80% Sp, at least about 85% Sp, at least about 90% Sp, or at least about 95% Sp, with the remainder Rp, or about 100% Sp. In embodiments, an ASO used in the methods of the disclosure, including, but not limited to, any of the ASOs set forth herein comprise a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of any one of SEQ ID NOs: 2-5, comprises about 10% to about 100% Sp, about 15% to about 100% Sp, about 20% to about 100% Sp, about 25% to about 100% Sp, about 30% to about 100% Sp, about 35% to about 100% Sp, about 40% to about 100% Sp, about 45% to about 100% Sp, about 50% to about 100% Sp, about 55% to about 100% Sp, about 60% to about 100% Sp, about 65% to about 100% Sp, about 70% to about 100% Sp, about 75% to about 100% Sp, about 80% to about 100% Sp, about 85% to about 100% Sp, about 90% to about 100% Sp, or about 95% to about 100% Sp, about 20% to about 80% Sp, about 25% to about 75% Sp, about 30% to about 70% Sp, about 40% to about 60% Sp, or about 45% to about 55% Sp, with the remainder Rp.

[00285] Any of the ASOs described herein may contain a sugar moiety that comprises ribose or deoxyribose, as present in naturally occurring nucleotides, or a modified sugar moiety or sugar analog, including a morpholine ring. Non-limiting examples of modified sugar moieties include 2' substitutions such as 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'MOE), 2'-O-aminoethyl, 2'F; N3'->P5' phosphoramidate, 2'-dimethylaminooxyethoxy,

2'-dimethylaminoethoxyethoxy, 2'-guanidinidinium, 2'-O-guanidinium ethyl, carbamate modified sugars, and bicyclic modified sugars. In some embodiments, the sugar moiety modification is selected from 2'-O-Me, 2'-F, and 2'-MOE. In some embodiments, the sugar moiety modification is an extra bridge bond, such as in a locked nucleic acid (LNA). In some embodiments the sugar analog contains a morpholine ring, such as phosphorodiamidate morpholino (PMO). In some embodiments, the sugar moiety comprises a ribofuranosyl or 2'-deoxyribofuranosyl modification. In some embodiments, the sugar moiety comprises 2',4'-constrained 2'-O-methoxyethyl (cMOE) modifications. In some embodiments, the sugar moiety comprises cEt 2',4' constrained 2'-O ethyl BNA modifications. In some embodiments, the sugar moiety comprises tricycloDNA (tcDNA) modifications. In some embodiments, the sugar moiety comprises ethylene nucleic acid (ENA) modifications. In some embodiments, the sugar moiety comprises MCE modifications. Modifications are known in the art and described in the literature, *e.g.*, by Jarver, *et al.*, 2014, "A Chemical View of Oligonucleotides for Exon Skipping and Related Drug Applications," Nucleic Acid Therapeutics 24(1): 37-47, incorporated by reference for this purpose herein.

[00286] In some embodiments, each monomer of the ASO is modified in the same way, for example each linkage of the backbone of the ASO comprises a phosphorothioate linkage or each ribose sugar moiety comprises a 2'-O-methyl modification. Such modifications that are present on each of the monomer components of an ASO are referred to as "uniform modifications." In some examples, a combination of different modifications may be desired, for example, an ASO may comprise a combination of phosphorodiamidate linkages and sugar moieties comprising morpholine rings (morpholinos). Combinations of different modifications to an ASO are referred to as "mixed modifications" or "mixed chemistries."

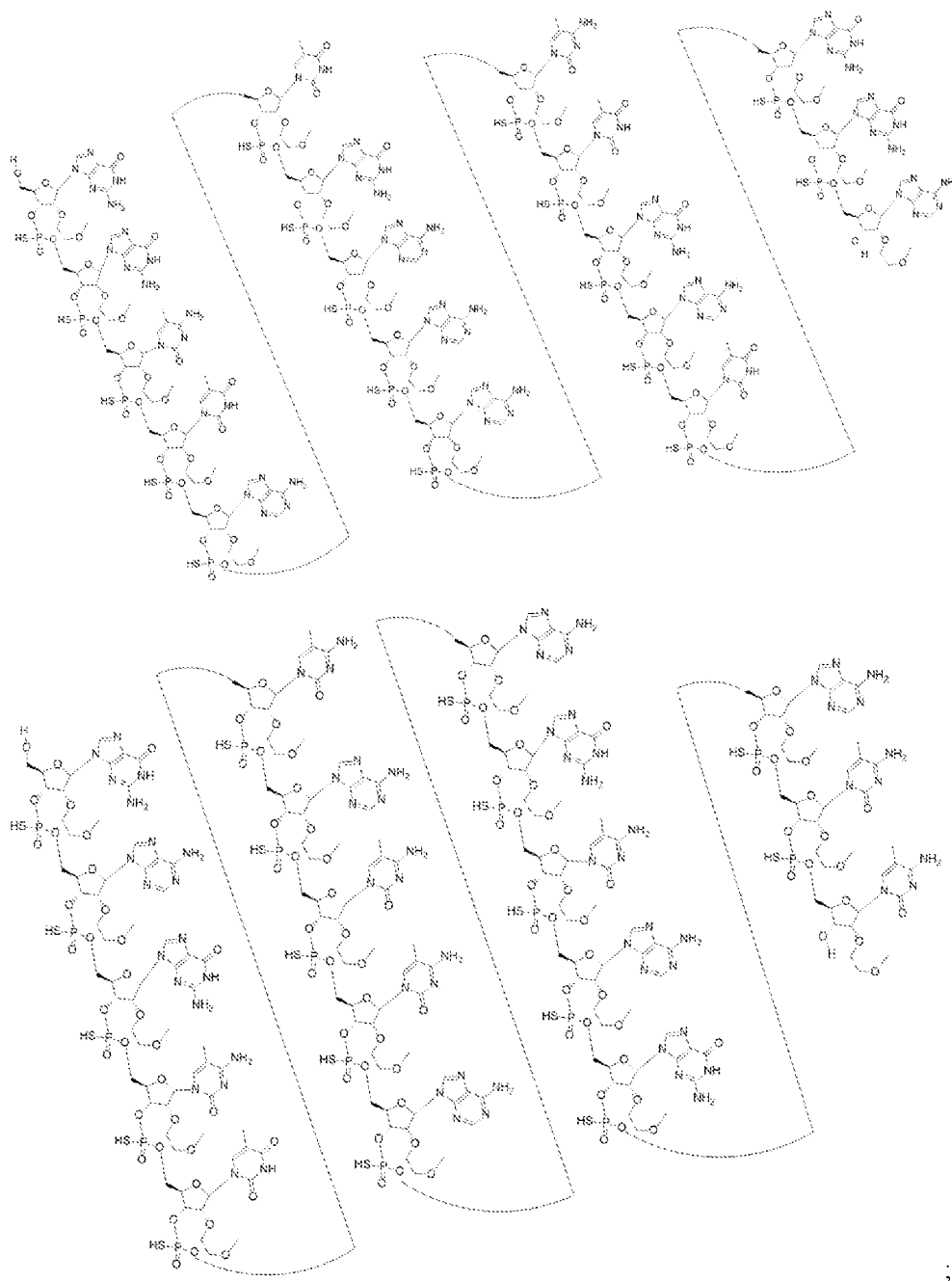
[00287] In some embodiments, the ASO comprises one or more backbone modifications. In some embodiments, the ASO comprises one or more sugar moiety modifications. In some embodiments, the ASO comprises one or more backbone modifications and one or more sugar moiety modifications. In some embodiments, the ASO comprises a 2'-MOE modification and a phosphorothioate backbone. In some embodiments, the ASO comprises a phosphorodiamidate morpholino (PMO). In some embodiments, the ASO comprises a peptide nucleic acid (PNA). Any of the ASOs or any component of an ASO (*e.g.*, a nucleobase, sugar moiety, backbone) described herein may be modified in order to achieve desired properties or activities of the ASO or reduce undesired properties or activities of the ASO. For example, an ASO or one or more components of any ASO may be modified to enhance binding affinity to a target sequence on a pre-mRNA transcript; reduce binding to any non-target sequence; reduce degradation by cellular nucleases (*i.e.*, RNase H); improve uptake of the ASO into a cell and/or into the nucleus of a cell; alter the pharmacokinetics or pharmacodynamics of the ASO; and/or modulate the half-life of the ASO.

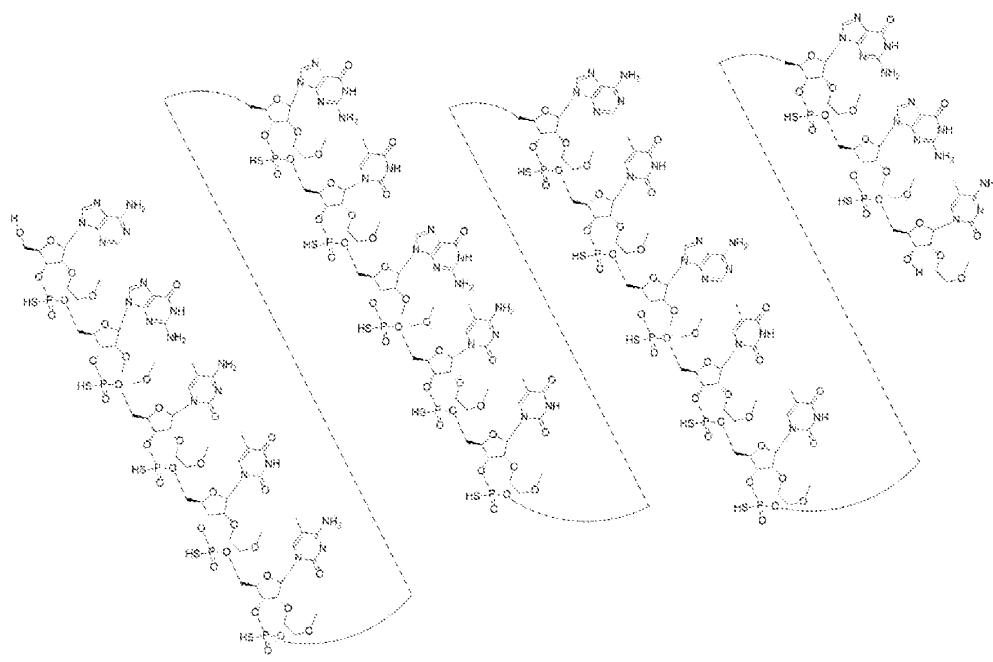
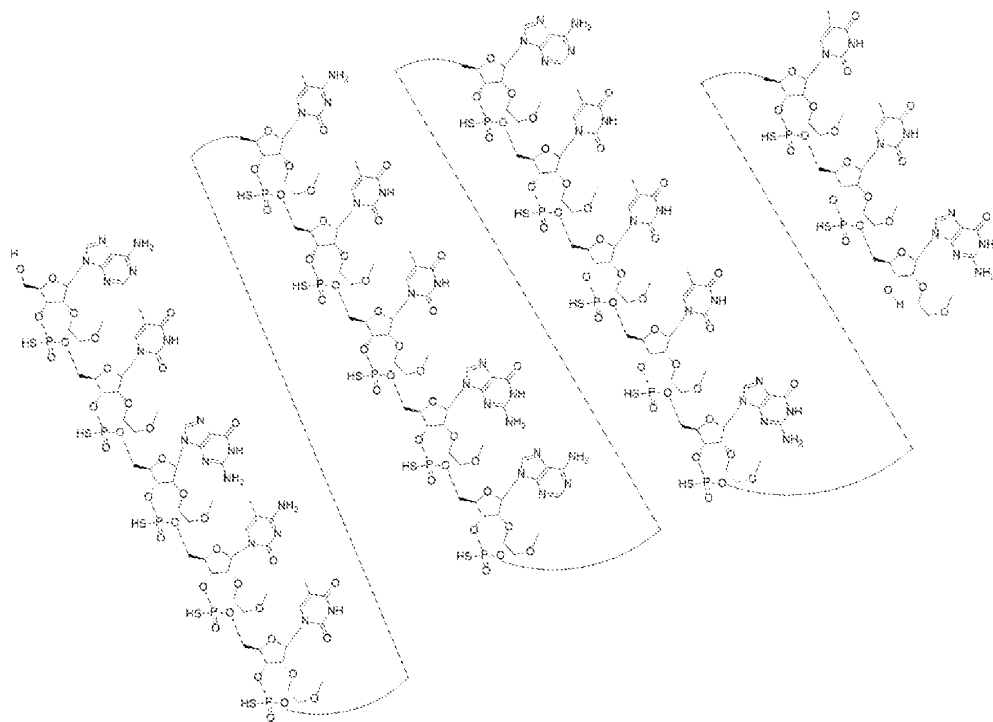
[00288] In some embodiments, the ASOs are comprised of 2'-O-(2-methoxyethyl) (MOE) phosphorothioate-modified nucleotides. ASOs comprised of such nucleotides are especially well-suited to the methods disclosed herein; oligomers having such modifications have been shown to have significantly enhanced resistance to nuclease degradation and increased bioavailability, making them suitable, for example, for oral delivery in some embodiments described herein. See *e.g.*, Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):890-7; Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):898-904.

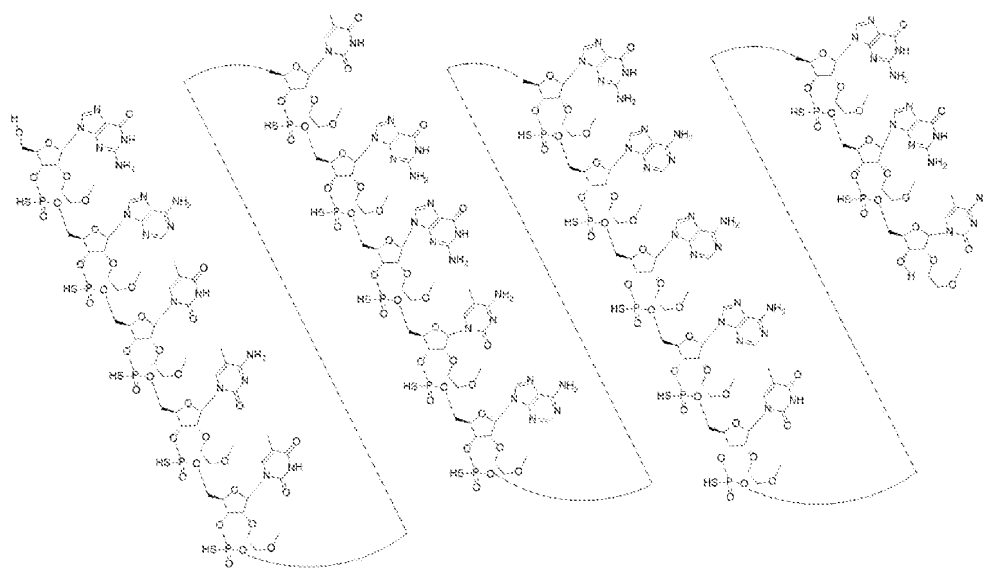
[00289] In some embodiments, the ASOs comprise 5'-methylcytosine (5'-MeC) nucleotides. In some embodiments, the ASOs comprise of at least one 5'-methylcytosine (5'-MeC) nucleotide. In some embodiments, each cytosine of the ASO is a 5'-methylcytosine (5'-MeC). In some embodiments, the ASOs comprise a 5'-methyluracil (5'-MeU). In some embodiments, the ASOs comprise at least one 5'-methyluracil (5'-MeU). In some embodiments, at least one cytosine or thymidine of the antisense oligomer is a 5'-methyluracil (5'-MeU). In some embodiments, each cytosine or thymidine of the antisense oligomer is a 5'-methyluracil (5'-MeU).

[00290] In any of the structural formulae (graphic representation of a chemical compound) presented herein, where two curved lines and a straight line in between them are used to connect a phosphorus atom ("P") and an oxygen atom ("O"), the two curved line and the straight line therebetween should be seen as a single integral segment, representing the covalent bond between the phosphorus atom and the oxygen atom, which is part of a backbone linkage (*e.g.*, phosphodiester linkage or phosphorothioate linkage) between two neighboring nucleotides. Any of the vertices (corners) in any of those structural formulae where a curved line and a straight-line join does not represent carbon atom or presence of -CH₂- at the relevant location in the compound the structural formula represents.

[00291] In some embodiments, the ASO has any one of the following chemical structures:



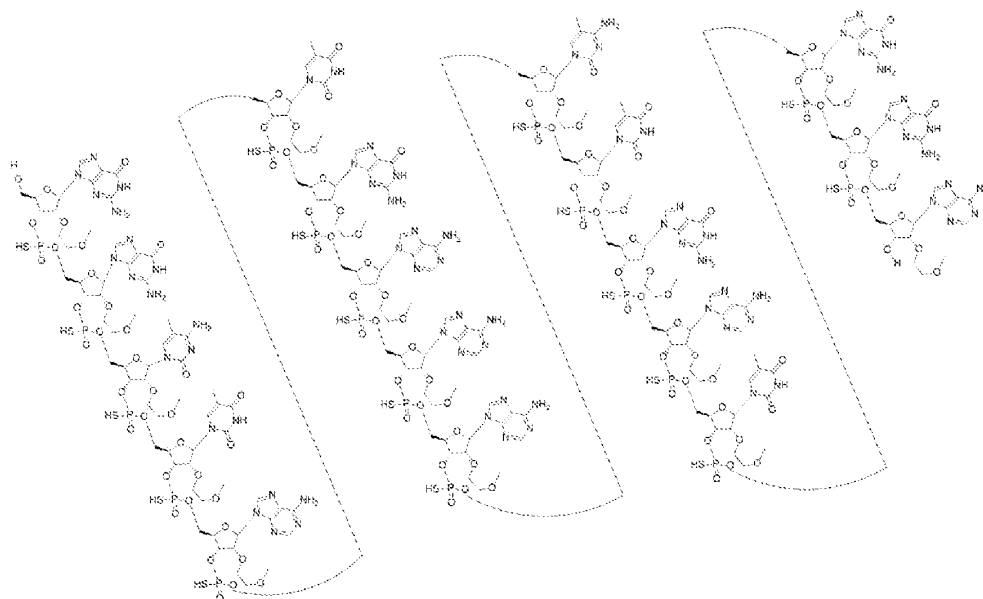




or a pharmaceutically acceptable salt thereof.

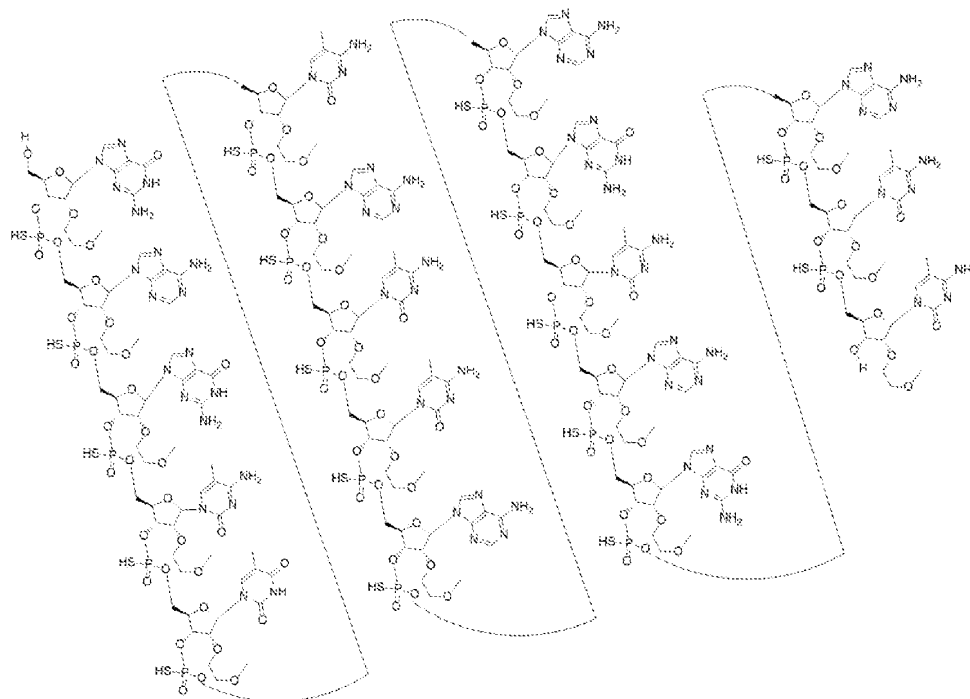
[00292]

In some embodiments, the ASO has the following structure:



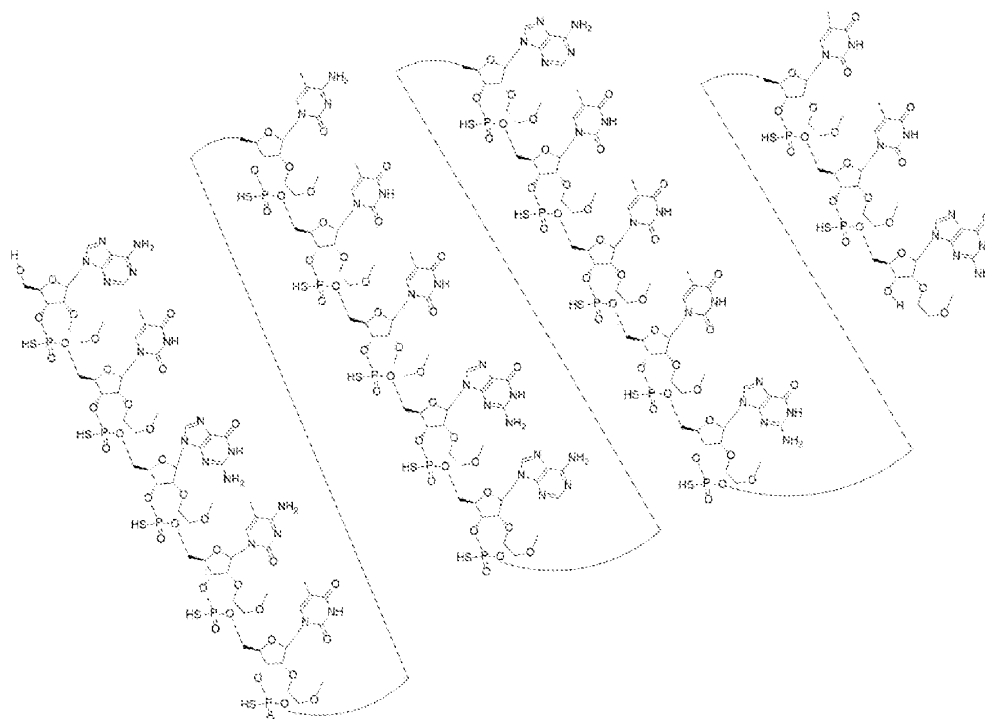
or a pharmaceutically acceptable salt thereof.

[00293] In some embodiments, the ASO has the following structure:



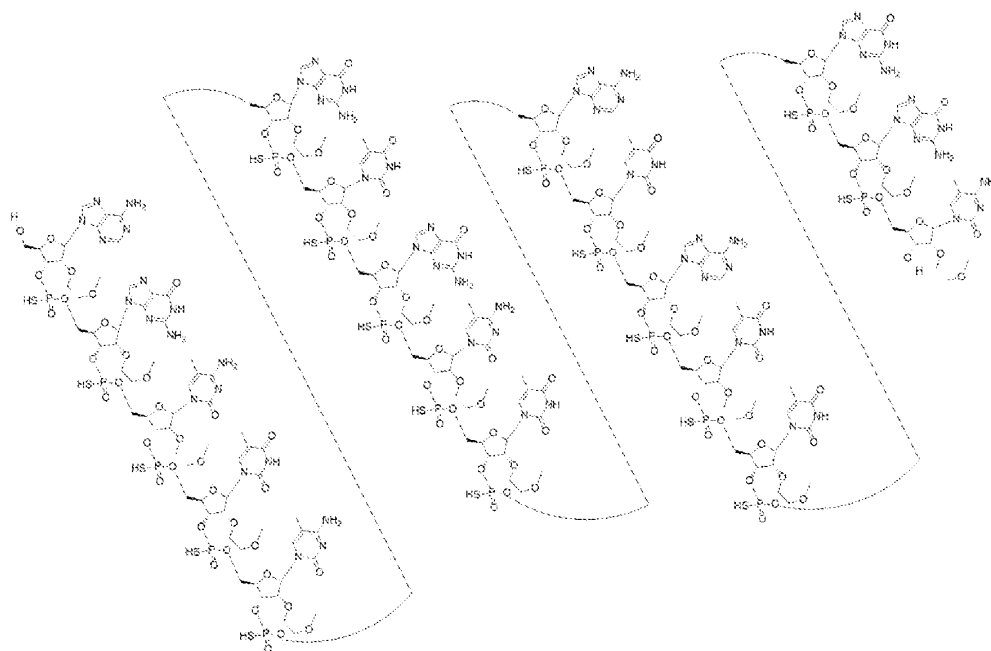
or a pharmaceutically acceptable salt thereof.

[00294] In some embodiments, the ASO has the following structure:



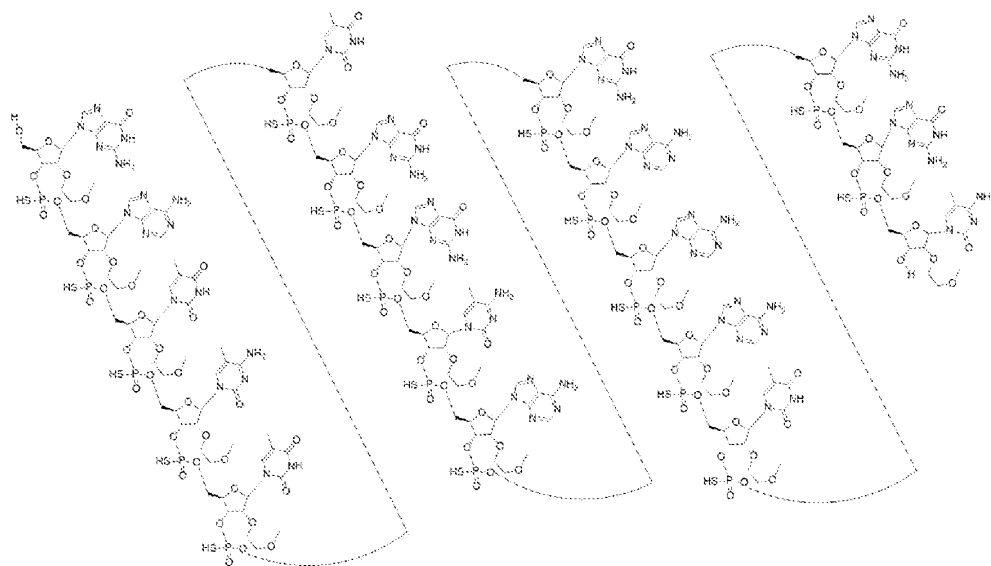
or a pharmaceutically acceptable salt thereof.

[00295] In some embodiments, the ASO has the following structure:



or a pharmaceutically acceptable salt thereof.

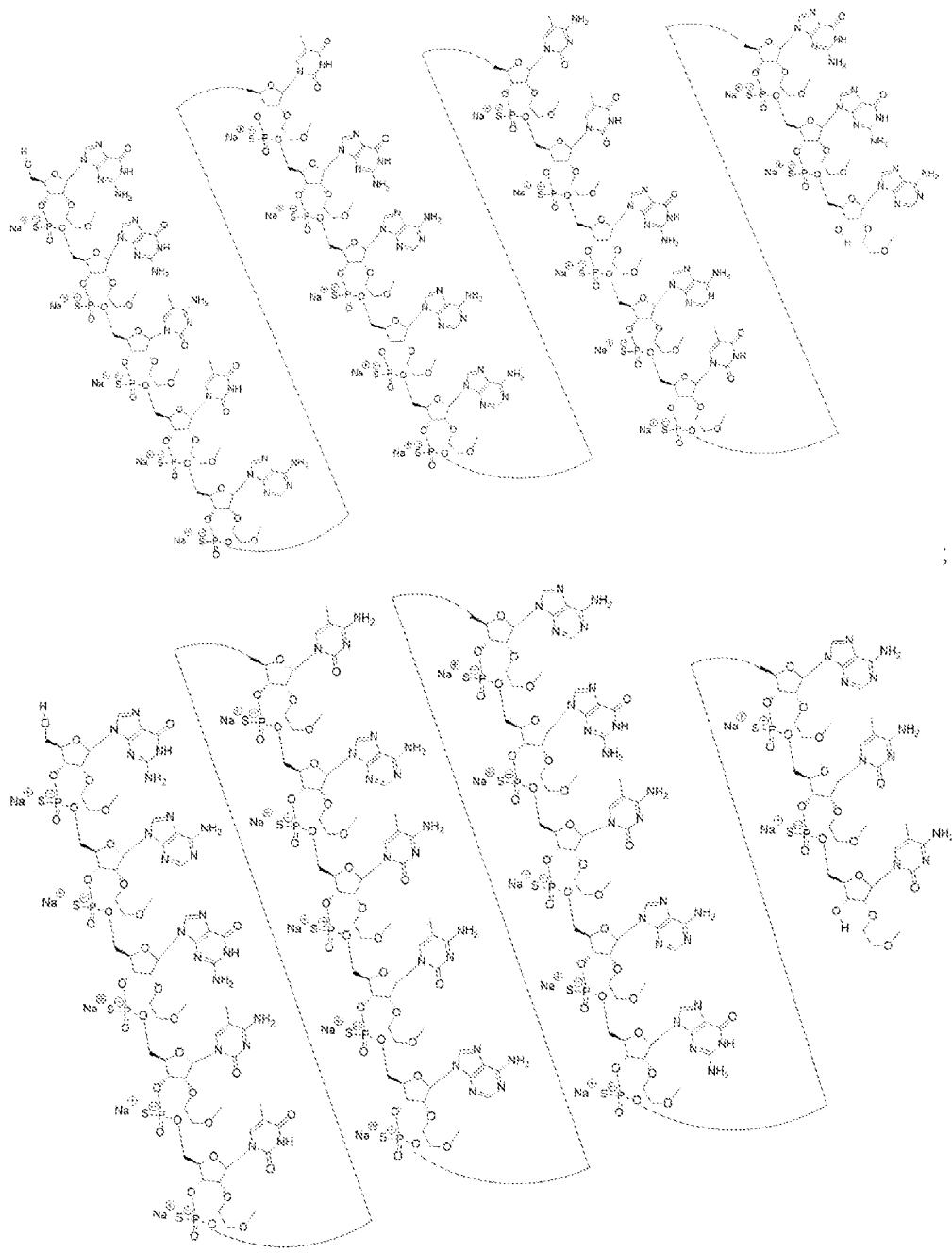
[00296] In some embodiments, the ASO has the following structure:

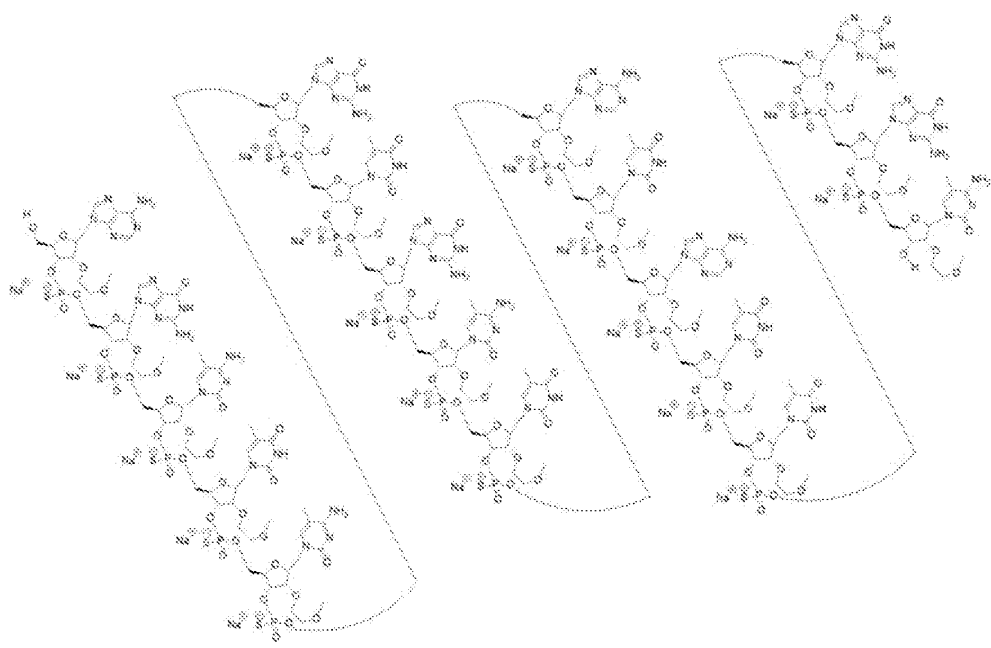
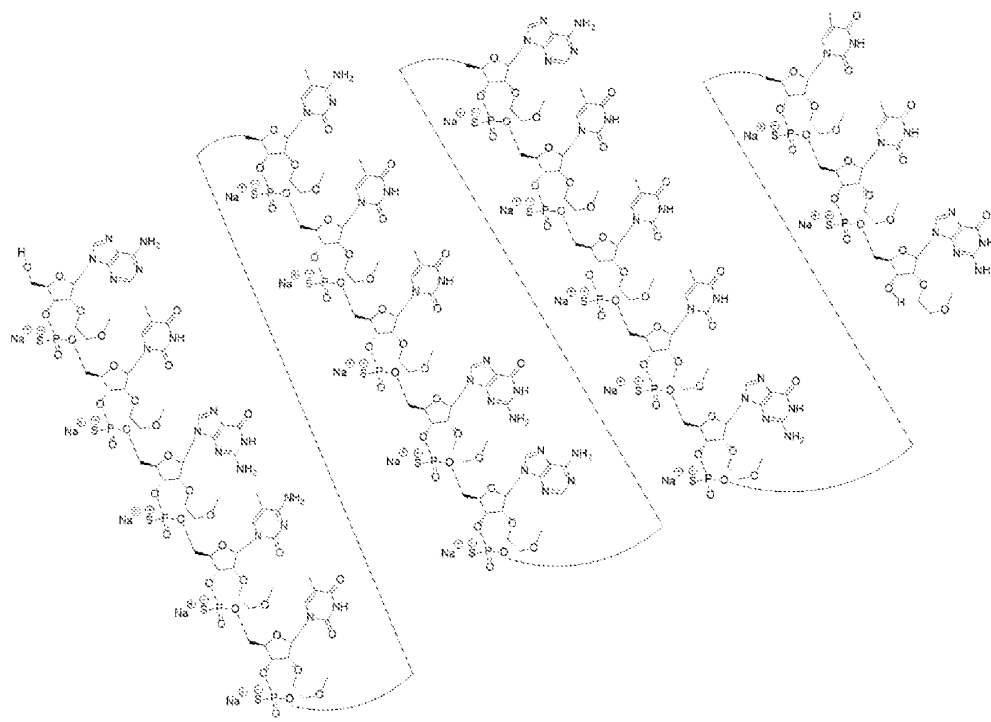


or a pharmaceutically acceptable salt thereof.

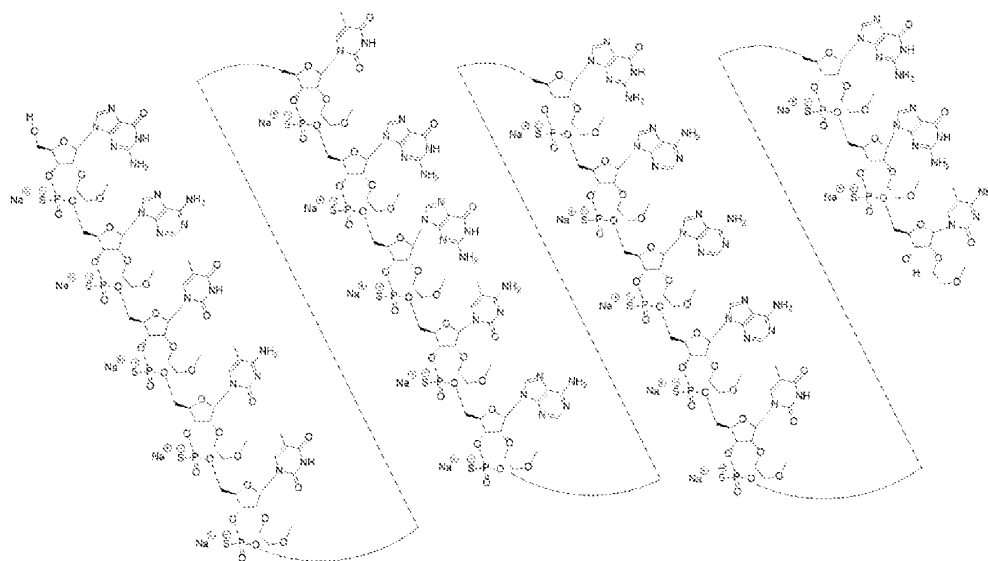
[00297]

In some embodiments, the ASO has any one of the following structures:

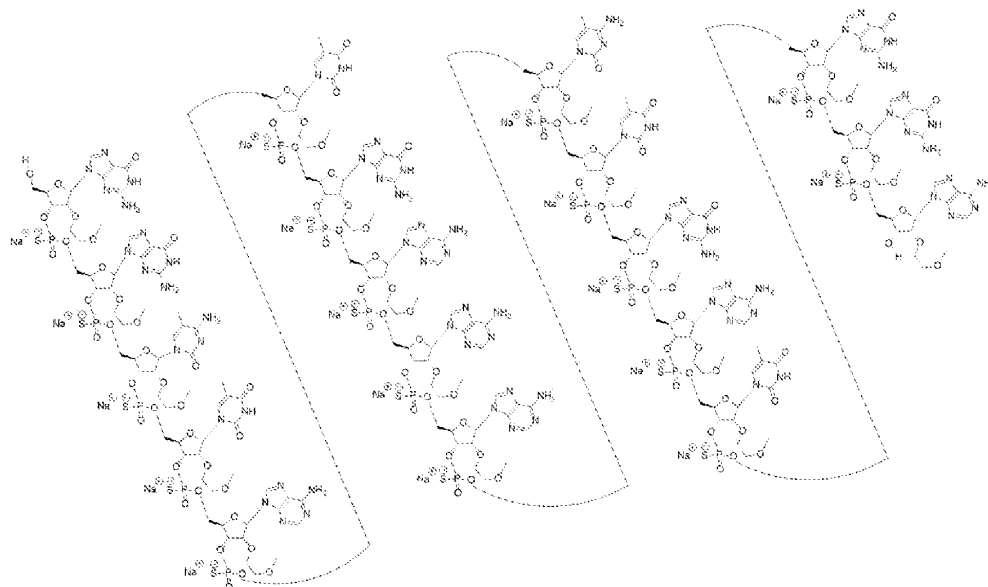




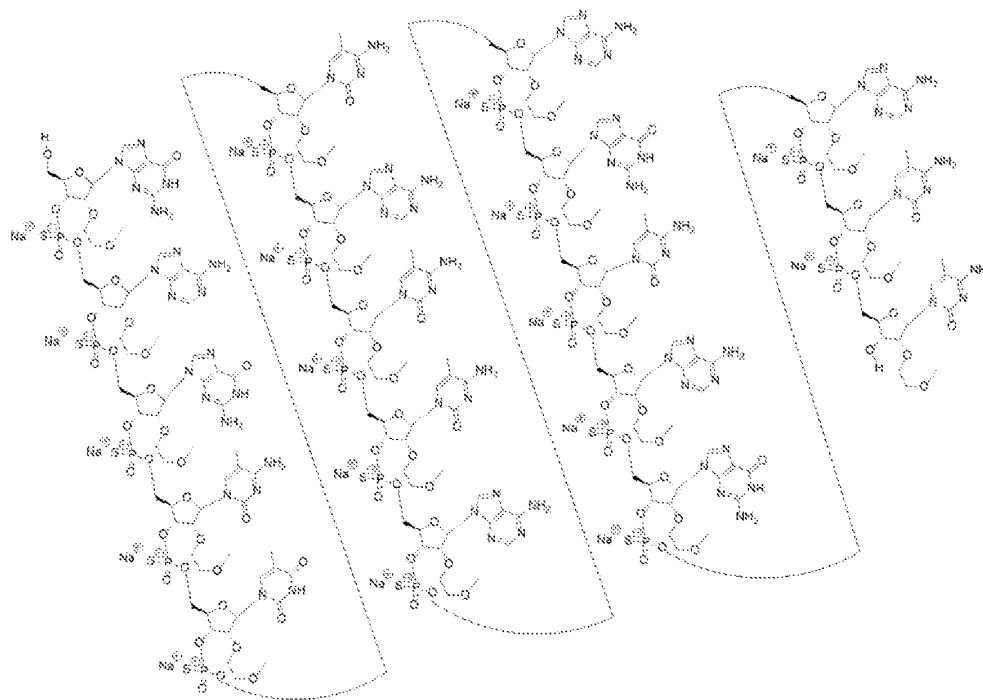
; or



[00298] In some embodiments, the ASO is in a salt form. In some embodiments, the salt form is sodium salt. In some embodiments, the sodium salt form of the ASO has the following structure:



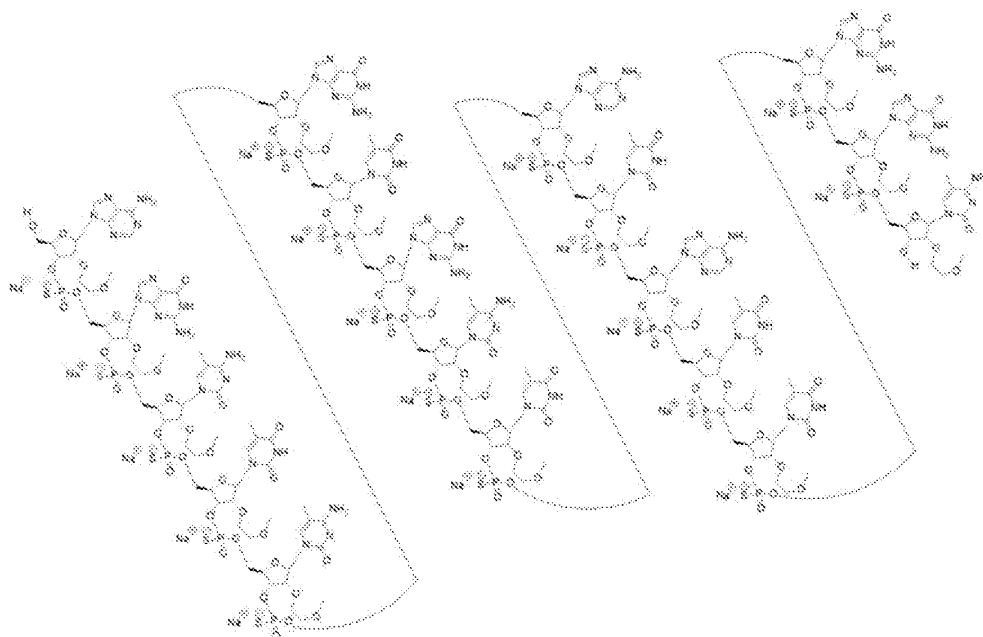
[00299] In some embodiments, the ASO is in a salt form. In some embodiments, the salt form is sodium salt. In some embodiments, the sodium salt form of the ASO has the following structure:



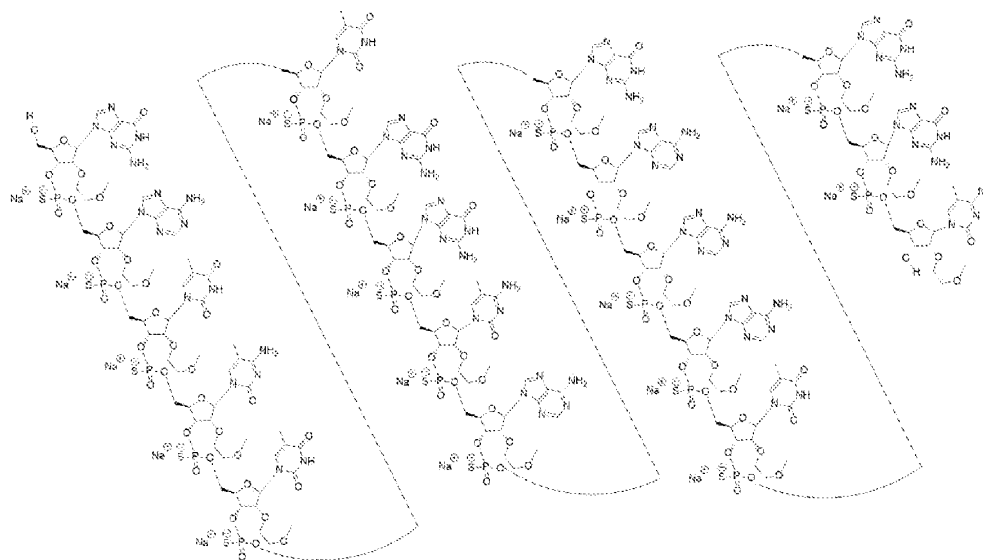
[00300] In some embodiments, the ASO is in a salt form. In some embodiments, the salt form is sodium salt. In some embodiments, the sodium salt form of the ASO has the following structure:



[00301] In some embodiments, the ASO is in a salt form. In some embodiments, the salt form is sodium salt. In some embodiments, the sodium salt form of the ASO has the following structure:



[00302] In some embodiments, the ASO is in a salt form. In some embodiments, the salt form is sodium salt. In some embodiments, the sodium salt form of the ASO has the following structure:



[00303] Methods of synthesizing ASOs will be known to one of skill in the art. Alternatively or in addition, ASOs may be obtained from a commercial source.

[00304] Unless specified otherwise, the left-hand end of single-stranded nucleic acid (*e.g.*, pre-mRNA transcript, oligonucleotide, ASO, etc.) sequences is the 5' end and the left-hand direction of single or double-stranded nucleic acid sequences is referred to as the 5' direction. Similarly, the right-hand end or direction of a nucleic acid sequence (single or double stranded) is the 3' end or direction. Generally, a region or sequence that is 5' to a reference point in a nucleic acid is referred to as "upstream," and a region or sequence that is 3' to a reference point in a nucleic acid is referred to as "downstream." Generally, the 5' direction or end of an mRNA is where the initiation or start codon is located, while the 3' end or direction is where the termination codon is located. In some aspects, nucleotides that are upstream of a reference point in a nucleic acid may be designated by a negative number, while nucleotides that are downstream of a reference point may be designated by a positive number. For example, a reference point (*e.g.*, an exon-exon junction in mRNA) may be designated as the "zero" site, and a nucleotide that is directly adjacent and upstream of the reference point is designated "minus one," *e.g.*, "-1," while a nucleotide that is directly adjacent and downstream of the reference point is designated "plus one," *e.g.*, "+1."

[00305] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is downstream (in the 3' direction) of the 5' splice site (or 3' end of the NMD exon) of the included exon in an *OPAI* pre-mRNA (*e.g.*, the direction designated by positive numbers relative to the 5' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA that is within the region about +1 to about +500 relative to the 5' splice site (or 3' end) of the included exon. In some embodiments, the ASOs may be complementary to a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is within the region between nucleotides +6 and +40,000 relative to the 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +1 to about +40,000, about +1 to about +30,000, about +1 to about +20,000, about +1 to about +15,000, about +1 to about +10,000, about +1 to about +5,000, about +1 to about +4,000, about +1 to about +3,000, about +1 to about +2,000, about +1 to about +1,000, about +1 to about +500, about +1 to about +490, about +1 to about +480, about +1 to about +470, about +1 to about +460, about +1 to about +450, about +1 to about +440, about +1 to about +430, about +1 to about +420, about +1 to about +410, about +1 to about +400, about +1 to about +390, about +1 to about +380, about +1 to about +370, about +1 to about +360, about +1 to about +350, about +1 to about +340, about +1 to about +330, about +1 to about +320, about +1 to about +310, about +1 to about +300, about +1 to about +290, about +1 to about +280, about +1 to about +270, about +1 to about +260, about +1 to about +250, about +1 to about +240, about +1 to about +230, about +1 to about +220, about +1 to about +210, about

+1 to about +200, about +1 to about +190, about +1 to about +180, about +1 to about +170, about +1 to about +160, about +1 to about +150, about +1 to about +140, about +1 to about +130, about +1 to about +120, about +1 to about +110, about +1 to about +100, about +1 to about +90, about +1 to about +80, about +1 to about +70, about +1 to about +60, about +1 to about +50, about +1 to about +40, about +1 to about +30, or about +1 to about +20 relative to 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region from about +1 to about +100, from about +100 to about +200, from about +200 to about +300, from about +300 to about +400, or from about +400 to about +500 relative to 5' splice site (or 3' end) of the included exon.

[00306] In some embodiments, the ASOs are complementary to (and bind to) a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is upstream (in the 5' direction) of the 5' splice site (or 3' end) of the included exon in an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA (*e.g.*, the direction designated by negative numbers relative to the 5' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA, that is within the region about -4 to about -270 relative to the 5' splice site (or 3' end) of the included exon. In some embodiments, the ASOs may be complementary to a targeted portion of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is within the region between nucleotides -1 and -40,000 relative to the 5' splice site (or 3' end) of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -1 to about -40,000, about -1 to about -30,000, about -1 to about -20,000, about -1 to about -15,000, about -1 to about -10,000, about -1 to about -5,000, about -1 to about -4,000, about -1 to about -3,000, about -1 to about -2,000, about -1 to about -1,000, about -1 to about -500, about -1 to about -490, about -1 to about -480, about -1 to about -470, about -1 to about -460, about -1 to about -450, about -1 to about -440, about -1 to about -430, about -1 to about -420, about -1 to about -410, about -1 to about -400, about -1 to about -390, about -1 to about -380, about -1 to about -370, about -1 to about -360, about -1 to about -350, about -1 to about -340, about -1 to about -330, about -1 to about -320, about -1 to about -310, about -1 to about -300, about -1 to about -290, about -1 to about -280, about -1 to about -270, about -1 to about -260, about -1 to about -250, about -1 to about -240, about -1 to about -230, about -1 to about -220, about -1 to about -210, about -1 to about -200, about -1 to about -190, about -1 to about -180, about -1 to about -170, about -1 to about -160, about -1 to about -150, about -1 to about -140, about -1 to about -130, about -1 to about -120, about -1 to about -110, about -1 to about -100, about -1 to about -90, about -1 to about -80, about -1 to about -70, about -1 to about -60, about -1 to about -50, about -1 to about -40, about -1 to about -30, or about -1 to about -20 relative to 5' splice site (or 3' end) of the included exon.

[00307] In some embodiments, the ASOs are complementary to a targeted region of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is upstream (in the 5' direction) of the 3' splice site (or 5' end) of the included exon in an *OPAI* pre-mRNA (*e.g.*, in the direction designated by negative numbers). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA, that is within the region about -1 to about -500 relative to the 3' splice site (or 5' end) of the included exon. In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA that is within the region -1 to -40,000 relative to the 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about -1 to about -40,000, about -1 to about -30,000, -1 to about -20,000, about -1 to about -15,000, about -1 to about -10,000, about -1 to about -5,000, about -1 to about -4,000, about -1 to about -3,000, about -1 to about -2,000, about -1 to about -1,000, about -1 to about -500, about -1 to about -490, about -1 to about -480, about -1 to about -470, about -1 to about -460, about -1 to about -450, about -1 to about -440, about -1 to about -430, about -1 to about -420, about -1 to about -410, about -1 to about -400, about -1 to about -390, about -1 to about -380, about -1 to about -370, about -1 to about -360, about -1 to about -350, about -1 to about -340, about -1 to about -330, about -1 to about -320, about -1 to about -310, about -1 to about -300, about -1 to about -290, about -1 to about -280, about -1 to about -270, about -1 to about -260, about -1 to about -250, about -1 to about -240, about -1 to about -230, about -1 to about -220, about -1 to about -210, about -1 to about -200, about -1 to about -190, about -1 to about -180, about -1 to about -170, about -1 to about -160, about -1 to about -150, about -1 to about -140, about -1 to about -130, about -1 to about -120, about -1 to about -110, about -1 to about -100, about -1 to about -90, about -1 to about -80, about -1 to about -70, about -1 to about -60, about -1 to about -50, about -1 to about -40, about -1 to about -30, or about -1 to about -20 relative to 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region from about -1 to about -100, from about -100 to about -200, from about -200 to about -300, from about -300 to about -400, or from about -400 to about -500 relative to 3' splice site of the included exon.

[00308] In some embodiments, the ASOs are complementary to a targeted region of an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA, that is downstream (in the 3' direction) of the 3' splice site (5' end) of the included exon in an *OPAI* pre-mRNA, *e.g.*, an *OPAI* NMD exon-containing pre-mRNA (*e.g.*, in the direction designated by positive numbers). In some embodiments, the ASOs are complementary to a targeted portion of the *OPAI* pre-mRNA that is within the region of about +1 to about +40,000 relative to the 3' splice site of the included exon. In some aspects, the ASOs are complementary to a targeted portion that is within the region about +1 to about +40,000, about +1 to about +30,000, about +1 to about +20,000, about +1 to about

+15,000, about +1 to about +10,000, about +1 to about +5,000, about +1 to about +4,000, about +1 to about +3,000, about +1 to about +2,000, about +1 to about +1,000, about +1 to about +500, about +1 to about +490, about +1 to about +480, about +1 to about +470, about +1 to about +460, about +1 to about +450, about +1 to about +440, about +1 to about +430, about +1 to about +420, about +1 to about +410, about +1 to about +400, about +1 to about +390, about +1 to about +380, about +1 to about +370, about +1 to about +360, about +1 to about +350, about +1 to about +340, about +1 to about +330, about +1 to about +320, about +1 to about +310, about +1 to about +300, about +1 to about +290, about +1 to about +280, about +1 to about +270, about +1 to about +260, about +1 to about +250, about +1 to about +240, about +1 to about +230, about +1 to about +220, about +1 to about +210, about +1 to about +200, about +1 to about +190, about +1 to about +180, about +1 to about +170, about +1 to about +160, about +1 to about +150, about +1 to about +140, about +1 to about +130, about +1 to about +120, about +1 to about +110, about +1 to about +100, about +1 to about +90, about +1 to about +80, about +1 to about +70, about +1 to about +60, about +1 to about +50, about +1 to about +40, about +1 to about +30, or about +1 to about +20, or about +1 to about +10 relative to 3' splice site of the included exon.

[00309] In some embodiments, the targeted portion of the *OPAI* pre-mRNA, *e.g.*, the *OPAI* NMD exon-containing pre-mRNA, is within the region +100 relative to the 5' splice site (3' end) of the included exon to -100 relative to the 3' splice site (5' end) of the included exon. In some embodiments, the targeted portion of the *OPAI* NMD exon-containing pre-mRNA is within the NMD exon. In some embodiments, the target portion of the *OPAI* NMD exon-containing pre-mRNA comprises a pseudo-exon and intron boundary.

[00310] The ASOs may be of any length suitable for specific binding and effective enhancement of splicing. In some embodiments, the ASOs consist of 8 to 50 nucleobases. For example, the ASO may be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, or 50 nucleobases in length. In some embodiments, the ASOs consist of more than 50 nucleobases. In some embodiments, the ASO is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, 12 to 15 nucleobases, 13 to 50 nucleobases, 13 to 40 nucleobases, 13 to 35 nucleobases, 13 to 30

nucleobases, 13 to 25 nucleobases, 13 to 20 nucleobases, 14 to 50 nucleobases, 14 to 40 nucleobases, 14 to 35 nucleobases, 14 to 30 nucleobases, 14 to 25 nucleobases, 14 to 20 nucleobases, 15 to 50 nucleobases, 15 to 40 nucleobases, 15 to 35 nucleobases, 15 to 30 nucleobases, 15 to 25 nucleobases, 15 to 20 nucleobases, 20 to 50 nucleobases, 20 to 40 nucleobases, 20 to 35 nucleobases, 20 to 30 nucleobases, 20 to 25 nucleobases, 25 to 50 nucleobases, 25 to 40 nucleobases, 25 to 35 nucleobases, or 25 to 30 nucleobases in length. In some embodiments, the ASOs are 18 nucleotides in length. In some embodiments, the ASOs are 15 nucleotides in length. In some embodiments, the ASOs are 25 nucleotides in length.

[00311] In some embodiments, two or more ASOs with different chemistries but complementary to the same targeted portion of the pre-mRNA, *e.g.*, NMD exon-containing pre-mRNA, are used. In some embodiments, two or more ASOs that are complementary to different targeted portions of the pre-mRNA, *e.g.*, the NMD exon-containing pre-mRNA, are used.

[00312] In some embodiments, the antisense oligonucleotides of the disclosure are chemically linked to one or more moieties or conjugates, *e.g.*, a targeting moiety or other conjugate that enhances the activity or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, a lipid moiety, *e.g.*, as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Oligonucleotides comprising lipophilic moieties and preparation methods have been described in the published literature. In embodiments, the antisense oligonucleotide is conjugated with a moiety including, but not limited to, an abasic nucleotide, a polyether, a polyamine, a polyamide, a peptides, a carbohydrate, *e.g.*, N-acetylgalactosamine (GalNAc), N-Ac-Glucosamine (GluNAc), or mannose (*e.g.*, mannose-6-phosphate), a lipid, or a polyhydrocarbon compound. Conjugates can be linked to one or more of any nucleotides comprising the antisense oligonucleotide at any of several positions on the sugar, base or phosphate group, as understood in the art and described in the literature, *e.g.*, using a linker. Linkers can include a bivalent or trivalent branched linker. In embodiments, the conjugate is attached to the 3' end of the antisense oligonucleotide. Methods of preparing oligonucleotide conjugates are described, *e.g.*, in U.S. Pat. No. 8,450,467, "Carbohydrate conjugates as delivery agents for oligonucleotides," incorporated by reference herein.

[00313] In some embodiments, the nucleic acid to be targeted by an ASO is an *OPAI* pre-mRNA, *e.g.*, NMD exon-containing pre-mRNA expressed in a cell, such as a eukaryotic cell. In some embodiments, the term "cell" may refer to a population of cells. In some embodiments, the cell is in a subject. In some embodiments, the cell is isolated from a subject. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is a condition or disease-relevant cell or a cell line. In some embodiments, the cell is *in vitro* (*e.g.*, in cell culture).

Pharmaceutical Compositions

[00314] Pharmaceutical compositions or formulations comprising the agent, *e.g.*, antisense oligonucleotide, of the described compositions and for use in any of the described methods can be prepared according to conventional techniques well known in the pharmaceutical industry and described in the published literature. In embodiments, a pharmaceutical composition or formulation for treating a subject comprises an effective amount of any antisense oligomer as described herein, or a pharmaceutically acceptable salt, solvate, hydrate or ester thereof. The pharmaceutical formulation comprising an antisense oligomer may further comprise a pharmaceutically acceptable excipient, diluent or carrier.

[00315] Pharmaceutically acceptable salts are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, etc., and are commensurate with a reasonable benefit/risk ratio. (See, *e.g.*, S. M. Berge, *et al.*, J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference for this purpose. The salts can be prepared *in situ* during the final isolation and purification of the compounds, or separately by reacting the free base form with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other documented methodologies such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[00316] In some embodiments, the compositions are formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. In embodiments, the compositions are formulated as suspensions in

aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers. In embodiments, a pharmaceutical formulation or composition of the present disclosure includes, but is not limited to, a solution, emulsion, microemulsion, foam or liposome-containing formulation (*e.g.*, cationic or noncationic liposomes).

[00317] The pharmaceutical composition or formulation described herein may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients as appropriate and well known to those of skill in the art or described in the published literature. In embodiments, liposomes also include sterically stabilized liposomes, *e.g.*, liposomes comprising one or more specialized lipids. These specialized lipids result in liposomes with enhanced circulation lifetimes. In embodiments, a sterically stabilized liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. In some embodiments, a surfactant is included in the pharmaceutical formulation or compositions. The use of surfactants in drug products, formulations and emulsions is well known in the art. In embodiments, the present disclosure employs a penetration enhancer to effect the efficient delivery of the antisense oligonucleotide, *e.g.*, to aid diffusion across cell membranes and /or enhance the permeability of a lipophilic drug. In some embodiments, the penetration enhancers are a surfactant, fatty acid, bile salt, chelating agent, or non-chelating nonsurfactant.

[00318] In some embodiments, the pharmaceutical formulation comprises multiple antisense oligonucleotides. In embodiments, the antisense oligonucleotide is administered in combination with another drug or therapeutic agent.

[00319] In some embodiments, the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer.

[00320] In some embodiments, the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to

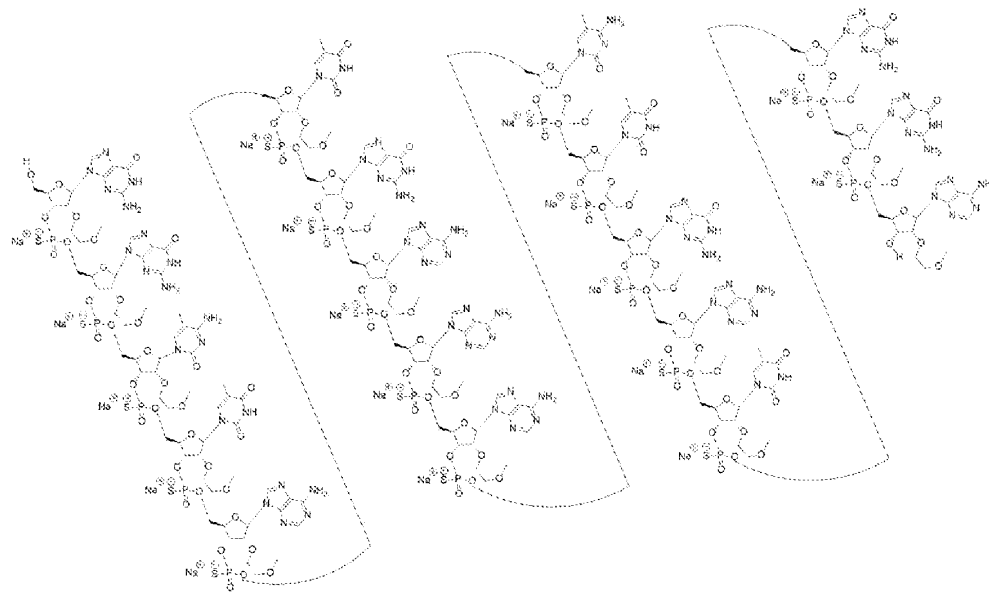
about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00321] In some embodiments, the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

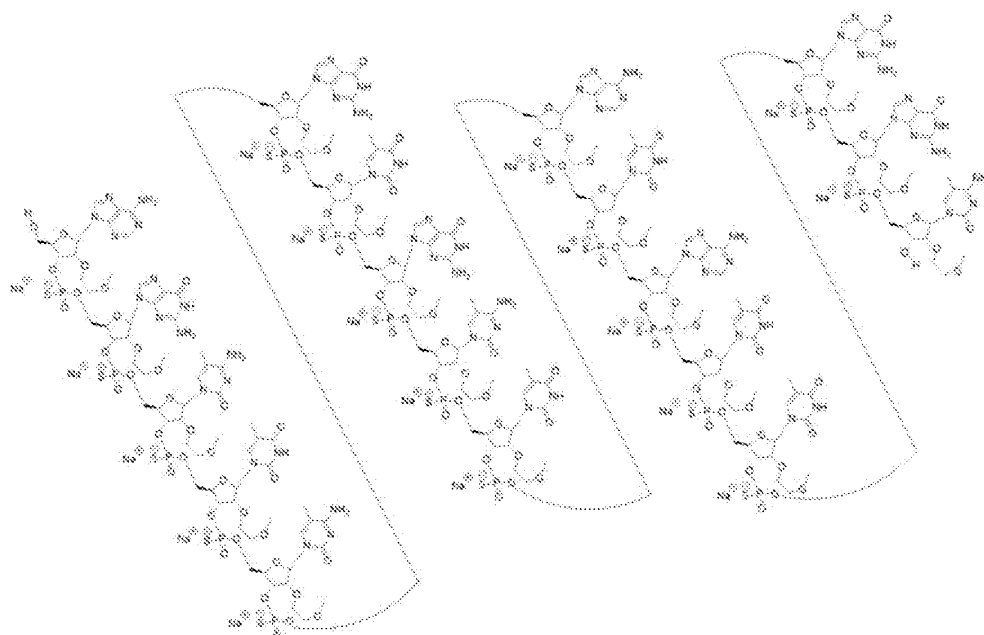
[00322] In some embodiments, the concentrate is a phosphate-buffered solution.

[00323] In some embodiments, the pharmaceutical formulation comprises: (a) an antisense oligomer, wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and (b) a pharmaceutically acceptable diluent; wherein the antisense oligomer is dissolved or suspended in a solution at a concentration of about 1 mg/mL to about 200 mg/mL.

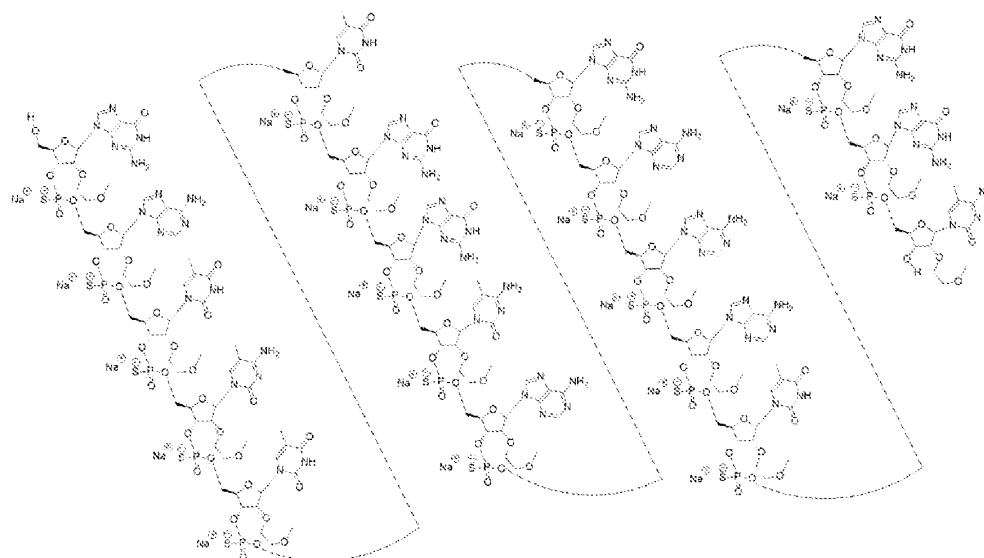
[00324] In some embodiments, the pharmaceutical formulation comprises (a) an antisense oligomer, wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and (b) a pharmaceutically acceptable diluent; wherein the antisense oligomer is dissolved or suspended in a solution, and wherein the antisense oligomer is a compound according to any one of the following chemical structures:



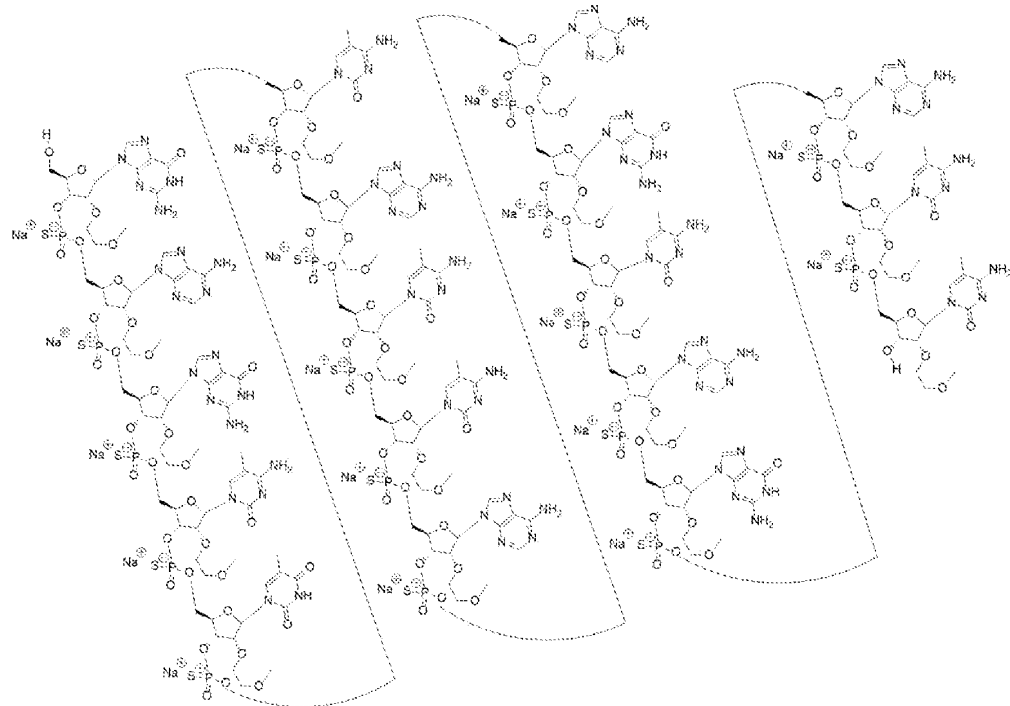
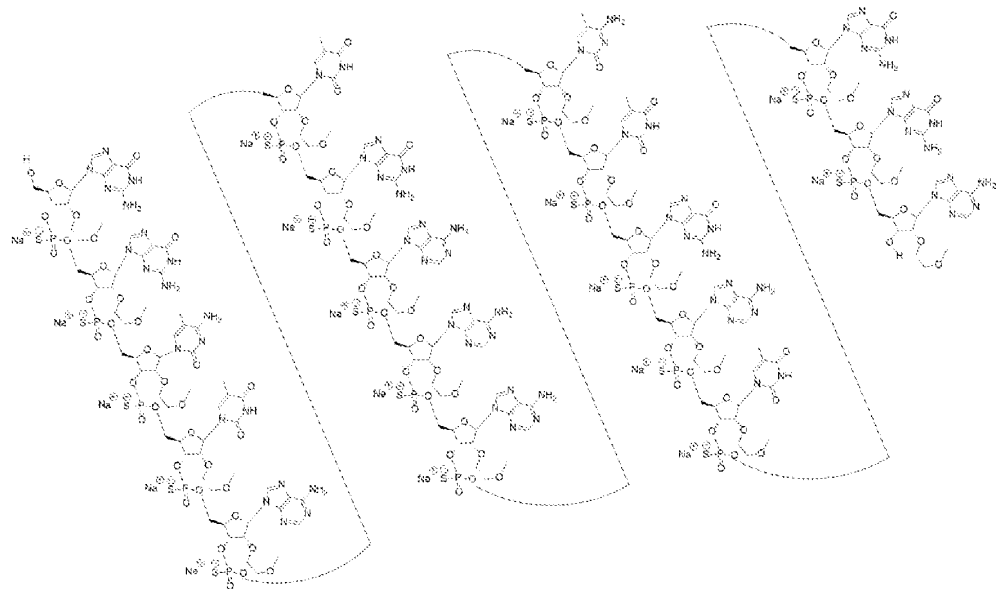


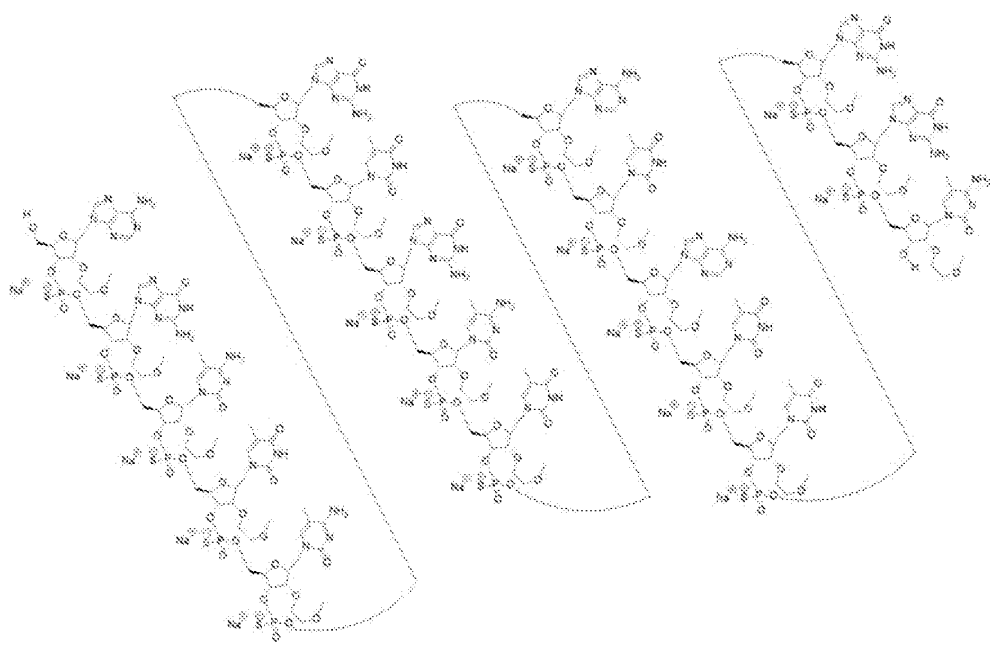
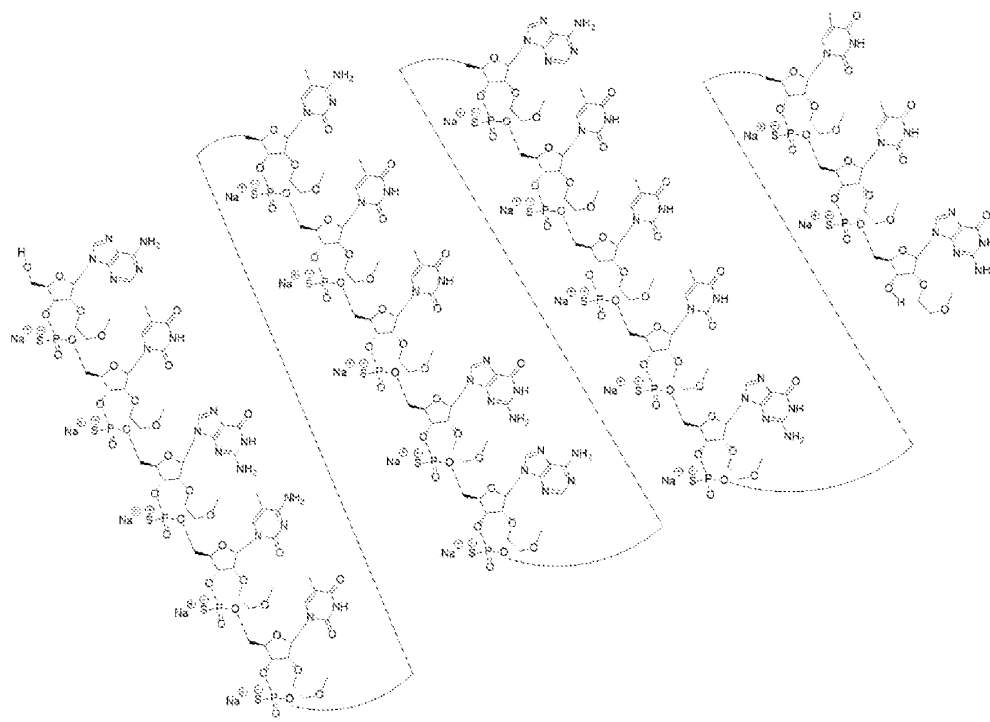


; or

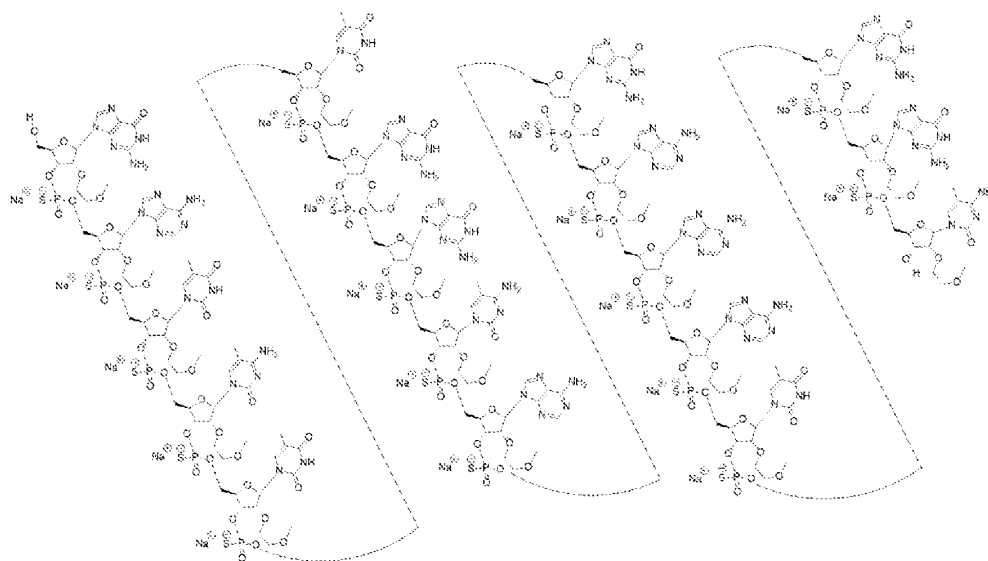


[00325] In some embodiments, the pharmaceutical formulation comprises (a) an antisense oligomer, wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOS: 6-275 or 280-299; and (b) a pharmaceutically acceptable diluent; wherein the antisense oligomer is dissolved or suspended in a solution, and wherein the antisense oligomer is a compound according to any one of the following chemical structures:





; or



or a pharmaceutically acceptable salt thereof.

[00326] In some embodiments, the antisense oligomer is present in the solution at a concentration of about 1 mg/mL to about 200 mg/mL.

[00327] In some embodiments, the antisense oligomer is present in the solution at a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80

mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

[00328] In some embodiments, the antisense oligomer is present in the solution at a concentration of about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00329] In some embodiments, the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer. In some embodiments, the pharmaceutical composition is prepared by diluting a concentrate consisting of the antisense oligomer.

[00330] In some embodiments, the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00331] In some embodiments, the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00332] In some embodiments, the concentrate is phosphate buffered. In some embodiments, the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection. In some embodiments, the antisense oligomer is solubilized or diluted in a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection. In some embodiments, the antisense oligomer is solubilized or diluted in an isotonic solution. In some embodiments, the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8. In some embodiments, the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution. In some embodiments, the pharmaceutical formulation does not comprise a preservative. In some embodiments, the pharmaceutical formulation is suitable for an intravitreal injection. In some embodiments, the pharmaceutical formulation is packaged in a single use vial.

[00333] In some embodiments, the pharmaceutical compositions are assembled into a kit comprising: (i) a concentrate comprising an antisense oligomer (ASO), wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and (ii) a diluent, wherein the concentrate is miscible with the diluent; and (iii) instructions for diluting the concentrate with the diluent.

[00334] In some embodiments, the pharmaceutical compositions are assembled into a kit consisting of: (i) a concentrate comprising an antisense oligomer (ASO), wherein the ASO consists of a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and (ii) a diluent, wherein the concentrate is miscible with the diluent; and (iii) instructions for diluting the concentrate with the diluent.

[00335] In some embodiments, the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00336] In some embodiments, the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00337] In some embodiments, the concentrate is phosphate buffered. In some embodiments, the diluent is a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection. In some embodiments, the diluent is a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection. In some embodiments, the diluent comprises an isotonic solution. In some embodiments, the diluent comprises a phosphate-buffered solution with at least pH 5.8. In some embodiments, the diluent comprises a phosphate-buffered (pH 6.6 – 7.6) solution. In some embodiments, the concentrate or the diluent does not comprise a preservative.

[00338] In some embodiments, the diluent is a solution consisting of one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection. In some embodiments, the diluent is a solution consisting of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection. In some embodiments, the diluent consists of an isotonic solution. In some embodiments, the diluent consists of a phosphate-buffered solution with at least pH 5.8. In some embodiments, the diluent consists of a phosphate-buffered (pH 6.6 – 7.6) solution. In some embodiments, the concentrate or the diluent does not consist of a preservative.

[00339] In some embodiments, the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 2 mg/mL to 200 mg/mL in the diluent.

[00340] In some embodiments, the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about

150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL in the diluent.

[00341] In some embodiments, the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the antisense oligomer to a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL in the diluent.

[00342] In some embodiments, the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 or 280-299.

[00343] In some embodiments, the antisense oligomer consists of a nucleotide sequence having at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 or 280-299.

[00344] In some embodiments, the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

[00345] In some embodiments, the antisense oligomer consists of a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

[00346] In some embodiments, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage. In some embodiments, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety. In some embodiments, the antisense oligomer comprises at least one modified sugar moiety. In some embodiments, each sugar moiety is a modified sugar moiety. In some embodiments, the antisense oligomer comprises a 5'-methylcytosine (5'-MeC). In some embodiments, each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC). In some embodiments, the antisense oligomer comprises a 5'-methyluracil (5'-MeU).

[00347] In some embodiments, the antisense oligomer consists of a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage. In some embodiments, the antisense oligomer consists of a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety. In some embodiments, the antisense oligomer consists of at least one modified sugar moiety. In some embodiments, each sugar moiety is a modified sugar moiety. In some embodiments, the antisense oligomer consists of a 5'-methylcytosine (5'-MeC). In some embodiments, each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC). In some embodiments, the antisense oligomer consists of a 5'-methyluracil (5'-MeU).

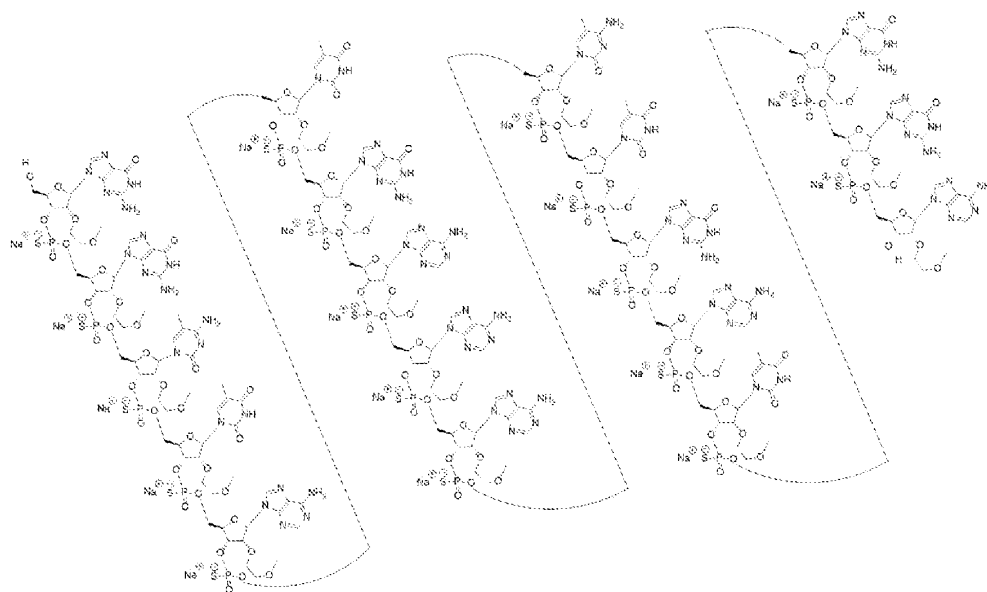
[00348] In some embodiments, each cytosine or thymidine of the antisense oligomer is a 5'-methyluracil (5'-MeU). In some embodiments, the antisense oligomer consists of a phosphorothioate linkage. In some embodiments, each internucleoside linkage of the ASO is a phosphorothioate linkage. In some embodiments, the antisense oligomer consists of a locked nucleic acid (LNA).

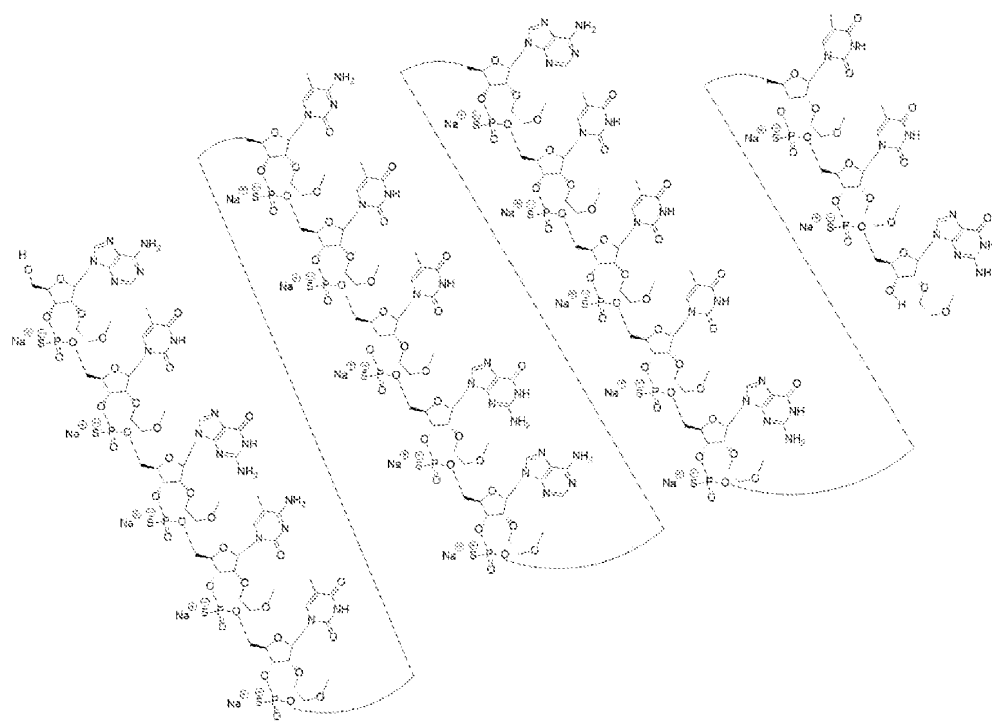
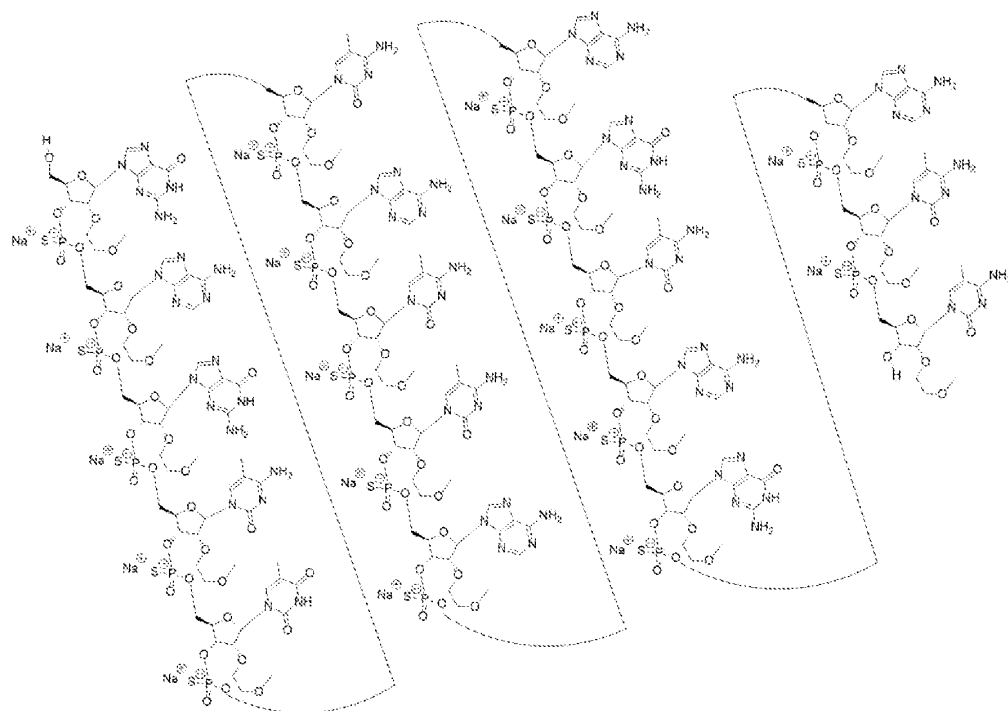
[00349] In some embodiments, the antisense oligomer comprises from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

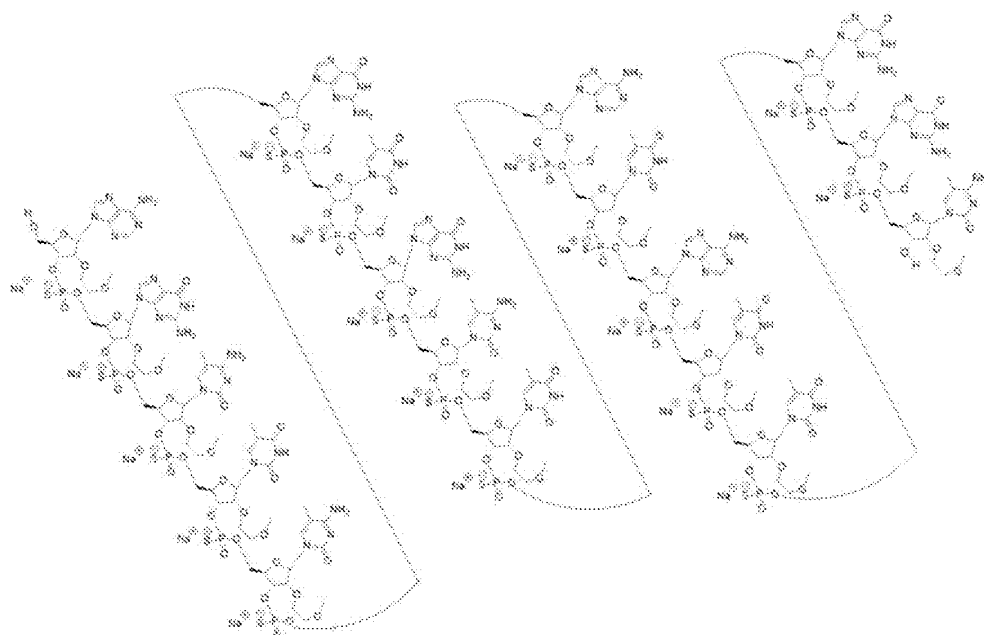
[00350] In some embodiments, the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20

nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

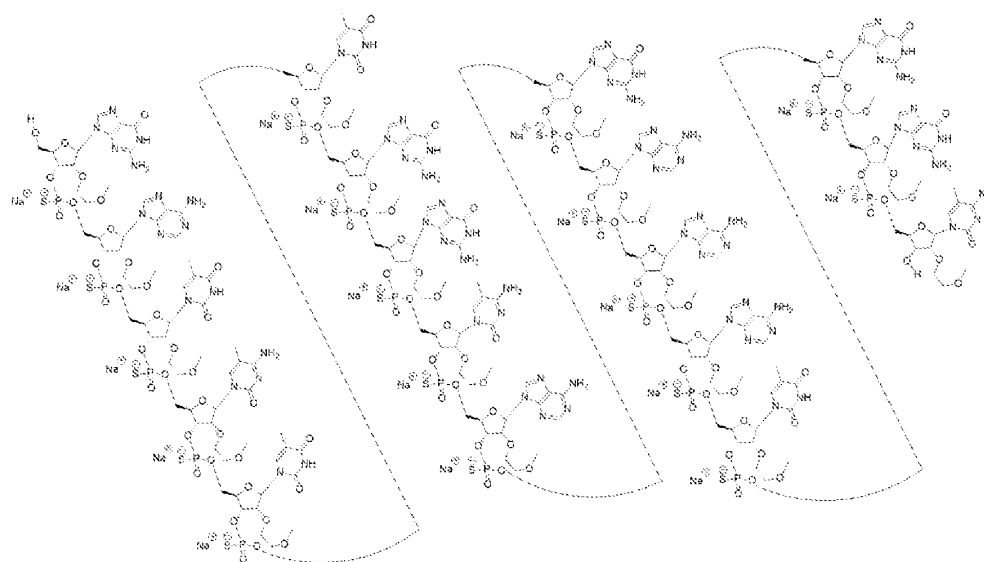
[00351] In some embodiments, the antisense oligomer has any one of the following chemical structures:



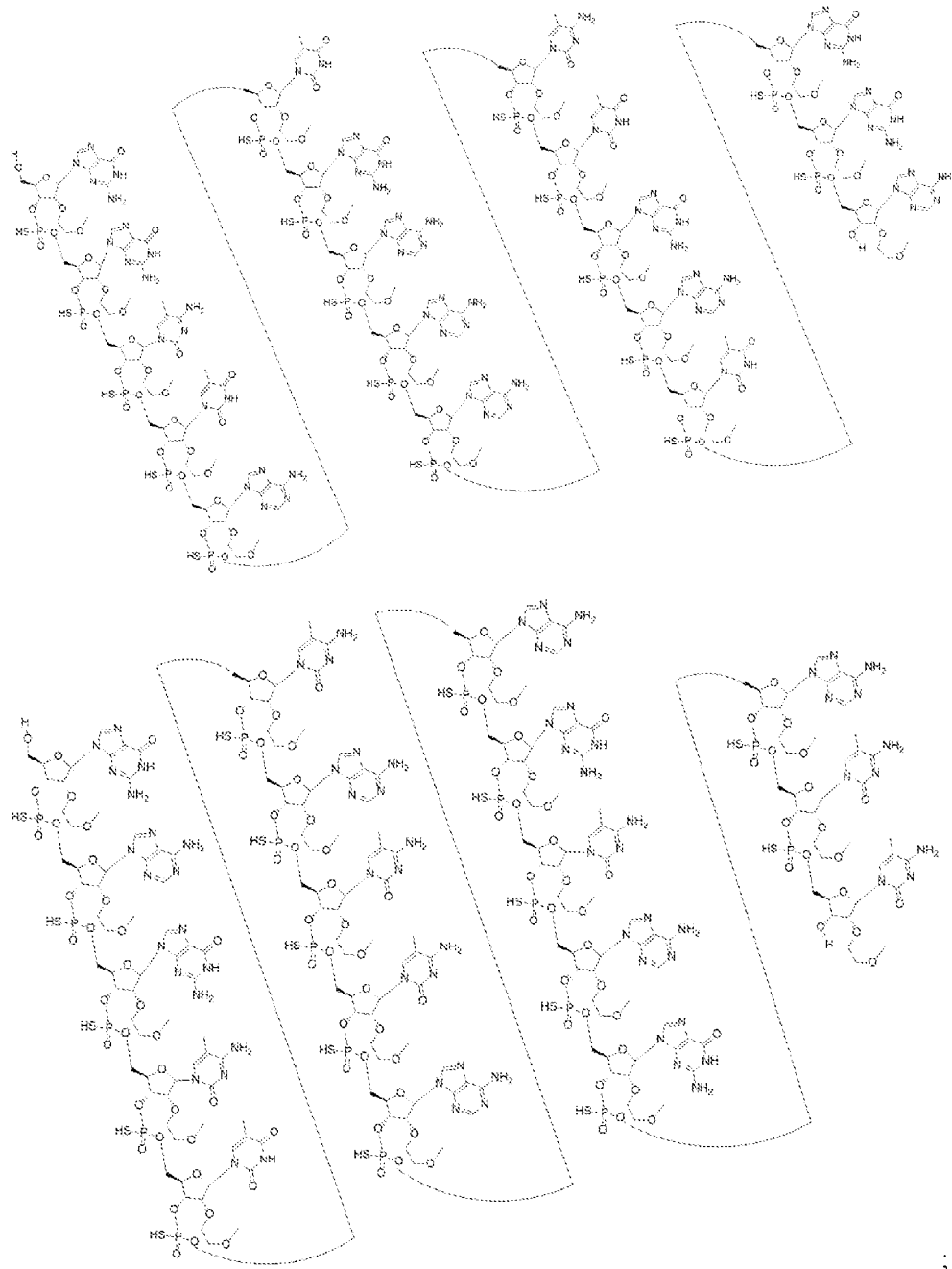


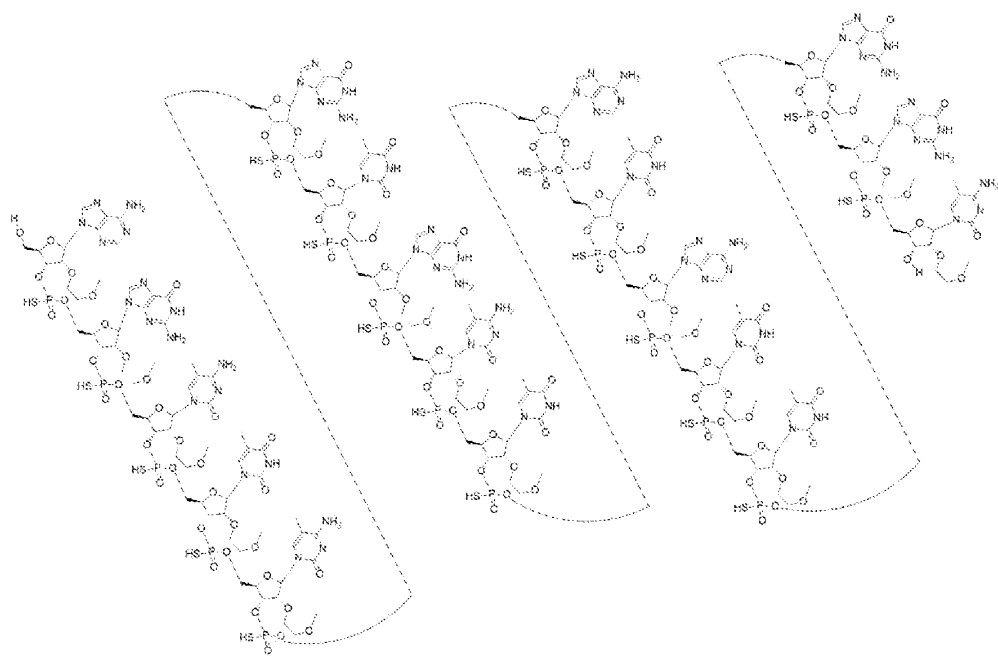
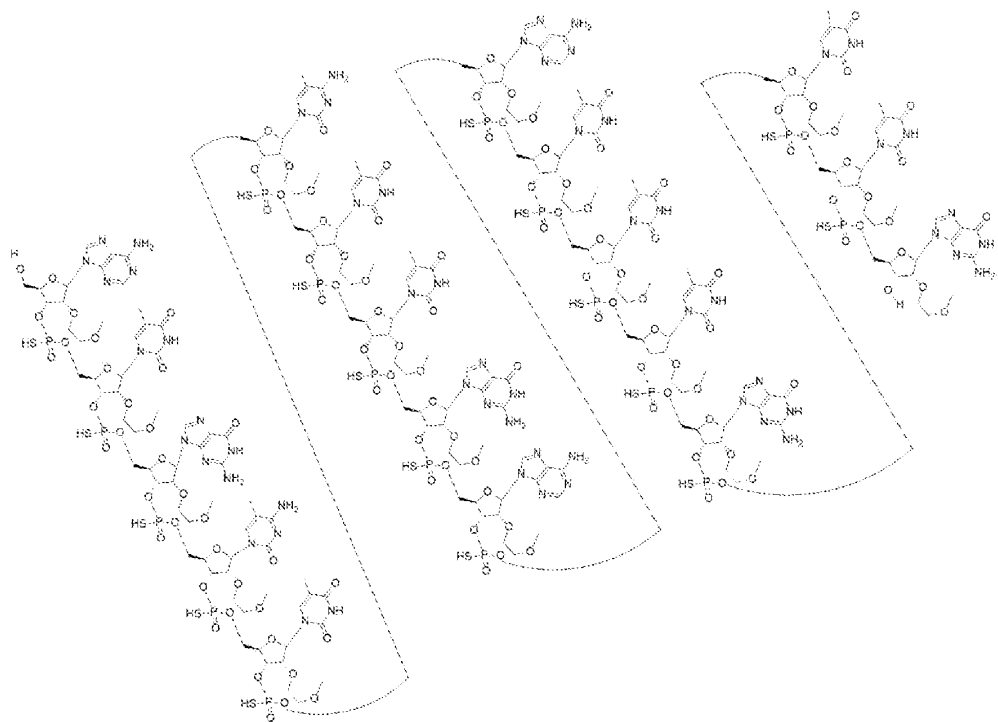


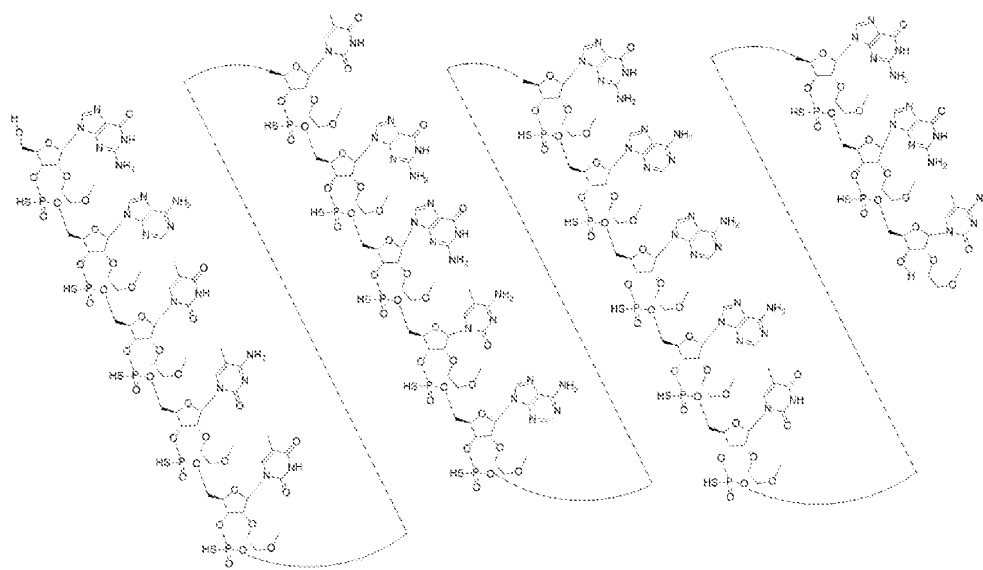
; or



[00352] In some embodiments, the antisense oligomer has any one of the following chemical structures:







or a pharmaceutically acceptable salt thereof.

[00353] Described herein, in some aspects, are uses of an antisense oligomer for the manufacture of a medicament for treating a disease or condition characterized by a reduced expression or function of OPA1 protein in a human subject in need thereof, or reducing likelihood of the subject developing the disease or condition, wherein the medicament is administered to one eye of the subject at a dose of about 0.005 mg to about 20 mg, and wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299.

[00354] Described herein, in some aspects, are uses of an antisense oligomer for the manufacture of a medicament for treating a disease or condition characterized by a reduced expression or function of OPA1 protein in a human subject in need thereof, or reducing likelihood of the subject developing the disease or condition, wherein the medicament is administered to one eye of the subject at a dose of about 0.005 mg to about 20 mg, and wherein the antisense oligomer consists of a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299.

[00355] In some embodiments, the medicament is administered to the one eye of the subject at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg to about 15 mg, about 0.01 mg to about 10 mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg, about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about

0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

[00356] In some embodiments, the medicament is administered to the one eye of the subject at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

[00357] In some embodiments, the medicament is administered to the one eye of the subject at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

[00358] In some embodiments, the medicament is administered to the one eye of the subject at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer. In some cases, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg to about 1.2 mg.

Combination Therapies

[00359] In some embodiments, the ASOs disclosed in the present disclosure can be used in combination with one or more additional therapeutic agents. In some embodiments, the one or more additional therapeutic agents can comprise a small molecule. For example, the one or more additional therapeutic agents can comprise a small molecule described in WO2016128343A1,

WO2017053982A1, WO2016196386A1, WO201428459A1, WO201524876A2, WO2013119916A2, and WO2014209841A2, which are incorporated by reference herein in their entirety. In some embodiments, the one or more additional therapeutic agents comprise an ASO that can be used to correct intron retention.

Treatment of Subjects

[00360] Any of the compositions provided herein may be administered to an individual. “Individual” may be used interchangeably with “subject” or “patient.” An individual may be a mammal, for example, a human or animal such as a non-human primate, a rodent, a rabbit, a rat, a mouse, a horse, a donkey, a goat, a cat, a dog, a cow, a pig, or a sheep. In embodiments, the individual is a human. In embodiments, the individual is a fetus, an embryo, or a child. In other embodiments, the individual may be another eukaryotic organism, such as a plant. In some embodiments, the compositions provided herein are administered to a cell *ex vivo*.

[00361] In some embodiments, the compositions provided herein are administered to an individual as a method of treating a disease or disorder. In some embodiments, the individual has a genetic disease, such as any of the diseases described herein. In some embodiments, the individual is at risk of having a disease, such as any of the diseases described herein. In some embodiments, the individual is at increased risk of having a disease or disorder caused by insufficient amount of a protein or insufficient activity of a protein. If an individual is “at an increased risk” of having a disease or disorder caused insufficient amount of a protein or insufficient activity of a protein, the method involves preventative or prophylactic treatment. For example, an individual may be at an increased risk of having such a disease or disorder because of family history of the disease. Typically, individuals at an increased risk of having such a disease or disorder benefit from prophylactic treatment (*e.g.*, by preventing or delaying the onset or progression of the disease or disorder). In embodiments, a fetus is treated *in utero*, *e.g.*, by administering the ASO composition to the fetus directly or indirectly (*e.g.*, via the mother).

[00362] In some cases, the subject pharmaceutical composition and method are applicable for treatment of a condition or disease associated with OPA1 deficiency. In some cases, the subject pharmaceutical composition and method are applicable for treatment of an eye disease or condition. In some cases, the subject pharmaceutical composition and method are applicable for treatment of Optic atrophy type 1, autosomal dominant optic atrophy (ADOA), ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile

mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[00363] In some embodiments, the disease or condition comprises Optic atrophy type 1. In some embodiments, the disease or condition comprises autosomal dominant optic atrophy (ADOA).

[00364] In some embodiments, the subject is characterized by having: (i) a heterozygous *OPA1* gene variant; (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments; (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity; (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or (v) any combination of (i)-(iv).

[00365] In some embodiments, the subject is additionally characterized by: (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPA1* gene; (2) not having only benign or likely benign variants in the *OPA1* gene; (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus); (4) not having been diagnosed with Behr syndrome; (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases; (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy; (7) not having or having a history of any ocular condition in either eye; (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering; (9) not having a history of retinal photocoagulation; (10) not having a history or presence of retinal vein occlusion; (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering; (12) not having dry age-related macular degeneration in either eye; (13) not having high myopia (>6 diopters); (14) not having a history of cancer (except

a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated); (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy; (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery; (17) any combination of (1)-(16).

[00366] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition and comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, and wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer, and wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

[00367] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition and comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 90% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, and wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer, and wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

[00368] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition and comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 100% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, and wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer, and wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

[00369] In some embodiments, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg

to about 15 mg, about 0.01 mg to about 10 mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg, about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

[00370] In some embodiments, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

[00371] In some embodiments, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

[00372] In some embodiments, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer. In

some cases, the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg to about 1.2 mg.

[00373] In some embodiments, the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μ L to about 250 μ L, about 10 μ L to about 250 μ L, about 20 μ L to about 250 μ L, about 30 μ L to about 250 μ L, about 40 μ L to about 250 μ L, about 50 μ L to about 250 μ L, about 60 μ L to about 250 μ L, about 70 μ L to about 250 μ L, about 80 μ L to about 250 μ L, about 100 μ L to about 250 μ L, about 120 μ L to about 250 μ L, about 150 μ L to about 250 μ L, about 160 μ L to about 250 μ L, about 180 μ L to about 500 μ L, about 200 μ L to about 250 μ L, about 220 μ L to about 250 μ L, about 5 μ L to about 220 μ L, about 10 μ L to about 220 μ L, about 20 μ L to about 220 μ L, about 30 μ L to about 220 μ L, about 40 μ L to about 220 μ L, about 50 μ L to about 220 μ L, about 60 μ L to about 220 μ L, about 70 μ L to about 220 μ L, about 80 μ L to about 220 μ L, about 100 μ L to about 220 μ L, about 120 μ L to about 220 μ L, about 150 μ L to about 220 μ L, about 160 μ L to about 220 μ L, about 180 μ L to about 220 μ L, about 5 μ L to about 200 μ L, about 10 μ L to about 200 μ L, about 20 μ L to about 200 μ L, about 30 μ L to about 200 μ L, about 40 μ L to about 200 μ L, about 50 μ L to about 200 μ L, about 60 μ L to about 200 μ L, about 70 μ L to about 200 μ L, about 80 μ L to about 200 μ L, about 100 μ L to about 200 μ L, about 120 μ L to about 200 μ L, about 150 μ L to about 200 μ L, about 160 μ L to about 200 μ L, about 180 μ L to about 200 μ L, about 5 μ L to about 180 μ L, about 10 μ L to about 180 μ L, about 20 μ L to about 180 μ L, about 30 μ L to about 180 μ L, about 40 μ L to about 180 μ L, about 50 μ L to about 180 μ L, about 60 μ L to about 180 μ L, about 70 μ L to about 180 μ L, about 80 μ L to about 180 μ L, about 100 μ L to about 180 μ L, about 120 μ L to about 180 μ L, about 150 μ L to about 180 μ L, about 5 μ L to about 150 μ L, about 10 μ L to about 150 μ L, about 20 μ L to about 150 μ L, about 30 μ L to about 150 μ L, about 40 μ L to about 150 μ L, about 50 μ L to about 150 μ L, about 60 μ L to about 150 μ L, about 70 μ L to about 150 μ L, about 80 μ L to about 150 μ L, about 100 μ L to about 150 μ L, about 120 μ L to about 150 μ L, about 5 μ L to about 120 μ L, about 10 μ L to about 120 μ L, about 20 μ L to about 120 μ L, about 30 μ L to about 120 μ L, about 40 μ L to about 120 μ L, about 50 μ L to about 120 μ L, about 60 μ L to about 120 μ L, about 70 μ L to about 120 μ L, about 80 μ L to about 120 μ L, about 100 μ L to about 120 μ L, about 5 μ L to about 100 μ L, about 10 μ L to about 100 μ L, about 20 μ L to about 100 μ L, about 30 μ L to about 100 μ L, about 40 μ L to about 100 μ L, about 50 μ L to about 100 μ L, about 60 μ L to about 100 μ L, about 70 μ L to about 100 μ L, about 80 μ L to about 100 μ L, about 5 μ L to about 80 μ L, about 10 μ L to about 80 μ L, about 20 μ L to about 80 μ L, about 30 μ L to about 80 μ L, about 40 μ L to about 80 μ L, about 50 μ L to about 80 μ L, about 60 μ L to about 80 μ L, about 5 μ L to about 60 μ L, about 10 μ L to about 60 μ L, about 20 μ L to about 60 μ L, about 30 μ L to about 60 μ L, about 40 μ L to about 60 μ L, or about 50 μ L to about 60 μ L.

[00374] In some embodiments, the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μ L, about 8 μ L, about 10 μ L, about 12 μ L, about 15 μ L, about 18 μ L, about 20 μ L, about 25 μ L, about 28 μ L, about 30 μ L, about 35 μ L, about 40 μ L, about 45 μ L, about 48 μ L, about 50 μ L, about 55 μ L, about 60 μ L, about 65 μ L, about 70 μ L, about 75 μ L, about 80 μ L, about 90 μ L, about 100 μ L, about 120 μ L, about 150 μ L, about 160 μ L, about 180 μ L, about 200 μ L, about 220 μ L, or about 250 μ L. In some cases, the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 50 μ L.

[00375] In some embodiments, the method comprises administering the pharmaceutical composition to both left eye and right eye of the subject. In some embodiments, the method comprises administering the pharmaceutical composition at the same dose to both the left eye and the right eye of the subject. In some embodiments, the method comprises administering the pharmaceutical composition at different doses to the left eye and the right eye of the subject. In some embodiments, the method comprises administering the pharmaceutical composition to at least one eye of the subject.

[00376] In some embodiments, the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 or 280-299. In some embodiments, the antisense oligomer consists of a nucleotide sequence having at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 or 280-299.

[00377] In some embodiments, the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292. In some embodiments, the antisense oligomer consists of a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

[00378] In some embodiments, administering comprises administering multiple doses of the pharmaceutical composition to the human subject. In some embodiments, administering comprises administering a first dose of the pharmaceutical composition to the human subject and a subsequent dose of the pharmaceutical composition to the human subject. In some embodiments, the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is not tolerated. In some embodiments, the subsequent dose is the same as the previous dose following an indication that administration of the previous dose is tolerated. In some embodiments, the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is tolerated. In some embodiments, the subsequent dose is the

same as the previous dose following an indication that administration of the previous dose is effective. In some embodiments, the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is effective. In some embodiments, the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is not effective.

[00379] In some embodiments, the pharmaceutical composition is a liquid composition. In some embodiments, the method comprises administering the pharmaceutical composition as a bolus injection over 1 to 60 minutes, 1 to 50 minutes, 1 to 40 minutes, 1 to 30 minutes, 1 to 20 minutes, 1 to 10 minutes, 1 to 5 minutes, or 1 to 3 minutes.

[00380] In some embodiments, the method comprises administering the pharmaceutical composition as a bolus injection. In some embodiments, the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection. In some embodiments, the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection. In some embodiments, the antisense oligomer is solubilized or diluted in an isotonic solution. In some embodiments, the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8. In some embodiments, the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution. In some embodiments, the pharmaceutical formulation does not comprise a preservative.

[00381] In some embodiments, the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL.

[00382] In some embodiments, the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL, about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL

to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

[00383] In some embodiments, the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL. Autosomal dominant optic atrophy (ADOA) is the most common inherited optic nerve disorder and is characterized by retinal ganglion cell loss. In some cases, 65-90% of ADOA cases are caused by mutations in one allele of the *OPA1* gene. *OPA1* gene encodes an OPA1 protein that is a mitochondrial GTPase, which can have a critical maintenance role in mitochondria structure and function. Most *OPA1* mutations can lead to a haploinsufficiency, resulting in about a 50% decrease of normal OPA1 protein levels. Approximately 1 out of 30,000 people are affected globally with a higher incidence of ~1 out of 10,000 in Denmark due to a founder effect. ADOA can present within the first decade of life. 80% of ADOA patients are symptomatic before 10 years of age. The disease can cause progressive and irreversible vision loss and up to 46% of patients are registered as legally blind.

[00384] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence

identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by having: (i) a heterozygous *OPA1* gene variant; (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments; (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity; (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or (v) any combination of (i)-(iv).

[00385] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 90% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by having: (i) a heterozygous *OPA1* gene variant; (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments; (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity; (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or (v) any combination of (i)-(iv).

[00386] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 100% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by having: (i) a heterozygous *OPA1* gene variant; (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments; (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity; (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or (v) any combination of (i)-(iv).

[00387] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is

characterized by: (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPAI* gene; (2) not having only benign or likely benign variants in the *OPAI* gene; (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus); (4) not having been diagnosed with Behr syndrome; (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases; (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy; (7) not having or having a history of any ocular condition in either eye; (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering; (9) not having a history of retinal photocoagulation; (10) not having a history or presence of retinal vein occlusion; (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering; (12) not having dry age-related macular degeneration in either eye; (13) not having high myopia (>6 diopters); (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated); (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy; (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery; or (17) any combination of (1)-(16).

[00388] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 90% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by: (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPAI* gene; (2) not having only benign or likely benign variants in the *OPAI* gene; (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus); (4) not having been diagnosed with Behr syndrome; (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases; (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy; (7) not having or having a history of any ocular condition in either eye; (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the

administering; (9) not having a history of retinal photocoagulation; (10) not having a history or presence of retinal vein occlusion; (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering; (12) not having dry age-related macular degeneration in either eye; (13) not having high myopia (>6 diopters); (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated); (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy; (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery; or (17) any combination of (1)-(16).

[00389] In some embodiments, the method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition comprises administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer comprises a nucleotide sequence having at least 100% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by: (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPA1* gene; (2) not having only benign or likely benign variants in the *OPA1* gene; (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus); (4) not having been diagnosed with Behr syndrome; (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases; (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy; (7) not having or having a history of any ocular condition in either eye; (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering; (9) not having a history of retinal photocoagulation; (10) not having a history or presence of retinal vein occlusion; (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering; (12) not having dry age-related macular degeneration in either eye; (13) not having high myopia (>6 diopters); (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated); (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy; (16) not having any history of nutritional deficiency (including B12 and/or

folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery; or (17) any combination of (1)-(16).

[00390] In some cases, a therapeutic agent comprises an oligonucleotide. In some cases, a therapeutic agent comprises a vector, *e.g.*, a viral vector, expressing an oligonucleotide that binds to the targeted region of a pre-mRNA that encodes the target peptide sequence. The methods provided herein can be adapted to contacting a vector that encodes an agent, *e.g.*, an oligonucleotide, to a cell, so that the agent binds to a pre-mRNA in the cell and modulates the processing of the pre-mRNA. In some cases, the viral vector comprises an adenoviral vector, adeno-associated viral (AAV) vector, lentiviral vector, Herpes Simplex Virus (HSV) viral vector, retroviral vector, or any applicable viral vector. In some cases, a therapeutic agent comprises a gene editing tool that is configured to modify a gene encoding the target peptide sequence such that a gene region that encodes the inefficient translation region is deleted. In some cases, a gene editing tool comprises vector, *e.g.*, viral vector, for gene editing based on CRISPR-Cas9, TALEN, Zinc Finger, or other applicable technologies.

[00391] Suitable routes for administration of ASOs of the present disclosure may vary depending on cell type to which delivery of the ASOs is desired. Multiple tissues and organs are affected by ADOA, with the eye being the most significantly affected tissue. The ASOs of the present disclosure may be administered to patients parenterally, for example, by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[00392] In some embodiments, the pharmaceutical composition is administered via intravitreal injection. In some embodiments, the method further comprises administering an additional therapeutic agent. In some embodiments, the additional therapeutic agent comprises a small molecule. In some embodiments, the additional therapeutic agent comprises an antisense oligomer. In some embodiments, the additional therapeutic agent comprises an ophthalmologic drug. In some embodiments, the additional therapeutic agent consists of an antisense oligomer. In some embodiments, the additional therapeutic agent consists of an ophthalmologic drug.

[00393] In embodiments, the antisense oligonucleotide is administered with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier by any method known in the art. For example, delivery of agents by administration of an adenovirus vector to motor neurons in muscle tissue is described in U.S. Pat. No. 6,632,427, "Adenoviral-vector-mediated gene transfer into medullary motor neurons," incorporated herein by reference. Delivery of vectors directly to the brain, *e.g.*, the striatum, the thalamus, the hippocampus, or the substantia nigra, is described, *e.g.*, in U.S. Pat. No. 6,756,523, "Adenovirus

vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain,” incorporated herein by reference.

[00394] In some embodiments, the antisense oligonucleotides are linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. In embodiments, the antisense oligonucleotide is coupled to a substance, known in the art to promote penetration or transport across the blood-brain barrier, *e.g.*, an antibody to the transferrin receptor. In embodiments, the antisense oligonucleotide is linked with a viral vector, *e.g.*, to render the antisense compound more effective or increase transport across the blood-brain barrier. In embodiments, osmotic blood brain barrier disruption is assisted by infusion of sugars, *e.g.*, meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids, *e.g.*, glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, *e.g.*, in U.S. Pat. No. 9,193,969, “Compositions and methods for selective delivery of oligonucleotide molecules to specific neuron types,” U.S. Pat. No. 4,866,042, “Method for the delivery of genetic material across the blood brain barrier,” U.S. Pat. No. 6,294,520, “Material for passage through the blood-brain barrier,” and U.S. Pat. No. 6,936,589, “Parenteral delivery systems,” each incorporated herein by reference.

[00395] In some embodiments, subjects treated using the methods and compositions are evaluated for improvement in condition using any methods known and described in the art.

Methods of Identifying Additional ASOs that Induce Exon Skipping

[00396] Also within the scope of the present disclosure are methods for identifying or determining ASOs that induce exon skipping of an *OPAI* NMD exon-containing pre-mRNA. For example, a method can comprise identifying or determining ASOs that induce pseudo-exon skipping of an *OPAI* NMD exon-containing pre-mRNA. ASOs that specifically hybridize to different nucleotides within the target region of the pre-mRNA may be screened to identify or determine ASOs that improve the rate and/or extent of splicing of the target intron. In some embodiments, the ASO may block or interfere with the binding site(s) of a splicing repressor(s)/silencer. Any method known in the art may be used to identify (determine) an ASO that when hybridized to the target region of the exon results in the desired effect (*e.g.*, pseudo-exon skipping, protein or functional RNA production). These methods also can be used for identifying ASOs that induce exon skipping of the included exon by binding to a targeted region

in an intron flanking the included exon, or in a non-included exon. An example of a method that may be used is provided below.

[00397] A round of screening, referred to as an ASO “walk” may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. For example, the ASOs used in the ASO walk can be tiled every 5 nucleotides from approximately 100 nucleotides upstream of the 3’ splice site of the included exon (*e.g.*, a portion of sequence of the exon located upstream of the target/included exon) to approximately 100 nucleotides downstream of the 3’ splice site of the target/included exon and/or from approximately 100 nucleotides upstream of the 5’ splice site of the included exon to approximately 100 nucleotides downstream of the 5’ splice site of the target/included exon (*e.g.*, a portion of sequence of the exon located downstream of the target/included exon). For example, a first ASO of 15 nucleotides in length may be designed to specifically hybridize to nucleotides +6 to +20 relative to the 3’ splice site of the target/included exon. A second ASO may be designed to specifically hybridize to nucleotides +11 to +25 relative to the 3’ splice site of the target/included exon. ASOs are designed as such spanning the target region of the pre-mRNA. In embodiments, the ASOs can be tiled more closely, *e.g.*, every 1, 2, 3, or 4 nucleotides. Further, the ASOs can be tiled from 100 nucleotides downstream of the 5’ splice site, to 100 nucleotides upstream of the 3’ splice site. In some embodiments, the ASOs can be tiled from about 1,160 nucleotides upstream of the 3’ splice site, to about 500 nucleotides downstream of the 5’ splice site. In some embodiments, the ASOs can be tiled from about 500 nucleotides upstream of the 3’ splice site, to about 1,920 nucleotides downstream of the 3’ splice site.

[00398] One or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region) are delivered, for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA (*e.g.*, a NMD exon-containing pre-mRNA described herein). The exon skipping effects of each of the ASOs may be assessed by any method known in the art, for example, by reverse transcriptase (RT)-PCR using primers that span the splice junction, as described in Example 4. A reduction or absence of a longer RT-PCR product produced using the primers spanning the region containing the included exon (*e.g.*, including the flanking exons of the NMD exon) in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target NMD exon has been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NMD exon), the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced functional protein production). Any method

known in the art for assessing and/or quantifying protein production, such as Western Blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00399] A second round of screening, referred to as an ASO “micro-walk” may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. The ASOs used in the ASO micro-walk are tiled every 1 nucleotide to further refine the nucleotide acid sequence of the pre-mRNA that when hybridized with an ASO results in exon skipping (or enhanced splicing of NMD exon).

[00400] Regions defined by ASOs that promote splicing of the target intron are explored in greater detail by means of an ASO “micro-walk”, involving ASOs spaced in 1-nt steps, as well as longer ASOs, typically 18-25 nt.

[00401] As described for the ASO walk above, the ASO micro-walk is performed by delivering one or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region), for example, by transfection, into a disease-relevant cell line that expresses the target pre-mRNA. The splicing-inducing effects of each of the ASOs may be assessed by any method known in the art, for example, by reverse transcriptase (RT)-PCR using primers that span the NMD exon, as described herein (see, *e.g.*, **Example 4**). A reduction or absence of a longer RT-PCR product produced using the primers spanning the NMD exon in ASO-treated cells as compared to in control ASO-treated cells indicates that exon skipping (or splicing of the target intron containing an NMD exon) has been enhanced. In some embodiments, the exon skipping efficiency (or the splicing efficiency to splice the intron containing the NMD exon), the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced functional protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western Blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00402] ASOs that when hybridized to a region of a pre-mRNA result in exon skipping (or enhanced splicing of the intron containing a NMD exon) and increased protein production may be tested *in vivo* using animal models, for example, transgenic mouse models in which the full-length human gene has been knocked-in or in humanized mouse models of disease. Suitable routes for administration of ASOs may vary depending on the disease and/or the cell types to which delivery of the ASOs is desired. ASOs may be administered, for example, by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection. Following administration, the cells, tissues, and/or organs of the model animals may be assessed to determine the effect of the ASO

treatment by, for example, evaluating splicing (*e.g.*, efficiency, rate, extent) and protein production by methods known in the art and described herein. The animal models may also be any phenotypic or behavioral indication of the disease or disease severity.

[00403] Also within the scope of the present disclosure is a method to identify or validate an NMD-inducing exon in the presence of an NMD inhibitor, for example, cycloheximide. An exemplary method is provided in Example 2.

SPECIFIC EMBODIMENTS

[00404] Embodiment A1. A method of treating Optic atrophy type 1 in a subject in need thereof, by increasing the expression of a target protein or functional RNA by a cell of the subject, wherein the cell has an mRNA that contains a nonsense-mediated RNA decay-inducing exon (NMD exon mRNA), and wherein the NMD exon mRNA encodes the target protein or functional RNA, the method comprising contacting the cell of the subject with a therapeutic agent that binds to a targeted portion of the NMD exon mRNA encoding the target protein or functional RNA, whereby the nonsense-mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding the target protein or functional RNA, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cell of the subject.

[00405] Embodiment A2. The method of embodiment A1, wherein the target protein is OPA1.

[00406] Embodiment A3. A method of increasing expression of OPA1 protein by a cell having an mRNA that contains a nonsense-mediated RNA decay-inducing exon (NMD exon mRNA) and encodes OPA1 protein, the method comprising contacting the cell with an agent that binds to a targeted portion of the NMD exon mRNA encoding OPA1 protein, whereby the nonsense-mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding OPA1 protein, thereby increasing the level of mRNA encoding OPA1 protein, and increasing the expression of OPA1 protein in the cell.

[00407] Embodiment A4. The method of any one of embodiments A1 to A3, wherein the nonsense-mediated RNA decay-inducing exon is spliced out from the NMD exon mRNA encoding the target protein or functional RNA.

[00408] Embodiment A5. The method of any one of embodiments A1 to A4, wherein the target protein does not comprise an amino acid sequence encoded by the nonsense-mediated RNA decay-inducing exon.

[00409] Embodiment A6. The method of any one of embodiments A1 to A5, wherein the target protein is a full-length target protein.

[00410] Embodiment A7. The method of any one of embodiments A1 to A6, wherein the agent is an antisense oligomer (ASO) complementary to the targeted portion of the NMD exon mRNA.

[00411] Embodiment A8. The method of any one of embodiments A1 to A7, wherein the mRNA is pre-mRNA.

[00412] Embodiment A9. The method of any one of embodiments A1 to A8, wherein the contacting comprises contacting the therapeutic agent to the mRNA, wherein the mRNA is in a nucleus of the cell.

[00413] Embodiment A10. The method of any one of embodiments A1 to A9, wherein the target protein or the functional RNA corrects a deficiency in the target protein or functional RNA in the subject.

[00414] Embodiment A11. The method of any one of embodiments A1 to A10, wherein the cells are in or from a subject with a condition caused by a deficient amount or activity of an OPA1 protein.

[00415] Embodiment A12. The method of any one of embodiments A1 to A11, wherein the deficient amount of the target protein is caused by haploinsufficiency of the target protein, wherein the subject has a first allele encoding a functional target protein, and a second allele from which the target protein is not produced, or a second allele encoding a nonfunctional target protein, and wherein the antisense oligomer binds to a targeted portion of a NMD exon mRNA transcribed from the first allele.

[00416] Embodiment A13. The method of any one of embodiments A1 to A11, wherein the subject has a condition caused by a disorder resulting from a deficiency in the amount or function of the target protein, wherein the subject has a first mutant allele from which

(i) the target protein is produced at a reduced level compared to production from a wild-type allele,

(ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

(iii) the target protein is not produced, and

(b) a second mutant allele from which

(i) the target protein is produced at a reduced level compared to production from a wild-type allele,

(ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

(iii) the target protein is not produced, and

wherein when the subject has a first mutant allele (a)(iii), the second mutant allele is (b)(i) or (b)(ii) and wherein when the subject has a second mutant allele (b)(iii), the first mutant allele is (a)(i) or (a)(ii), and wherein the NMD exon mRNA is transcribed from either the first mutant allele that is (a)(i) or (a)(ii), and/or the second allele that is (b)(i) or (b)(ii).

[00417] Embodiment A14. The method of embodiment A13, wherein the target protein is produced in a form having reduced function compared to the equivalent wild-type protein.

[00418] Embodiment A15. The method of embodiment A13, wherein the target protein is produced in a form that is fully-functional compared to the equivalent wild-type protein.

[00419] Embodiment A16. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is within the nonsense-mediated RNA decay-inducing exon.

[00420] Embodiment A17. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is either upstream or downstream of the nonsense-mediated RNA decay-inducing exon.

[00421] Embodiment A18. The method of any one of embodiments A1 to A17, wherein the NMD exon mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3.

[00422] Embodiment A19. The method of any one of embodiments A1 to A17, wherein the NMD exon mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1.

[00423] Embodiment A20. The method of any one of embodiments A1 to A17, wherein the targeted portion of the NMD exon mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[00424] Embodiment A21. The method of any one of embodiments A1 to A20, wherein the agent is an antisense oligomer (ASO) and wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[00425] Embodiment A22. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is within the nonsense-mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00426] Embodiment A23. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA is upstream or downstream of the nonsense-mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00427] Embodiment A24. The method of any one of embodiments A1 to A15, wherein the targeted portion of the NMD exon mRNA comprises an exon-intron junction exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00428] Embodiment A25. The method of any one of embodiments A1 to A24, wherein the target protein produced is full-length protein, or wild-type protein.

[00429] Embodiment A26. The method of any one of embodiments A1 to A25, wherein the total amount of the mRNA encoding the target protein or functional RNA produced in the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of the mRNA encoding the target protein or functional RNA produced in a control cell.

[00430] Embodiment A27. The method of any one of embodiments A1 to A25, wherein the total amount of the mRNA encoding the target protein or functional RNA produced in the cell contacted with the antisense oligomer is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300%, compared to the total amount of the mRNA encoding the target protein or functional RNA produced in a control cell.

[00431] Embodiment A28. The method of one any of embodiments A1 to A25, wherein the total amount of target protein produced by the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-

fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of target protein produced by a control cell.

[00432] Embodiment A29. The method of one any of embodiments A1 to A25, wherein the total amount of target protein produced by the cell contacted with the antisense oligomer is increased about 20% to about 300%, about 50% to about 300%, about 100% to about 300%, about 150% to about 300%, about 20% to about 50%, about 20% to about 100%, about 20% to about 150%, about 20% to about 200%, about 20% to about 250%, about 50% to about 100%, about 50% to about 150%, about 50% to about 200%, about 50% to about 250%, about 100% to about 150%, about 100% to about 200%, about 100% to about 250%, about 150% to about 200%, about 150% to about 250%, about 200% to about 250%, at least about 10%, at least about 20%, at least about 50%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300%, compared to the total amount of target protein produced by a control cell.

[00433] Embodiment A30. The method of any one of embodiments A1 to A29, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00434] Embodiment A31. The method of any one of embodiments A1 to A30, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[00435] Embodiment A32. The method of any one of embodiments A1 to A31, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[00436] Embodiment A33. The method of embodiment A32, wherein each sugar moiety is a modified sugar moiety.

[00437] Embodiment A34. The method of any one of embodiments A1 to A33, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35

nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[00438] Embodiment A35. The method of any one of embodiments A1 to A34, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted portion of the NMD exon mRNA encoding the protein.

[00439] Embodiment A36. The method of any one of embodiments A1 to A35, wherein the method further comprises assessing *OPAI* mRNA or protein expression.

[00440] Embodiment A37. The method of any one of embodiments A1 to A36, wherein Optic atrophy type 1 is treated and wherein the antisense oligomer binds to a targeted portion of an *OPAI* NMD exon mRNA, wherein the targeted portion is within SEQ ID NO: 2 or 3.

[00441] Embodiment A38. The method of any one of embodiments A1 to A37, wherein the subject is a human.

[00442] Embodiment A39. The method of any one of embodiments A1 to A38, wherein the subject is a non-human animal.

[00443] Embodiment A40. The method of any one of embodiments A1 to A39, wherein the subject is a fetus, an embryo, or a child.

[00444] Embodiment A41. The method of any one of embodiments A1 to A40, wherein the cells are *ex vivo*.

[00445] Embodiment A42. The method of any one of embodiments A1 to A41, wherein the therapeutic agent is administered by intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject.

[00446] Embodiment A43. The method of any of embodiments A1 to A42, wherein the method further comprises administering a second therapeutic agent to the subject.

[00447] Embodiment A44. The method of embodiment A43, wherein the second therapeutic agent is a small molecule.

[00448] Embodiment A45. The method of embodiment A43, wherein the second therapeutic agent is an ASO.

[00449] Embodiment A46. The method of any one of embodiments A43 to A45, wherein the second therapeutic agent corrects intron retention.

[00450] Embodiment A47. An antisense oligomer as used in a method of any of embodiments A1 to A46.

[00451] Embodiment A48. An antisense oligomer comprising a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[00452] Embodiment A49. A pharmaceutical composition comprising the antisense oligomer of embodiment A47 or A48 and an excipient.

[00453] Embodiment A50. A method of treating a subject in need thereof, comprising administering the pharmaceutical composition of embodiment A49 to the subject, wherein the administering is by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[00454] Embodiment A51. A composition comprising a therapeutic agent for use in a method of increasing expression of a target protein or a functional RNA by cells to treat Optic atrophy type 1 in a subject in need thereof, associated with a deficient protein or deficient functional RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject, wherein the target protein is:

- (a) the deficient protein; or
- (b) a compensating protein which functionally augments or replaces the deficient protein or in the subject;

and wherein the functional RNA is:

- (c) the deficient RNA; or
- (d) a compensating functional RNA which functionally augments or replaces the deficient functional RNA in the subject;

wherein the therapeutic agent enhances exclusion of the nonsense-mediated RNA decay-inducing exon from the NMD exon mRNA encoding the target protein or functional RNA, thereby increasing production or activity of the target protein or the functional RNA in the subject.

[00455] Embodiment A52. A composition comprising a therapeutic agent for use in a method of treating a condition associated with OPA1 protein in a subject in need thereof, the method comprising the step of increasing expression of OPA1 protein by cells of the subject, wherein the cells have an mRNA that contains a nonsense-mediated RNA decay-inducing exon (NMD exon mRNA) and encodes OPA1 protein, the method comprising contacting the cells with the therapeutic agent, whereby the nonsense-mediated RNA decay-inducing exon is excluded from the NMD exon mRNA that encodes OPA1 protein, thereby increasing the level of mRNA encoding OPA1 protein, and increasing the expression of OPA1 protein in the cells of the subject.

[00456] Embodiment A53. The composition of embodiment A52, wherein the condition is a disease or disorder.

[00457] Embodiment A54. The composition of embodiment A53, wherein the disease or disorder is Optic atrophy type 1.

[00458] Embodiment A55. The composition of any one of embodiments A52 to 54, wherein the OPA1 protein and NMD exon mRNA are encoded by the *OPA1* gene.

[00459] Embodiment A56. The composition of any one of embodiments A51 to A55, wherein the nonsense-mediated RNA decay-inducing exon is spliced out from the NMD exon mRNA encoding the OPA1 protein.

[00460] Embodiment A57. The composition of any one of embodiments A51 to A56, wherein the OPA1 protein does not comprise an amino acid sequence encoded by the nonsense-mediated RNA decay-inducing exon.

[00461] Embodiment A58. The composition of any one of embodiments A51 to A57, wherein the OPA1 protein is a full-length OPA1 protein.

[00462] Embodiment A59. The composition of any one of embodiments A51 to A58, wherein the therapeutic agent is an antisense oligomer (ASO) complementary to the targeted portion of the NMD exon mRNA.

[00463] Embodiment A60. The composition of any of embodiments A51 to A59, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer targets a portion of the NMD exon mRNA that is within the nonsense-mediated RNA decay-inducing exon.

[00464] Embodiment A61. The composition of any of embodiments A51 to A59, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer targets a portion of the NMD exon mRNA that is upstream or downstream of the nonsense-mediated RNA decay-inducing exon.

[00465] Embodiment A62. The composition of any one of embodiments A51 to A61, wherein the target protein is OPA1.

[00466] Embodiment A63. The composition of embodiment A62, wherein the NMD exon mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 2 or 3.

[00467] Embodiment A64. The composition of embodiment A62, wherein the NMD exon mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1.

[00468] Embodiment A65. The composition of embodiment A62, wherein the targeted portion of the NMD exon mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[00469] Embodiment A66. The composition of any one of embodiments A62 to A65, wherein the targeted portion of the NMD exon mRNA is within the nonsense-mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00470] Embodiment A67. The composition of any one of embodiments A62 to A65, wherein the targeted portion of the NMD exon mRNA is upstream or downstream of the nonsense-mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00471] Embodiment A68. The composition of any one of embodiments A62 to A65, wherein the targeted portion of the NMD exon mRNA comprises an exon-intron junction of exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00472] Embodiment A69. The composition of any one of embodiments A62 to A68, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[00473] Embodiment A70. The composition of any one of embodiments A51 to A69, wherein the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA.

[00474] Embodiment A71. The composition of any one of embodiments A51 to A70, wherein the target protein produced is full-length protein, or wild-type protein.

[00475] Embodiment A72. The composition of any one of embodiments A51 to A71, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00476] Embodiment A73. The composition of any of embodiments A51 to A72, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein said antisense oligomer is an antisense oligonucleotide.

[00477] Embodiment A74. The composition of any of embodiments A51 to A73, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[00478] Embodiment A75. The composition of any of embodiments A51 to A74, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[00479] Embodiment A76. The composition of embodiment A75, wherein each sugar moiety is a modified sugar moiety.

[00480] Embodiment A77. The composition of any of embodiments A51 to A76, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[00481] Embodiment A78. A pharmaceutical composition comprising the therapeutic agent of any of the compositions of embodiments A51 to A77, and an excipient.

[00482] Embodiment A79. A method of treating a subject in need thereof, comprising administering the pharmaceutical composition of embodiment A78 to the subject, wherein the administering is by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[00483] Embodiment A80. The method of any of embodiments A51 to A79, wherein the method further comprises administering a second therapeutic agent to the subject.

[00484] Embodiment A81. The method of embodiment A80, wherein the second therapeutic agent is a small molecule.

[00485] Embodiment A82. The method of embodiment A80, wherein the second therapeutic agent is an ASO.

[00486] Embodiment A83. The method of any one of embodiments A80 to A82, wherein the second therapeutic agent corrects intron retention.

[00487] Embodiment A84. A pharmaceutical composition comprising: an antisense oligomer that hybridizes to a target sequence of an *OPAI* mRNA transcript, wherein the *OPAI* mRNA transcript comprises a nonsense-mediated RNA decay-inducing exon, wherein the antisense oligomer induces exclusion of the nonsense-mediated RNA decay-inducing exon from the *OPAI* mRNA transcript; and a pharmaceutical acceptable excipient.

[00488] Embodiment A85. The pharmaceutical composition of embodiment A84, wherein the *OPAI* mRNA transcript is an *OPAI* NMD exon mRNA transcript.

[00489] Embodiment A86. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the NMD exon mRNA is within the nonsense-mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00490] Embodiment A87. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the NMD exon mRNA is upstream or downstream of the nonsense-mediated RNA decay-inducing exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00491] Embodiment A88. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the NMD exon mRNA comprises an exon-intron junction exon 6x of *OPAI*, exon 7x of *OPAI*, or exon 28x of *OPAI*.

[00492] Embodiment A89. The pharmaceutical composition of any one of embodiments A84 to A88, wherein the *OPAI* NMD exon mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 1.

[00493] Embodiment A90. The pharmaceutical composition of embodiment A84 or A88, wherein the *OPAI* NMD exon mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 2 or 3.

[00494] Embodiment A91. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00495] Embodiment A92. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer is an antisense oligonucleotide.

[00496] Embodiment A93. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[00497] Embodiment A94. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises at least one modified sugar moiety.

[00498] Embodiment A95. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[00499] Embodiment A96. The pharmaceutical composition of embodiment A84 or A85, wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least

98%, at least 99%, or is 100% complementary to a targeted portion of the *OPAI* NMD exon mRNA transcript.

[00500] Embodiment A97. The pharmaceutical composition of embodiment A84 or A85, wherein the targeted portion of the *OPAI* NMD exon mRNA transcript is within SEQ ID NO: 2 or 3.

[00501] Embodiment A98. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a nucleotide sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[00502] Embodiment A99. The pharmaceutical composition of embodiment A84, wherein the antisense oligomer comprises a nucleotide sequence that is identical a region comprising at least 8 contiguous nucleic acids SEQ ID NO: 2 or 3.

[00503] Embodiment A100. The pharmaceutical composition of any one of the embodiments A84 to A99, wherein the pharmaceutical composition is formulated for intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[00504] Embodiment A101. The method of any of embodiments A84 to A100, wherein the method further comprises administering a second therapeutic agent to the subject.

[00505] Embodiment A102. The method of embodiment A101, wherein the second therapeutic agent is a small molecule.

[00506] Embodiment A103. The method of embodiment A101, wherein the second therapeutic agent is an ASO.

[00507] Embodiment A104. The method of any one of embodiments A101 to A103, wherein the second therapeutic agent corrects intron retention.

[00508] Embodiment A105. A method of inducing processing of a deficient *OPAI* mRNA transcript to facilitate removal of a nonsense-mediated RNA decay-inducing exon to produce a fully processed *OPAI* mRNA transcript that encodes a functional form of an OPA1 protein, the method comprising:

- (a) contacting an antisense oligomer to a target cell of a subject;
- (b) hybridizing the antisense oligomer to the deficient *OPAI* mRNA transcript, wherein the deficient *OPAI* mRNA transcript is capable of encoding the functional form of an OPA1 protein and comprises at least one nonsense-mediated RNA decay-inducing exon;
- (c) removing the at least one nonsense-mediated RNA decay-inducing exon from the deficient *OPAI* mRNA transcript to produce the fully processed *OPAI* mRNA transcript that encodes the functional form of OPA1 protein; and

(d) translating the functional form of OPA1 protein from the fully processed *OPA1* mRNA transcript.

[00509] Embodiment A106. A method of treating a subject having a condition caused by a deficient amount or activity of OPA1 protein comprising administering to the subject an antisense oligomer comprising a nucleotide sequence with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 2 or 3.

[00510] Embodiment A107. A method of treating Optic atrophy type 1 in a subject in need thereof, by increasing the expression of a target protein or functional RNA by a cell of the subject, wherein the cell has an mRNA that contains a nonsense-mediated RNA decay-inducing exon (NMD exon mRNA), and wherein the NMD exon mRNA encodes the target protein or functional RNA, the method comprising contacting the cell of the subject with a therapeutic agent that modulates splicing of the NMD exon mRNA encoding the target protein or functional RNA, whereby the nonsense-mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding the target protein or functional RNA, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cell of the subject.

[00511] Embodiment A108. A method of increasing expression of OPA1 protein by a cell having an mRNA that contains a nonsense-mediated RNA decay-inducing exon (NMD exon mRNA) and encodes OPA1 protein, the method comprising contacting the cell with an agent that modulates splicing of the NMD exon mRNA encoding OPA1 protein, whereby the nonsense-mediated RNA decay-inducing exon is excluded from the NMD exon mRNA encoding OPA1 protein, thereby increasing the level of mRNA encoding OPA1 protein, and increasing the expression of OPA1 protein in the cell.

[00512] Embodiment A109. The method of embodiment A107 or A108, wherein the agent (a) binds to a targeted portion of the NMD exon mRNA encoding the target protein or functional RNA;

(b) binds to one or more components of a spliceosome; or

(c) a combination of (a) and (b).

[00513] Embodiment B1. A method of modulating expression of a target protein, by a cell having an mRNA that comprises a nonsense-mediated RNA decay-inducing exon (NMD exon) and encodes the target protein, the method comprising contacting a therapeutic agent to the cell, whereby the therapeutic agent modulates splicing of the NMD exon from the mRNA, thereby modulating level of processed mRNA encoding the target protein, and modulating the expression

of the target protein in the cell, wherein the target protein is selected from the group consisting of: OPA1 proteins.

[00514] Embodiment B2. A method of treating a disease or condition in a subject in need thereof by modulating expression of a target protein in a cell of the subject, comprising: contacting the cell of the subject with a therapeutic agent that modulates splicing of a nonsense-mediated mRNA decay-inducing exon (NMD exon) from an mRNA in the cell, wherein the mRNA comprises the NMD exon and encodes the target protein, thereby modulating level of processed mRNA encoding the target protein, and modulating expression of the target protein in the cell of the subject, wherein the target protein is selected from the group consisting of: OPA1 proteins.

[00515] Embodiment B3. The method of embodiment B1 or B2, wherein the therapeutic agent

- (a) binds to a targeted portion of the mRNA encoding the target protein;
- (b) modulates binding of a factor involved in splicing of the NMD exon; or
- (c) a combination of (a) and (b).

[00516] Embodiment B4. The method of embodiment B3, wherein the therapeutic agent interferes with binding of the factor involved in splicing of the NMD exon to a region of the targeted portion.

[00517] Embodiment B5. The method of embodiment B3 or B4, wherein the targeted portion is proximal to the NMD exon.

[00518] Embodiment B6. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.

[00519] Embodiment B7. The method of any one of embodiments B3 to B6, wherein the targeted portion is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.

[00520] Embodiment B8. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about

300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.

[00521] Embodiment B9. The method of any one of embodiments B3 to B5 or B8, wherein the targeted portion is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.

[00522] Embodiment B10. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site selected from the group consisting of: GRCh38/ hg38: chr3 193628509; and GRCh38/ hg38: chr3 193603500.

[00523] Embodiment B11. The method of any one of embodiments B3 to B5 or B10, wherein the targeted portion is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site selected from the group consisting of: GRCh38/ hg38: chr3 193628509; and GRCh38/ hg38: chr3 193603500.

[00524] Embodiment B12. The method of any one of embodiments B3 to B5, wherein the targeted portion is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site selected from the group consisting of: GRCh38/ hg38: chr3 193628616; and GRCh38/ hg38: chr3 193603557.

[00525] Embodiment B13. The method of any one of embodiments B3 to B5 or B12, wherein the targeted portion is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of

genomic site selected from the group consisting of: GRCh38/ hg38: chr3 193628616; and GRCh38/ hg38: chr3 193603557.

[00526] Embodiment B14. The method of any one of embodiments B3 to B13, wherein the targeted portion is located in an intronic region between two canonical exonic regions of the mRNA encoding the target protein, and wherein the intronic region contains the NMD exon.

[00527] Embodiment B15. The method of any one of embodiments B3 to B14, wherein the targeted portion at least partially overlaps with the NMD exon.

[00528] Embodiment B16. The method of any one of embodiments B3 to B15, wherein the targeted portion at least partially overlaps with an intron upstream or downstream of the NMD exon.

[00529] Embodiment B17. The method of any one of embodiments B3 to B16, wherein the targeted portion comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction.

[00530] Embodiment B18. The method of any one of embodiments B3 to B16, wherein the targeted portion is within the NMD exon.

[00531] Embodiment B19. The method of any one of embodiments B1 to B18, wherein the targeted portion comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[00532] Embodiment B20. The method of any one of embodiments B1 to B19, wherein the mRNA encoding the target protein comprises a sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 4 or 5.

[00533] Embodiment B21. The method of any one of embodiments B1 to B20, wherein the mRNA encoding the target protein is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to SEQ ID NO: 1.

[00534] Embodiment B22. The method of any one of embodiments B3 to B21, wherein the targeted portion of the mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 4 or 5.

[00535] Embodiment B23. The method of any one of embodiments B1 to B22, wherein the agent is an antisense oligomer (ASO) and wherein the ASO comprises a sequence that is at least about 80%, 85%, 90%, 95%, 97%, or 100% complementary to at least 8 contiguous nucleic acids of SEQ ID Ns: 4 or 5.

[00536] Embodiment B24. The method of any one of embodiments B3 to B23, wherein the targeted portion of the mRNA is within the nonsense-mediated RNA decay-inducing exon selected from the group consisting of: GRCh38/ hg38: chr3 193628509 193628616; and GRCh38/ hg38: chr3 193603500 193603557.

[00537] Embodiment B25. The method of any one of embodiments B3 to B23, wherein the targeted portion of the mRNA is upstream or downstream of the nonsense-mediated RNA decay-inducing exon selected from the group consisting of: GRCh38/ hg38: chr3 193628509 193628616; and GRCh38/ hg38: chr3 193603500 193603557.

[00538] Embodiment B26. The method of any one of embodiments B3 to B23, wherein the targeted portion of the mRNA comprises an exon-intron junction of exon selected from the group consisting of: GRCh38/ hg38: chr3 193628509 193628616; and GRCh38/ hg38: chr3 193603500 193603557.

[00539] Embodiment B27. The method of any one of embodiments B1 to B26, wherein the target protein produced is a full-length protein or a wild-type protein.

[00540] Embodiment B28. The method of any one of embodiments B1 to B27, wherein the therapeutic agent promotes exclusion of the NMD exon from the pre-mRNA encoding the target protein.

[00541] Embodiment B29. The method of embodiment B28, wherein exclusion of the NMD exon from the pre-mRNA encoding the target protein in the cell contacted with the therapeutic agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to exclusion of the NMD exon from the pre-mRNA encoding the target protein in a control cell.

[00542] Embodiment B30. The method of embodiment B28 or B29, wherein the therapeutic agent increases the level of the processed mRNA encoding the target protein in the cell.

[00543] Embodiment B31. The method of any one of embodiments B28 to B30, wherein the level of the processed mRNA encoding the target protein produced in the cell contacted with the therapeutic agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about

9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the processed mRNA encoding the target protein in a control cell.

[00544] Embodiment B32. The method of any one of embodiments B28 to B31, wherein the therapeutic agent increases the expression of the target protein in the cell.

[00545] Embodiment B33. The method of any one of embodiments B28 to B32, wherein a level of the target protein produced in the cell contacted with the therapeutic agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the target protein produced in a control cell.

[00546] Embodiment B34. The method of any one of embodiments B2 to B33, wherein the disease or condition is induced by a loss-of-function mutation in the target protein.

[00547] Embodiment B35. The method of embodiment B34, wherein the disease or condition is associated with haploinsufficiency of a gene encoding the target protein, and wherein the subject has a first allele encoding a functional target protein, and a second allele from which the target protein is not produced or produced at a reduced level, or a second allele encoding a nonfunctional target protein or a partially functional target protein.

[00548] Embodiment B36. The method of any one of embodiments B2 to B35, wherein the disease or condition is selected from the group consisting of: Optic atrophy type 1.

[00549] Embodiment B37. The method of any one of embodiments B34 to B36, wherein the therapeutic agent promotes exclusion of the NMD exon from the pre-mRNA encoding the target protein and increases the expression of the target protein in the cell.

[00550] Embodiment B38. The method of any one of embodiments B1 to B27, wherein the therapeutic agent inhibits exclusion of the NMD exon from the pre-mRNA encoding the target protein.

[00551] Embodiment B39. The method of embodiment B38, wherein exclusion of the NMD exon from the pre-mRNA encoding the target protein in the cell contacted with the therapeutic agent is decreased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-

fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to exclusion of the NMD exon from the pre-mRNA encoding the target protein in a control cell.

[00552] Embodiment B40. The method of embodiment B38 or B39, wherein the therapeutic agent decreases the level of the processed mRNA encoding the target protein in the cell.

[00553] Embodiment B41. The method of any one of embodiments B38 to B40, wherein the level of the processed mRNA encoding the target protein in the cell contacted with the therapeutic agent is decreased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the processed mRNA encoding the target protein in a control cell.

[00554] Embodiment B42. The method of any one of embodiments B38 to B41, wherein the therapeutic agent decreases the expression of the target protein in the cell.

[00555] Embodiment B43. The method of any one of embodiments B38 to B42, wherein a level of the target protein produced in the cell contacted with the therapeutic agent is decreased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to a level of the target protein produced in a control cell.

[00556] Embodiment B44. The method of any one of embodiments B2 to B27 or B38 to B43, wherein the disease or condition is induced by a gain-of-function mutation in the target protein.

[00557] Embodiment B45. The method of embodiment B44, wherein the subject has an allele from which the target protein is produced at an increased level, or an allele encoding a mutant target protein that exhibits increased activity in the cell.

[00558] Embodiment B46. The method of embodiment B44 or B45, wherein the therapeutic agent inhibits exclusion of the NMD exon from the pre-mRNA encoding the target protein and decreases the expression of the target protein in the cell.

[00559] Embodiment B47. The method of any one of embodiments B1 to B46, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00560] Embodiment B48. The method of any one of embodiments B1 to B47, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[00561] Embodiment B49. The method of any one of embodiments B1 to B48, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[00562] Embodiment B50. The method of embodiment B49, wherein each sugar moiety is a modified sugar moiety.

[00563] Embodiment B51. The method of any one of embodiments B1 to B50, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[00564] Embodiment B52. The method of any one of embodiments B3 to B51, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer is at least

80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted portion of the mRNA.

[00565] Embodiment B53. The method of any one of embodiments B1 to B52, wherein the method further comprises assessing mRNA level or expression level of the target protein.

[00566] Embodiment B54. The method of any one of embodiments B1 to B53, wherein the subject is a human.

[00567] Embodiment B55. The method of any one of embodiments B1 to B53, wherein the subject is a non-human animal.

[00568] Embodiment B56. The method of any one of embodiments B2 to B54, wherein the subject is a fetus, an embryo, or a child.

[00569] Embodiment B57. The method of any one of embodiments B1 to B56, wherein the cells are *ex vivo*.

[00570] Embodiment B58. The method of any one of embodiments B2 to B56, wherein the therapeutic agent is administered by intravitreal injection, intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, intravitreal, or intravenous injection of the subject.

[00571] Embodiment B59. The method of any one of embodiments B2 to B56 or B58, wherein the method further comprises administering a second therapeutic agent to the subject.

[00572] Embodiment B60. The method of any one of embodiments B1 to B59, wherein the second therapeutic agent is a small molecule.

[00573] Embodiment B61. The method of any one of embodiments B1 to B59, wherein the second therapeutic agent is an antisense oligomer.

[00574] Embodiment B62. The method of any one of embodiments B1 to B61, wherein the second therapeutic agent corrects intron retention.

[00575] Embodiment B63. The method of any one of embodiments B2 to B62, wherein the disease or condition is Optic atrophy type 1.

[00576] Embodiment C1. A method of modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an *OPA1* gene and that comprises a nonsense-mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent modulates splicing of the NMD exon from the pre-mRNA, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating the expression of the OPA1 protein in the cell, wherein the agent comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299.

[00577] Embodiment C2. The method of embodiment C1, wherein the agent:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the NMD exon; or
- (c) a combination of (a) and (b).

[00578] Embodiment C3. The method of embodiment C2, wherein the agent interferes with binding of the factor involved in splicing of the NMD exon to a region of the targeted portion.

[00579] Embodiment C4. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.

[00580] Embodiment C5. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.

[00581] Embodiment C6. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.

[00582] Embodiment C7. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.

[00583] Embodiment C8. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.

[00584] Embodiment C9. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70

nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

[00585] Embodiment C10. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

[00586] Embodiment C11. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[00587] Embodiment C12. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[00588] Embodiment C13. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon.

[00589] Embodiment C14. The method of embodiment C2, wherein the targeted portion of the pre-mRNA at least partially overlaps with the NMD exon.

[00590] Embodiment C15. The method of embodiment C2, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron upstream or downstream of the NMD exon.

[00591] Embodiment C16. The method of embodiment C2, wherein the targeted portion of the pre-mRNA comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction.

[00592] Embodiment C17. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is within the NMD exon.

[00593] Embodiment C18. The method of embodiment C2, wherein the targeted portion of the pre-mRNA comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[00594] Embodiment C19. The method of any one of embodiments C1 to C18, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.

[00595] Embodiment C20. The method of any one of embodiments C1 to C18, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.

[00596] Embodiment C21. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is within the nonsense-mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00597] Embodiment C22. The method of embodiment C2, wherein the targeted portion of the pre-mRNA is upstream or downstream of the nonsense-mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00598] Embodiment C23. The method of embodiment C2, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00599] Embodiment C24. The method of any one of embodiments C1 to C23, wherein the OPA1 protein expressed from the processed mRNA is a full-length OPA1 protein or a wild-type OPA1 protein.

[00600] Embodiment C25. The method of any one of embodiments C1 to C23, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.

[00601] Embodiment C26. The method of any one of embodiments C1 to C23, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.

[00602] Embodiment C27. The method of any one of embodiments C1 to C23, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

[00603] Embodiment C28. The method of any one of embodiments C1 to C23, or C25 to C27, wherein the OPA1 protein expressed from the processed mRNA is an OPA1 protein that lacks an amino acid sequence encoded by a nucleic acid sequence with at least 80% sequence identity to SEQ ID NO: 277.

[00604] Embodiment C29. The method of any one of embodiments C1 to C28, wherein the method promotes exclusion of the NMD exon from the pre-mRNA.

[00605] Embodiment C30. The method of embodiment C29, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold,

about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00606] Embodiment C31. The method of any one of embodiments C1 to C30, wherein the method results in an increase in the level of the processed mRNA in the cell.

[00607] Embodiment C32. The method of embodiment C31, wherein the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00608] Embodiment C33. The method of any one of embodiments C1 to C32, wherein the method results in an increase in the expression of the OPA1 protein in the cell.

[00609] Embodiment C34. The method of embodiment C33, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00610] Embodiment C35. The method of any one of embodiments C1 to C34, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity

to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

[00611] Embodiment C36. The method of any one of embodiments C1 to C34, wherein the agent further comprises a gene editing molecule.

[00612] Embodiment C37. The method of embodiment C36, wherein the gene editing molecule comprises CRISPR-Cas9.

[00613] Embodiment C38. A method of modulating expression of an *OPA1* protein in a cell having a pre-mRNA that is transcribed from an *OPA1* gene, wherein the pre-mRNA comprises a coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell, whereby the agent promotes exclusion of the coding exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in the cell.

[00614] Embodiment C39. The method of embodiment C38, wherein the agent:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the coding exon; or
- (c) a combination of (a) and (b).

[00615] Embodiment C40. The method of embodiment C39, wherein the agent interferes with binding of the factor involved in splicing of the coding exon to a region of the targeted portion.

[00616] Embodiment C41. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is proximal to the coding exon.

[00617] Embodiment C42. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon.

[00618] Embodiment C43. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 90 to 50, from 80 to 50, from 70 to 50, from 60 to 50, from 60 to 40, from 60 to 30, from 60 to 20, from 60 to 10, from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.

[00619] Embodiment C44. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.

[00620] Embodiment C45. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon.

[00621] Embodiment C46. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, from 1 to 19, from 10 to 60, from 20 to 60, from 30 to 60, from 40 to 60, from 50 to 60, from 50 to 70, from 50 to 80, from 50 to 90, or from 50 to 100 nucleotides downstream of 3' end of the coding exon.

[00622] Embodiment C47. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of 3' end of the coding exon.

[00623] Embodiment C48. The method of embodiment C39, wherein the targeted portion of the pre-mRNA at least partially overlaps with the coding exon.

[00624] Embodiment C49. The method of embodiment C39, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron immediately upstream or immediately downstream of the coding exon.

[00625] Embodiment C50. The method of embodiment C39, wherein the targeted portion of the pre-mRNA comprises 5' coding exon-intron junction or 3' coding exon-intron junction.

[00626] Embodiment C51. The method of embodiment C39, wherein the targeted portion is within the coding exon of the pre-mRNA.

[00627] Embodiment C52. The method of any one of embodiments C39 to C51, wherein the coding exon is an alternatively spliced exon.

[00628] Embodiment C53. The method of any one of embodiments C39 to C52, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.

[00629] Embodiment C54. The method of any one of embodiments C39 to C52, wherein the coding exon comprises SEQ ID NO: 277.

[00630] Embodiment C55. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00631] Embodiment C56. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092.

[00632] Embodiment C57. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00633] Embodiment C58. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is within a region spanning from 1 to 49, from 1 to 39, from 1 to 29, or from 1 to 19 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.

[00634] Embodiment C59. The method of embodiment C39, wherein the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00635] Embodiment C60. The method of embodiment C39, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00636] Embodiment C61. The method of embodiment C39, wherein the targeted portion comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.

[00637] Embodiment C62. The method of embodiment C39, wherein the targeted portion of the pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO: 277.

[00638] Embodiment C63. The method of any one of embodiments C38 to C62, wherein the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00639] Embodiment C64. The method of any one of embodiments C38 to C63, wherein the method results in an increase in expression of the OPA1 protein in the cell.

[00640] Embodiment C65. The method of embodiment C64, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00641] Embodiment C66. The method of embodiment C64, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent.

[00642] Embodiment C67. The method of any one of embodiments C64 to C66, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.

[00643] Embodiment C68. The method of any one of embodiments C64 to C66, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.

[00644] Embodiment C69. The method of any one of embodiments C64 to C66, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

[00645] Embodiment C70. The method of any one of embodiments C64 to C69, wherein the OPA1 protein expressed from the processed mRNA comprises fewer proteolytic cleavage sites than an OPA1 protein encoded by a corresponding mRNA containing the coding exon.

[00646] Embodiment C71. The method of any one of embodiments C38 to C70, wherein the agent promotes exclusion of a nonsense-mediated RNA decay-inducing exon (NMD exon) from the pre-mRNA.

[00647] Embodiment C72. The method of embodiment C71, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.

[00648] Embodiment C73. The method of embodiment C71, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.

[00649] Embodiment C74. The method of any one of embodiments C64 to C73, wherein the OPA1 protein expressed from the processed mRNA comprises fewer proteolytic cleavage sites than an OPA1 protein encoded by a corresponding mRNA containing the coding exon.

[00650] Embodiment C75. The method of any one of embodiments C38 to C74, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242, 250, 280-283, 288, and 290-292.

[00651] Embodiment C76. The method of any one of embodiments C38 to C74, wherein the agent comprises a gene editing molecule.

[00652] Embodiment C77. The method of embodiment C76, wherein the gene editing molecule comprises CRISPR-Cas9.

[00653] Embodiment C78. A method of modulating expression of an *OPAI* protein in a cell having a pre-mRNA that is transcribed from an *OPAI* gene, wherein the pre-mRNA comprises a coding exon, the method comprising contacting an agent or a vector encoding the agent to the cell, wherein the agent comprises an antisense oligomer that binds to:

(a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or

(b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA;

whereby the agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.

[00654] Embodiment C79. The method of embodiment C78, wherein the coding exon is an alternatively spliced exon.

[00655] Embodiment C80. The method of embodiments C78 or C79, wherein the method promotes inclusion of the coding exon in the processed mRNA during splicing of the pre-mRNA in the cell.

[00656] Embodiment C81. The method of any one of embodiments C78 to C80, wherein the target portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of a 5' end of the coding exon.

[00657] Embodiment C82. The method of any one of embodiments C78 to C80, wherein the target portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100, from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of a 3' end of the coding exon.

[00658] Embodiment C83. The method of any one of embodiments C78 to C80, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.

[00659] Embodiment C84. The method of any one of embodiments C78 to C80, wherein the coding exon comprises SEQ ID NO: 277.

[00660] Embodiment C85. The method of any one of embodiments C78 to C80, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 to 50, from 100 to 60, from 100 to 70, from 100 to 80, or from 100 to 90 nucleotides upstream of genomic site GRCh38/hg38: chr3 193626092.

[00661] Embodiment C86. The method of any one of embodiments C78 to C80, wherein the targeted portion of the pre-mRNA is within a region spanning from 40 to 100, from 50 to 100,

from 60 to 100, from 70 to 100, from 80 to 100, or from 90 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202.

[00662] Embodiment C87. The method of any one of embodiments C78 to C86, wherein the inclusion of the coding exon in the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00663] Embodiment C88. The method of any one of embodiments C78 to C87, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.

[00664] Embodiment C89. A method of modulating expression of a target protein in a cell having a pre-mRNA transcribed from a gene that encodes the target protein, wherein the pre-mRNA comprises a coding exon and a nonsense-mediated RNA decay-inducing exon (NMD exon), the method comprising contacting an agent or a vector encoding the agent to the cell, wherein the agent promotes exclusion of both the coding exon and the NMD exon from the pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks both the NMD exon and the coding exon in the cell

[00665] Embodiment C90. The method of embodiment C89, wherein the agent:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the coding exon, the NMD exon, or both; or
- (c) a combination of (a) and (b).

[00666] Embodiment C91. The method of embodiment C90, wherein the agent interferes with binding of the factor involved in splicing of the coding exon, the NMD exon, or both, to a region of the targeted portion.

[00667] Embodiment C92. The method of any one of embodiments C89 to C91, wherein the NMD exon is within an intronic region adjacent to the coding exon.

[00668] Embodiment C93. The method of embodiment C92, wherein the NMD exon is within an intronic region immediately upstream of the coding exon.

[00669] Embodiment C94. The method of embodiment C92, wherein the NMD exon is within an intronic region immediately downstream of the coding exon.

[00670] Embodiment C95. The method of any one of embodiments C90 to C94, wherein the targeted portion of the pre-mRNA is proximal to the coding exon.

[00671] Embodiment C96. The method of any one of embodiments C90 to C94, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the coding exon.

[00672] Embodiment C97. The method of any one of embodiments C90 to C94, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the coding exon.

[00673] Embodiment C98. The method of any one of embodiments C90 to C94, wherein the targeted portion of the pre-mRNA is located within the coding exon.

[00674] Embodiment C99. The method of any one of embodiments C90 to C94, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of 5' end of the coding exon.

[00675] Embodiment C100. The method of any one of embodiments C90 to C94, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the coding exon to 100 nucleotides downstream of the coding exon.

[00676] Embodiment C101. The method of any one of embodiments C89 to C100, wherein the coding exon is an alternatively spliced exon.

[00677] Embodiment C102. The method of any one of embodiments C89 to C101, wherein the coding exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 277.

[00678] Embodiment C103. The method of any one of embodiments C89 to C101, wherein the coding exon comprises SEQ ID NO: 277.

[00679] Embodiment C104. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is immediately upstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00680] Embodiment C105. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is immediately downstream of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00681] Embodiment C106. The method of any one of embodiments C90 to C94, wherein the targeted portion of the pre-mRNA is within a region spanning from 49 to 1, from 39 to 1, from 29 to 1, or from 19 to 1 nucleotides upstream of GRCh38/ hg38: chr3 193626092.

[00682] Embodiment C107. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193626092. to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193626202. .

[00683] Embodiment C108. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is within the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00684] Embodiment C109. The method of embodiment C90, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of the coding exon GRCh38/ hg38: chr3 193626092 to 193626202.

[00685] Embodiment C110. The method of embodiment C90, wherein the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the coding exon.

[00686] Embodiment C111. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.

[00687] Embodiment C112. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately upstream of the NMD exon.

[00688] Embodiment C113. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is located in an intronic region immediately downstream of the NMD exon.

[00689] Embodiment C114. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is located within the NMD exon.

[00690] Embodiment C115. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of the NMD exon to 100 nucleotides downstream of the NMD exon.

[00691] Embodiment C116. The method of any one of embodiments C89 to C115, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.

[00692] Embodiment C117. The method of embodiment C89, wherein the NMD exon comprises SEQ ID NO: 279.

[00693] Embodiment C118. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is immediately upstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00694] Embodiment C119. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is immediately downstream of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00695] Embodiment C120. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is within a region spanning from 100 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509 to 100 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[00696] Embodiment C121. The method of embodiment C90, wherein the targeted portion of the pre-mRNA is within the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00697] Embodiment C122. The method of embodiment C90, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of the NMD exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00698] Embodiment C123. The method of embodiment C90, wherein the targeted portion comprises about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[00699] Embodiment C124. The method of any one of embodiments C89 to C123, wherein the exclusion of the coding exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00700] Embodiment C125. The method of any one of embodiments C89 to C124, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00701] Embodiment C126. The method of any one of embodiments C89 to C125, wherein the agent results in an increase in the level of the processed mRNA in the cell.

[00702] Embodiment C127. The method of embodiment C126, wherein the level of the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00703] Embodiment C128. The method of any one of embodiments C89 to C127, wherein the method results in an increase in expression of the target protein in the cell.

[00704] Embodiment C129. The method of embodiment C128, wherein a level of the target protein expressed from the processed mRNA in the cell contacted with the agent is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the agent.

[00705] Embodiment C130. The method of any one of embodiments C89 to C128, wherein the target protein is an OPA1 protein.

[00706] Embodiment C131. The method of embodiment C130, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the agent is increased by at least about 1.5-fold compared to in the absence of the agent.

[00707] Embodiment C132. The method of embodiment C130, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.

[00708] Embodiment C133. The method of embodiment C130, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.

[00709] Embodiment C134. The method of embodiment C130, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

[00710] Embodiment C135. The method of any one of embodiments C89 to C127, wherein the agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 236, 242, 250, 280-283, 288, and 290-292.

[00711] Embodiment C136. The method of any one of embodiments C78 to C135, wherein the agent comprises a gene editing molecule.

[00712] Embodiment C137. The method of embodiment C136, wherein the gene editing molecule comprises CRISPR-Cas9.

[00713] Embodiment C138. The method of any one of embodiments C1 to C75 or C78 to C135, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00714] Embodiment C139. The method of any one of embodiments C1 to C75 or C78 to C138, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety.

[00715] Embodiment C140. The method of any one of embodiments C1 to C75 or C78 to C139, wherein the therapeutic agent is an antisense oligomer (ASO) and wherein the antisense oligomer comprises at least one modified sugar moiety.

[00716] Embodiment C141. The method of embodiment C140, wherein each sugar moiety is a modified sugar moiety.

[00717] Embodiment C142. The method of any one of embodiments C1 to C75 or C78 to C141, wherein the agent is an antisense oligomer (ASO) and wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

- [00718]** Embodiment C143. The method of any one of embodiments C1 to C142, wherein the vector comprises a viral vector encoding the agent.
- [00719]** Embodiment C144. The method of embodiment C143, wherein the viral vector comprises an adenoviral vector, adeno-associated viral (AAV) vector, lentiviral vector, Herpes Simplex Virus (HSV) viral vector, or retroviral vector.
- [00720]** Embodiment C145. The method of any one of embodiments C1 to C144, wherein the method further comprises assessing mRNA level or expression level of the OPA1 protein.
- [00721]** Embodiment C146. The method of any one of embodiments C1 to C145, wherein the agent is a therapeutic agent.
- [00722]** Embodiment C147. A pharmaceutical composition comprising the therapeutic agent of embodiment C146 or a vector encoding the therapeutic agent of embodiment C146, and a pharmaceutically acceptable excipient.
- [00723]** Embodiment C148. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding a therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299.
- [00724]** Embodiment C149. The pharmaceutical composition of embodiment C148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242 and 250.
- [00725]** Embodiment C150. The pharmaceutical composition of embodiment C148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.
- [00726]** Embodiment C151. The pharmaceutical composition of embodiment C148, wherein the therapeutic agent comprises an antisense oligomer with at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, and 280-299.
- [00727]** Embodiment C152. A composition, comprising an antisense oligomer with at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299, wherein the antisense oligomer comprises a backbone modification, a sugar moiety modification, or a combination thereof.
- [00728]** Embodiment C153. The composition of embodiment C152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 227-242 and 250.

[00729] Embodiment C154. The composition of embodiment C152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 267.

[00730] Embodiment C155. The composition of embodiment C152, wherein the antisense oligomer has at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, and 280-299.

[00731] Embodiment C156. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of a coding exon from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon in a cell, wherein the pre-mRNA is transcribed from an *OPAI* gene and that comprises the coding exon.

[00732] Embodiment C157. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent comprises an antisense oligomer that binds to a pre-mRNA that is transcribed from an *OPAI* gene in a cell, wherein the antisense oligomer binds to:

(a) a targeted portion of the pre-mRNA within an intronic region immediately upstream of a 5' end of the coding exon of the pre-mRNA; or

(b) a targeted portion of the pre-mRNA within an intronic region immediately downstream of a 3' end of the coding exon of the pre-mRNA;

whereby the therapeutic agent increases a level of a processed mRNA that is processed from the pre-mRNA and that contains the coding exon in the cell.

[00733] Embodiment C158. A pharmaceutical composition, comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent promotes exclusion of both a coding exon and a nonsense-mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA, thereby increasing a level of a processed mRNA that is processed from the pre-mRNA and that lacks the coding exon and the NMD exon in a cell, wherein the pre-mRNA is transcribed from an *OPAI* gene in the cell and comprises the coding exon and the NMD exon.

[00734] Embodiment C159. The pharmaceutical composition of any one of embodiments C147 to C158, wherein the pharmaceutical composition is formulated for intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

[00735] Embodiment C160. The pharmaceutical composition of any one of embodiments C147 to C158, wherein the pharmaceutical composition is formulated for intravitreal injection.

[00736] Embodiment C161. The pharmaceutical composition of any one of embodiments C147 to C160, wherein the pharmaceutical composition further comprises a second therapeutic agent.

[00737] Embodiment C162. The pharmaceutical composition of embodiment C161, wherein the second therapeutic agent comprises a small molecule.

[00738] Embodiment C163. The pharmaceutical composition of embodiment C161, wherein the second therapeutic agent comprises an antisense oligomer.

[00739] Embodiment C164. The pharmaceutical composition of embodiment C161, wherein the second therapeutic agent corrects intron retention.

[00740] Embodiment C165. The pharmaceutical composition or composition of any one of embodiments C147 to C160, wherein the antisense oligomer is selected from the group consisting of Compound ID NOs: 1-303.

[00741] Embodiment C166. A method of treating or reducing the likelihood of developing a disease or condition in a subject in need thereof by modulating expression of an OPA1 protein in a cell of the subject, comprising contacting to cells of the subject the therapeutic agent of any one of embodiments C147 to C165.

[00742] Embodiment C167. The method of embodiment C166, wherein the disease or condition is associated with a loss-of-function mutation in an OPA1 gene.

[00743] Embodiment C168. The method of embodiment C166 or C167, wherein the disease or condition is associated with haploinsufficiency of the OPA1 gene, and wherein the subject has a first allele encoding a functional OPA1 protein, and a second allele from which the OPA1 protein is not produced or produced at a reduced level, or a second allele encoding a nonfunctional OPA1 protein or a partially functional OPA1 protein.

[00744] Embodiment C169. The method of any one of embodiments C166 to C168, wherein the disease or condition comprises an eye disease or condition.

[00745] Embodiment C170. The method of any one of embodiments C166 to C168, wherein the disease or condition comprises ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy;

Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[00746] Embodiment C171. The method of any one of embodiments C166 to C168, wherein the disease or condition comprises Optic atrophy type 1.

[00747] Embodiment C172. The method of any one of embodiments C166 to C168, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA).

[00748] Embodiment C173. The method of embodiment C166 or C167, wherein the disease or condition is associated with an autosomal recessive mutation of OPA1 gene, wherein the subject has a first allele encoding from which:

(i) OPA1 protein is not produced or produced at a reduced level compared to a wild-type allele; or

(ii) the OPA1 protein produced is nonfunctional or partially functional compared to a wild-type allele, and

a second allele from which:

(iii) the OPA1 protein is produced at a reduced level compared to a wild-type allele and the OPA1 protein produced is at least partially functional compared to a wild-type allele; or

(iv) the OPA1 protein produced is partially functional compared to a wild-type allele.

[00749] Embodiment C174. The method of any one of embodiments C166 to C173, wherein the subject is a human.

[00750] Embodiment C175. The method of any one of embodiments C166 to C173, wherein the subject is a non-human animal.

[00751] Embodiment C176. The method of any one of embodiments C166 to C173, wherein the subject is a fetus, an embryo, or a child.

[00752] Embodiment C177. The method of any one of embodiments C166 to C173, wherein the cells are *ex vivo*.

[00753] Embodiment C178. The method of any one of embodiments C166 to C173, wherein the therapeutic agent is administered by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

[00754] Embodiment C179. The method of any one of embodiments C166 to C173, wherein the therapeutic agent is administered by intravitreal injection.

[00755] Embodiment C180. The method of any one of embodiments C166 to C179, wherein the method treats the disease or condition.

FURTHER SPECIFIC EMBODIMENTS

[00756] Embodiment 1. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,

wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, and

wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

[00757] Embodiment 2. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,

wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299,

wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by having:

- (i) a heterozygous OPA1 gene variant;
- (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments;
- (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity;
- (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or
- (v) any combination of (i)-(iv).

[00758] Embodiment 3. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,

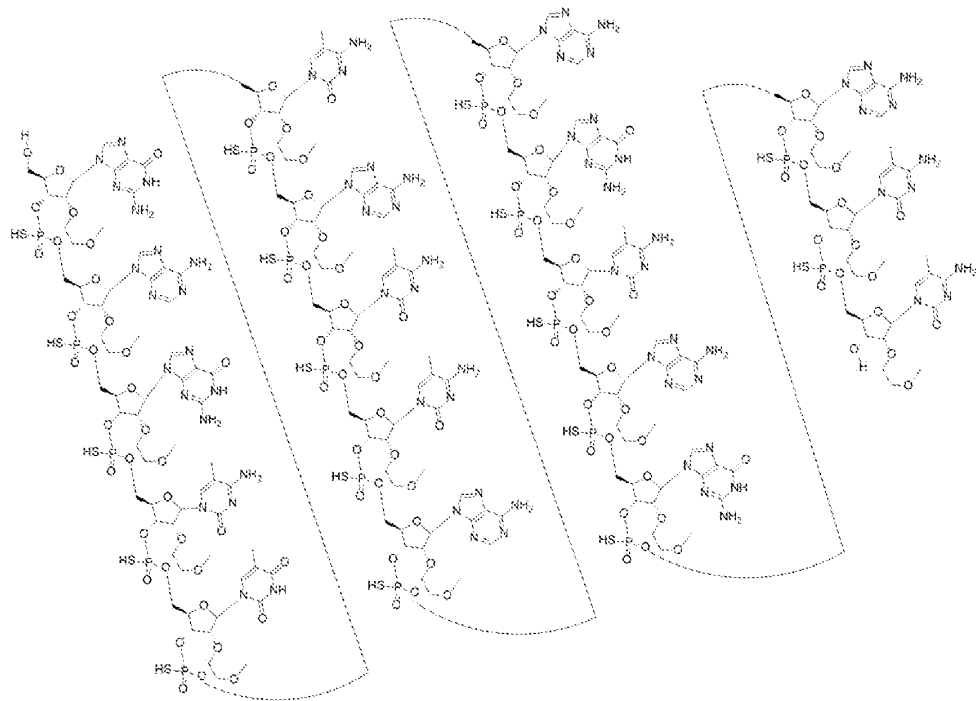
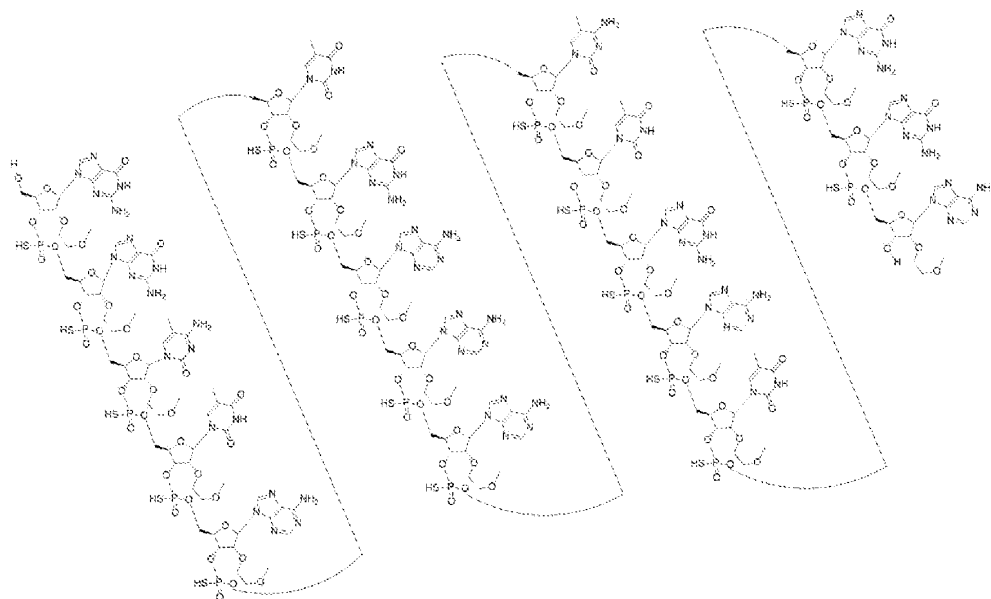
wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299,

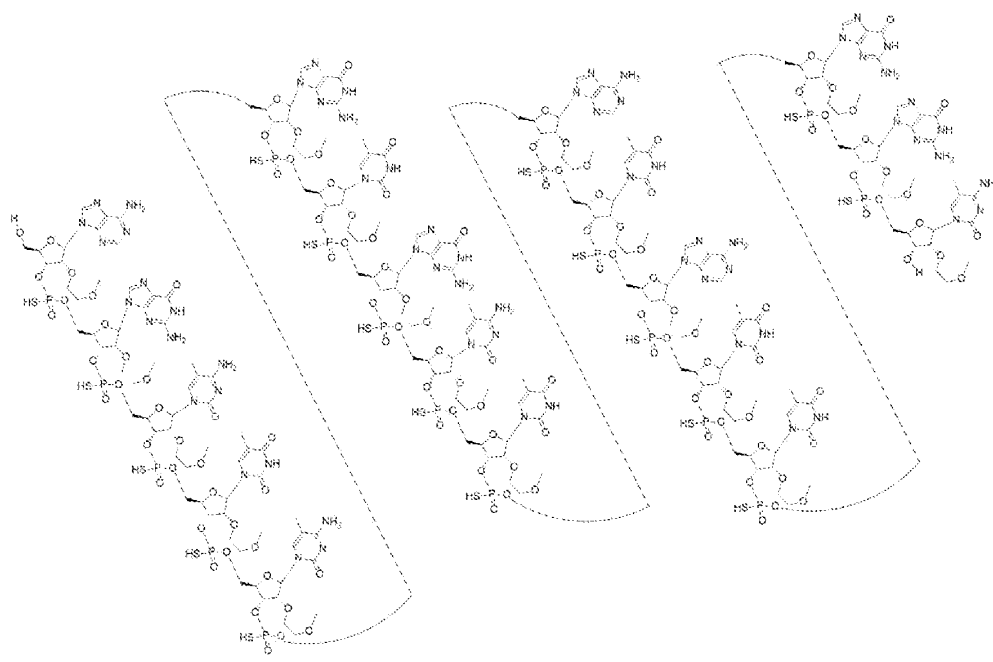
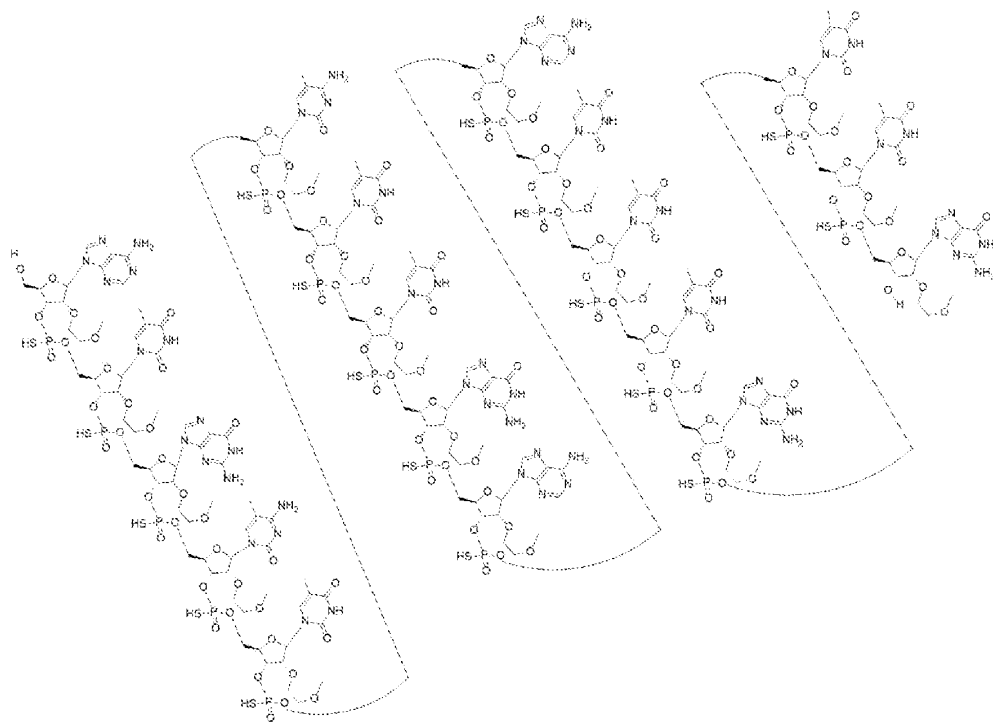
wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and wherein the subject is characterized by:

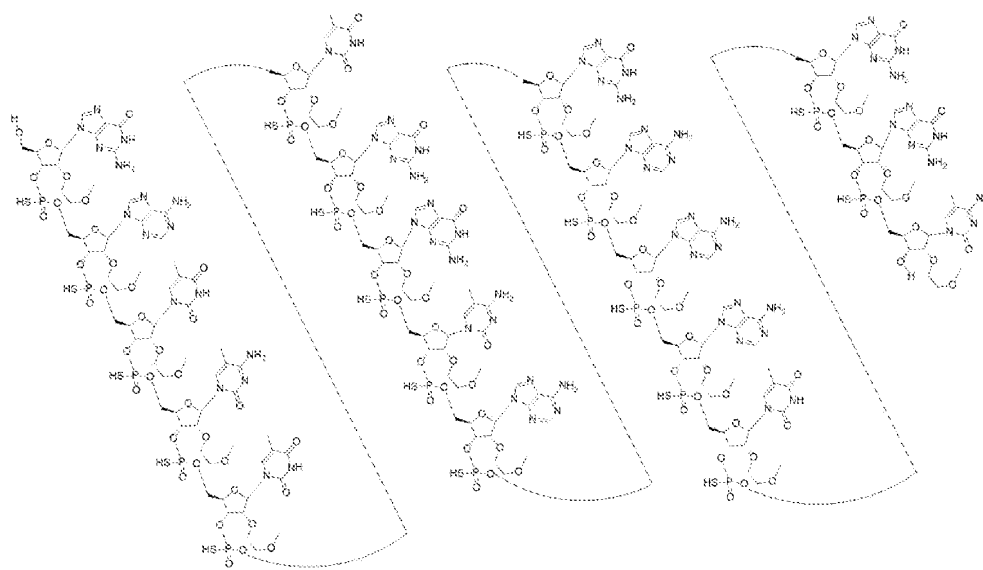
- (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in OPA1 gene;

- (2) not having only benign or likely benign variants in the OPA1 gene;
- (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus);
- (4) not having been diagnosed with Behr syndrome;
- (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases;
- (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy;
- (7) not having or having a history of any ocular condition in either eye;
- (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering;
- (9) not having a history of retinal photocoagulation;
- (10) not having a history or presence of retinal vein occlusion;
- (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering;
- (12) not having dry age-related macular degeneration in either eye;
- (13) not having high myopia (>6 diopters);
- (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated);
- (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy;
- (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery; or
- (17) any combination of (1)-(16).

[00759] Embodiment 4. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer is a compound according to any one of the following chemical structures:







or a pharmaceutically acceptable salt thereof.

[00760] Embodiment 5. The method of any one of embodiments 2-4, wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

[00761] Embodiment 6. The method of any one of embodiments 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg to about 15 mg, about 0.01 mg to about 10 mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg, about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

[00762] Embodiment 7. The method of any one of embodiments 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

[00763] Embodiment 8. The method of any one of embodiments 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

[00764] Embodiment 9. The method of any one of embodiments 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer, preferably about 0.1 mg to about 1.2 mg of the antisense oligomer.

[00765] Embodiment 10. The method of any one of embodiments 1-9, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μ L to about 250 μ L, about 10 μ L to about 250 μ L, about 20 μ L to about 250 μ L, about 30 μ L to about 250 μ L, about 40 μ L to about 250 μ L, about 50 μ L to about 250 μ L, about 60 μ L to about 250 μ L, about 70 μ L to about 250 μ L, about 80 μ L to about 250 μ L, about 100 μ L to about 250 μ L, about 120 μ L to about 250 μ L, about 150 μ L to about 250 μ L, about 160 μ L to about 250 μ L, about 180 μ L to about 500 μ L, about 200 μ L to about 250 μ L, about 220 μ L to about 250 μ L, about 5 μ L to about 220 μ L, about 10 μ L to about 220 μ L, about 20 μ L to about 220 μ L, about 30 μ L to about 220 μ L, about 40 μ L to about 220 μ L, about 50 μ L to about 220 μ L, about 60 μ L to about 220 μ L, about 70 μ L to about 220 μ L, about 80 μ L to about 220 μ L, about 100 μ L to about 220 μ L, about 120 μ L to about 220 μ L, about 150 μ L to about 220 μ L, about 160 μ L to about 220 μ L, about 180 μ L to about 220 μ L, about 5 μ L to about 200 μ L, about 10 μ L to about 200 μ L, about 20 μ L to about 200 μ L, about 30 μ L to about 200 μ L, about 40 μ L to about

200 μL , about 50 μL to about 200 μL , about 60 μL to about 200 μL , about 70 μL to about 200 μL , about 80 μL to about 200 μL , about 100 μL to about 200 μL , about 120 μL to about 200 μL , about 150 μL to about 200 μL , about 160 μL to about 200 μL , about 180 μL to about 200 μL , about 5 μL to about 180 μL , about 10 μL to about 180 μL , about 20 μL to about 180 μL , about 30 μL to about 180 μL , about 40 μL to about 180 μL , about 50 μL to about 180 μL , about 60 μL to about 180 μL , about 70 μL to about 180 μL , about 80 μL to about 180 μL , about 100 μL to about 180 μL , about 120 μL to about 180 μL , about 150 μL to about 180 μL , about 5 μL to about 150 μL , about 10 μL to about 150 μL , about 20 μL to about 150 μL , about 30 μL to about 150 μL , about 40 μL to about 150 μL , about 50 μL to about 150 μL , about 60 μL to about 150 μL , about 70 μL to about 150 μL , about 80 μL to about 150 μL , about 100 μL to about 150 μL , about 120 μL to about 150 μL , about 5 μL to about 150 μL , about 10 μL to about 120 μL , about 20 μL to about 120 μL , about 30 μL to about 120 μL , about 40 μL to about 120 μL , about 50 μL to about 120 μL , about 60 μL to about 120 μL , about 70 μL to about 120 μL , about 80 μL to about 120 μL , about 100 μL to about 120 μL , about 5 μL to about 100 μL , about 10 μL to about 100 μL , about 20 μL to about 100 μL , about 30 μL to about 100 μL , about 40 μL to about 100 μL , about 50 μL to about 100 μL , about 60 μL to about 100 μL , about 70 μL to about 100 μL , about 80 μL to about 100 μL , about 5 μL to about 80 μL , about 10 μL to about 80 μL , about 20 μL to about 80 μL , about 30 μL to about 80 μL , about 40 μL to about 80 μL , about 50 μL to about 80 μL , about 60 μL to about 80 μL , about 5 μL to about 60 μL , about 10 μL to about 60 μL , about 20 μL to about 60 μL , about 30 μL to about 60 μL , about 40 μL to about 60 μL , or about 50 μL to about 60 μL .

[00766] Embodiment 11. The method of any one of embodiments 1-9, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μL , about 8 μL , about 10 μL , about 12 μL , about 15 μL , about 18 μL , about 20 μL , about 25 μL , about 28 μL , about 30 μL , about 35 μL , about 40 μL , about 45 μL , about 48 μL , about 50 μL , about 55 μL , about 60 μL , about 65 μL , about 70 μL , about 75 μL , about 80 μL , about 90 μL , about 100 μL , about 120 μL , about 150 μL , about 160 μL , about 180 μL , about 200 μL , about 220 μL , or about 250 μL , preferably about 50 μL .

[00767] Embodiment 12. The method of any one of embodiments 1-11, wherein the method comprises administering the pharmaceutical composition to both left eye and right eye of the subject.

[00768] Embodiment 13. The method of embodiment 12, wherein the method comprises administering the pharmaceutical composition at the same dose to both the left eye and the right eye of the subject.

[00769] Embodiment 14. The method of embodiment 12, wherein the method comprises administering the pharmaceutical composition at different doses to the left eye and the right eye of the subject.

[00770] Embodiment 15. The method of any one of embodiments 1-3 or 5-14, wherein the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to the sequence set forth in any one of SEQ ID NOs: 6-275 or 280-299.

[00771] Embodiment 16. The method of any one of embodiments 1-3 or 5-14, wherein the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to the sequence set forth in any one of SEQ ID NO: 36, 236, 242, 250, 280-283, 288, or 290-292.

[00772] Embodiment 17. The method of any one of embodiments 1-3 or 5-14, wherein the antisense oligomer modulates splicing of a non-sense mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA in a cell of the subject, wherein the pre-mRNA encodes the OPA1 protein and comprises the NMD exon, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating expression of the OPA1 protein in the cell.

[00773] Embodiment 18. The method of embodiment 17, wherein the antisense oligomer:

- (a) binds to a targeted portion of the pre-mRNA;
- (b) modulates binding of a factor involved in splicing of the NMD exon; or
- (c) a combination of (a) and (b).

[00774] Embodiment 19. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.

[00775] Embodiment 20. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.

[00776] Embodiment 21. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.

[00777] Embodiment 22. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides,

about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.

[00778] Embodiment 23. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.

[00779] Embodiment 24. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

[00780] Embodiment 25. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

[00781] Embodiment 26. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[00782] Embodiment 27. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

[00783] Embodiment 28. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon.

[00784] Embodiment 29. The method of embodiment 18, wherein the targeted portion of the pre-mRNA at least partially overlaps with the NMD exon.

[00785] Embodiment 30. The method of embodiment 18, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron upstream or downstream of the NMD exon.

[00786] Embodiment 31. The method of embodiment 18, wherein the targeted portion of the pre-mRNA comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction.

[00787] Embodiment 32. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is within the NMD exon.

[00788] Embodiment 33. The method of embodiment 18, wherein the targeted portion of the pre-mRNA comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.

[00789] Embodiment 34. The method of any one of embodiments 17-33, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.

[00790] Embodiment 35. The method of any one of embodiments 17-33, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.

[00791] Embodiment 36. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is within the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00792] Embodiment 37. The method of embodiment 18, wherein the targeted portion of the pre-mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00793] Embodiment 38. The method of embodiment 18, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193628509 to 193628616.

[00794] Embodiment 39. The method of any one of embodiments 17-38, wherein the OPA1 protein expressed from the processed mRNA is a full-length OPA1 protein or a wild-type OPA1 protein.

[00795] Embodiment 40. The method of any one of embodiments 17-38, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.

[00796] Embodiment 41. The method of any one of embodiments 17-38, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.

[00797] Embodiment 42. The method of any one of embodiments 17-38, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.

[00798] Embodiment 43. The method of any one of embodiments 17-42, wherein the antisense oligomer promotes exclusion of the NMD exon from the pre-mRNA.

[00799] Embodiment 44. The method of embodiment 43, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.

[00800] Embodiment 45. The method of any one of embodiments 17-44, wherein the method results in an increase in the level of the processed mRNA in the cell.

[00801] Embodiment 46. The method of embodiment 45, wherein the level of the processed mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.

[00802] Embodiment 47. The method of any one of embodiments 17-46, wherein the method results in an increase in the expression of the OPA1 protein in the cell.

[00803] Embodiment 48. The method of embodiment 47, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the antisense oligomer is

increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.

[00804] Embodiment 49. The method of any one of embodiments 1-3 or 5-48, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00805] Embodiment 50. The method of any one of embodiments 1-3 or 5-48, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety.

[00806] Embodiment 51. The method of any one of embodiments 1-3 or 5-48, wherein the antisense oligomer comprises at least one modified sugar moiety.

[00807] Embodiment 52. The method of embodiment 51, wherein each sugar moiety is a modified sugar moiety.

[00808] Embodiment 53. The method of any one of embodiments 1-3 or 5-52, wherein the antisense oligomer comprises a 5'-methylcytosine (5'-MeC).

[00809] Embodiment 54. The method of any one of embodiments 1-3 or 5-53, wherein each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC).

[00810] Embodiment 55. The method of any one of embodiments 1-3 or 5-54, wherein the antisense oligomer comprises a 5'-methyluracil (5'-MeU).

[00811] Embodiment 56. The method of any one of embodiments 1-3 or 5-55, wherein the antisense oligomer comprises a phosphorothioate linkage.

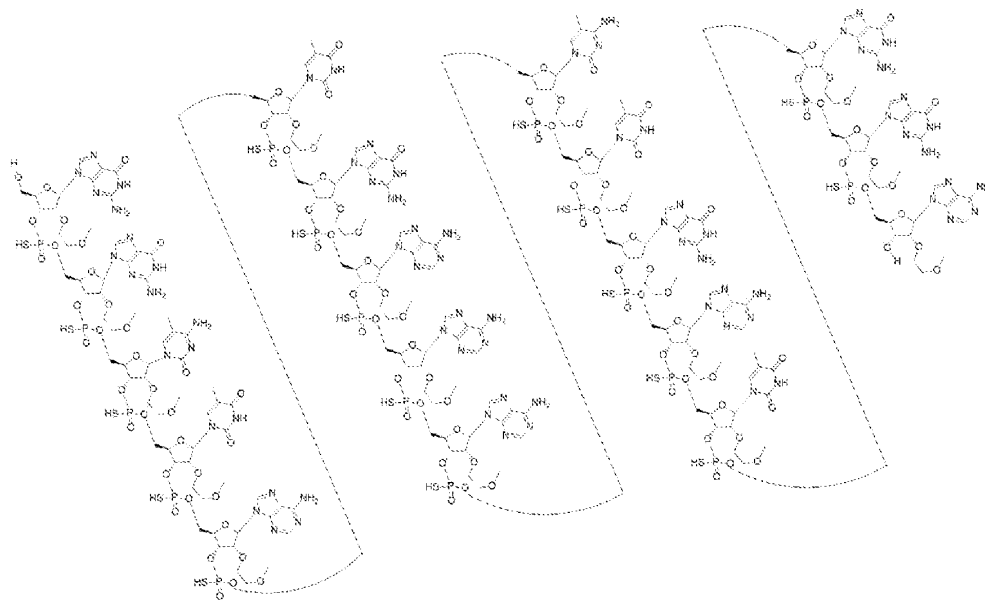
[00812] Embodiment 57. The method of any one of embodiments 1-3 or 5-56, wherein each internucleoside linkage of the ASO is a phosphorothioate linkage.

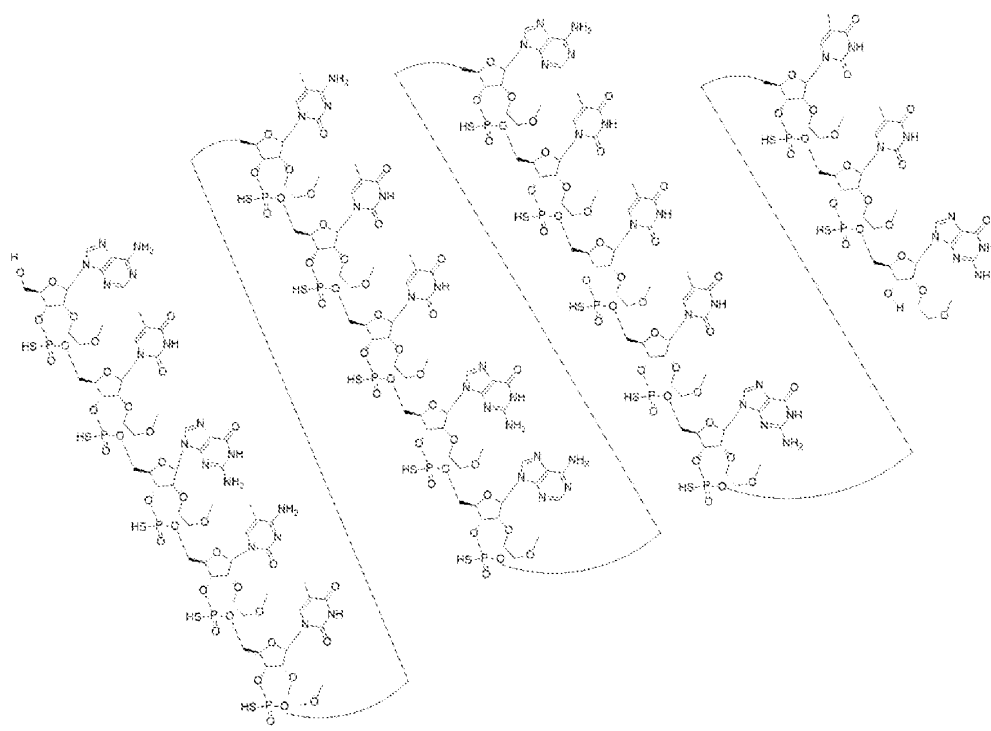
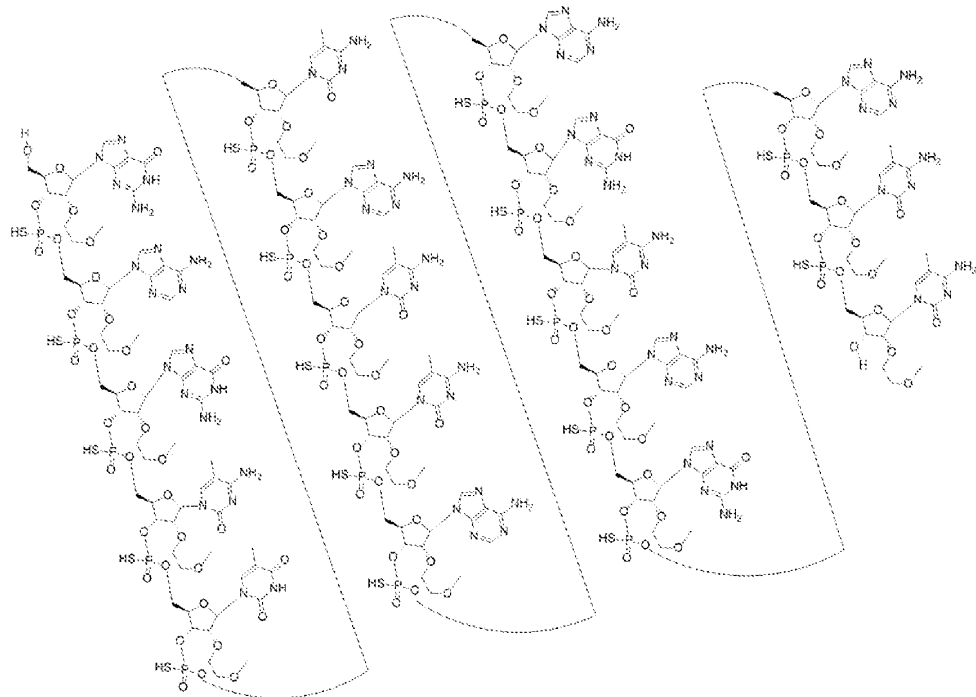
[00813] Embodiment 58. The method of any one of embodiments 1-3 or 5-57, wherein the antisense oligomer comprises a locked nucleic acid (LNA).

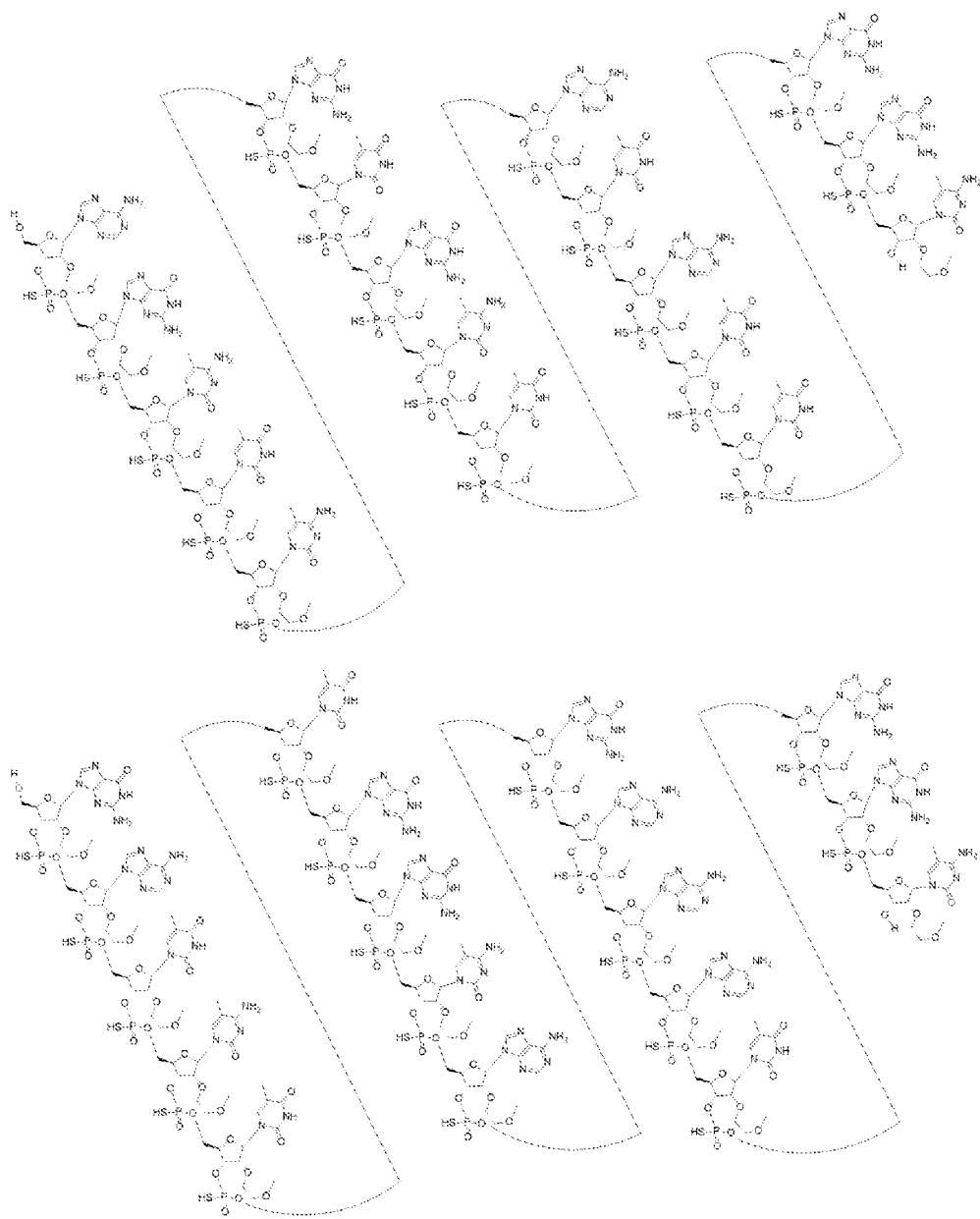
[00814] Embodiment 59. The method of any one of embodiments 1-3 or 5-58, wherein the antisense oligomer is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases,

9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases in length.

[00815] Embodiment 60. The method of any one of embodiments 1-3 or 5-59, wherein the antisense oligomer is a compound according to any one of the following chemical structures

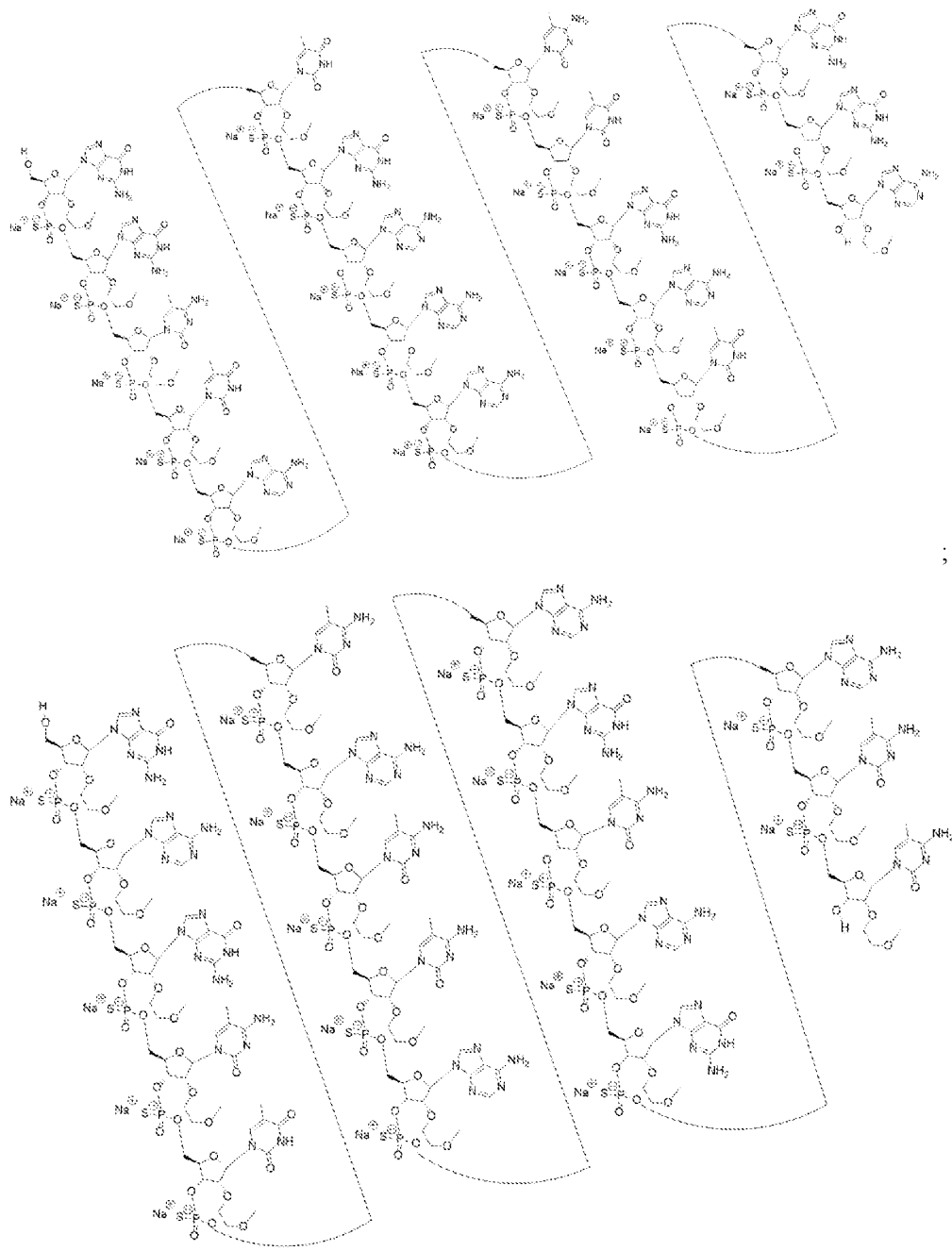


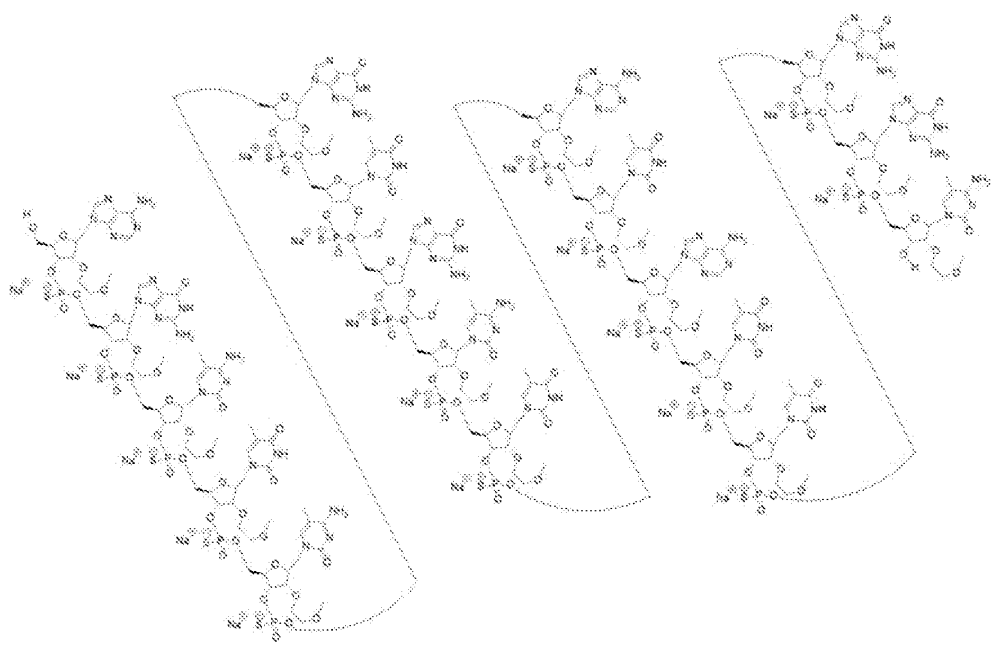
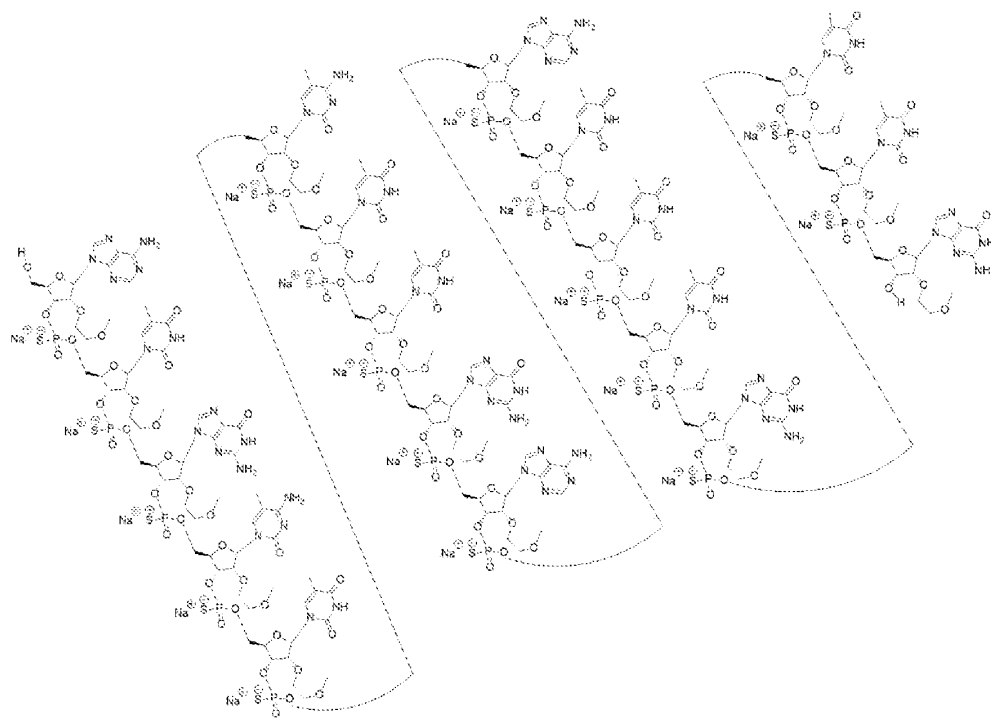




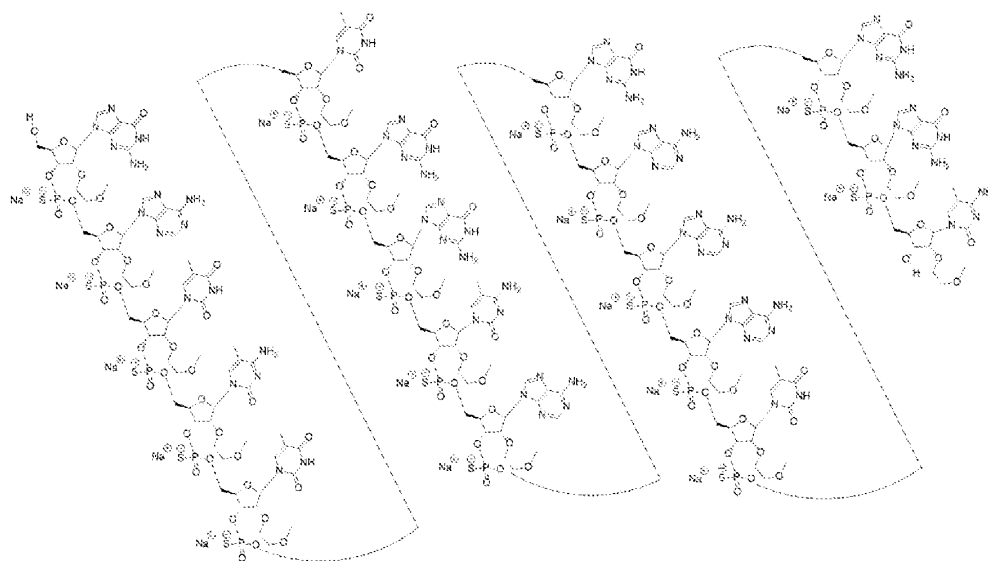
or a pharmaceutically acceptable salt thereof.

[00816] Embodiment 61. The method of embodiment 4 or 60, wherein the antisense oligomer is a compound according to any of the following structures:





; or



[00817] Embodiment 62. The method of any one of embodiments 1-61, wherein the pharmaceutical composition is a liquid composition.

[00818] Embodiment 63. The method of any one of embodiments 1-62, wherein the method comprises administering the pharmaceutical composition as a bolus injection.

[00819] Embodiment 64. The method of any one of embodiments 1-63, wherein the method comprises administering the pharmaceutical composition as a bolus injection over 1 to 60 minutes, 1 to 50 minutes, 1 to 40 minutes, 1 to 30 minutes, 1 to 20 minutes, 1 to 10 minutes, 1 to 5 minutes, or 1 to 3 minutes.

[00820] Embodiment 65. The method of any one of embodiments 1-64, wherein the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

[00821] Embodiment 66. The method of any one of embodiments 1-64, wherein the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

[00822] Embodiment 67. The method of any one of embodiments 1-66, wherein the antisense oligomer is solubilized or diluted in an isotonic solution.

[00823] Embodiment 68. The method of any one of embodiments 1-67, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8.

[00824] Embodiment 69. The method of any one of embodiments 1-67, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution.

[00825] Embodiment 70. The method of any one of embodiments 1-69, wherein the pharmaceutical formulation does not comprise a preservative.

[00826] Embodiment 71. The method of any one of embodiments 1-70, wherein the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL.

[00827] Embodiment 72. The method of any one of embodiments 1-70, wherein the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL, about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

[00828] Embodiment 73. The method of any one of embodiments 1-70, wherein the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL,

about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00829] Embodiment 74. The method of any one of embodiments 1-73, wherein the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer.

[00830] Embodiment 75. The method of embodiment 74, wherein the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00831] Embodiment 76. The method of embodiment 74, wherein the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00832] Embodiment 77. The method of any one of embodiments 74-76, wherein the concentrate is a phosphate-buffered solution.

[00833] Embodiment 78. The method of any one of embodiments 1-77, wherein the subject is a human subject.

[00834] Embodiment 79. The method of any one of embodiments 1 or 4-78, wherein the disease or condition is associated with a deficient amount or activity of the OPA1 protein.

[00835] Embodiment 80. The method of any one of embodiments 1 or 4-79, wherein the disease or condition comprises an eye disease or condition.

[00836] Embodiment 81. The method of any one of embodiments 1 or 4-79, wherein the disease or condition comprises a cardiovascular disease or condition.

[00837] Embodiment 82. The method of any one of embodiments 1 or 4-79, wherein the disease or condition comprises a neurological disease or condition.

[00838] Embodiment 83. The method of any one of embodiments 1 or 4-79, wherein the disease or condition comprises ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

[00839] Embodiment 84. The method of any one of embodiments 1 or 4-79, wherein the disease or condition comprises Optic atrophy type 1.

[00840] Embodiment 85. The method of any one of embodiments 1 or 4-79, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA).

[00841] Embodiment 86. The method of embodiment 85, wherein the subject is characterized by having:

- (i) a heterozygous OPA1 gene variant;
- (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments;
- (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity;
- (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or
- (v) any combination of (i)-(iv).

[00842] Embodiment 87. The method of embodiment 86, wherein the subject is additionally characterized by:

- (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in OPA1 gene;
- (2) not having only benign or likely benign variants in the OPA1 gene;
- (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus);
- (4) not having been diagnosed with Behr syndrome;
- (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases;
- (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy;
- (7) not having or having a history of any ocular condition in either eye;
- (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering;
- (9) not having a history of retinal photocoagulation;
- (10) not having a history or presence of retinal vein occlusion;
- (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering;
- (12) not having dry age-related macular degeneration in either eye;
- (13) not having high myopia (>6 diopters);
- (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated);
- (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy;
- (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery;
- (17) any combination of (1)-(16).

[00843] Embodiment 88. The method of any one of embodiments 1-87, wherein administering comprises administering multiple doses of the pharmaceutical composition to the human subject.

[00844] Embodiment 89. The method of embodiment 88, wherein administering comprises administering a first dose of the pharmaceutical composition to the human subject and a subsequent dose of the pharmaceutical composition to the human subject.

[00845] Embodiment 90. The method of embodiment 89, wherein the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is not tolerated.

[00846] Embodiment 91. The method of embodiment 89, wherein the subsequent dose is the same as the previous dose following an indication that administration of the previous dose is tolerated.

[00847] Embodiment 92. The method of embodiment 89, wherein the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is tolerated.

[00848] Embodiment 93. The method of embodiment 89, wherein the subsequent dose is the same as the previous dose following an indication that administration of the previous dose is effective.

[00849] Embodiment 94. The method of embodiment 89, wherein the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is effective.

[00850] Embodiment 95. The method of embodiment 89, wherein the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is not effective.

[00851] Embodiment 96. The method of any one of embodiments 1-95, wherein the pharmaceutical composition is administered via intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

[00852] Embodiment 97. The method of any one of embodiments 1-87, wherein the pharmaceutical composition is administered via intravitreal injection.

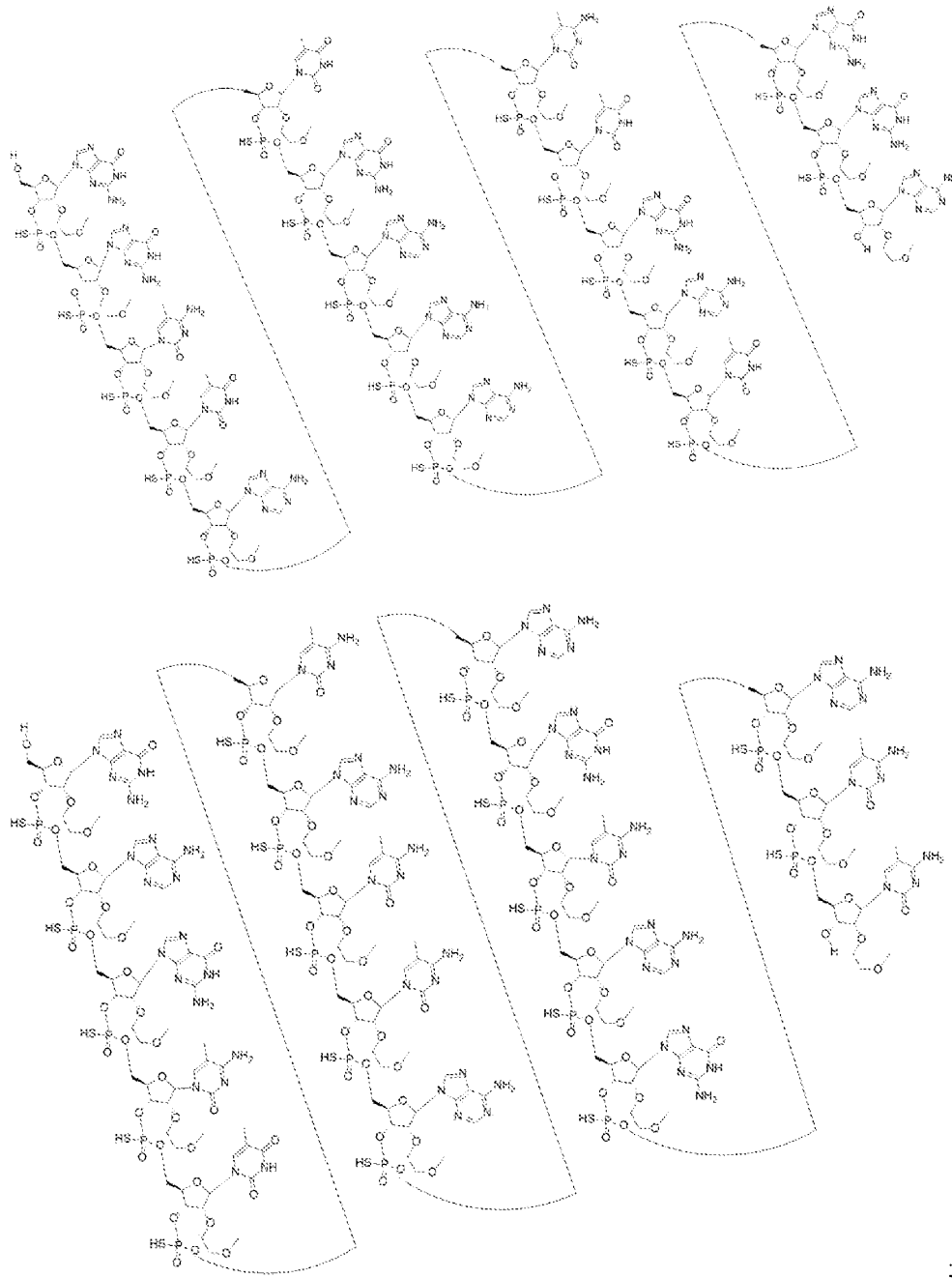
[00853] Embodiment 98. The method of any one of embodiments 1-97, wherein the method further comprises administering an additional therapeutic agent.

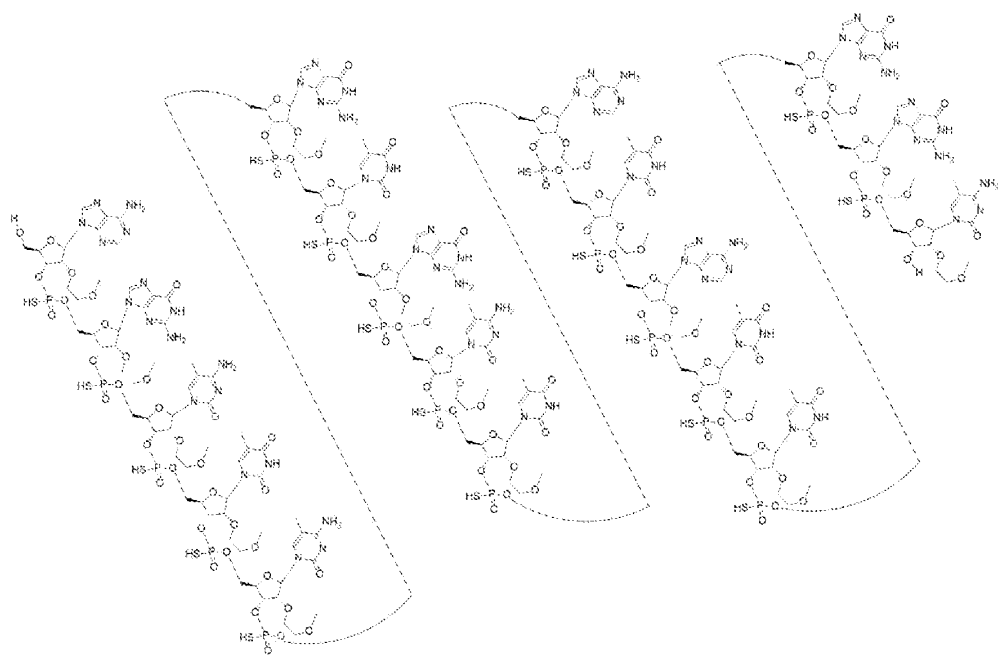
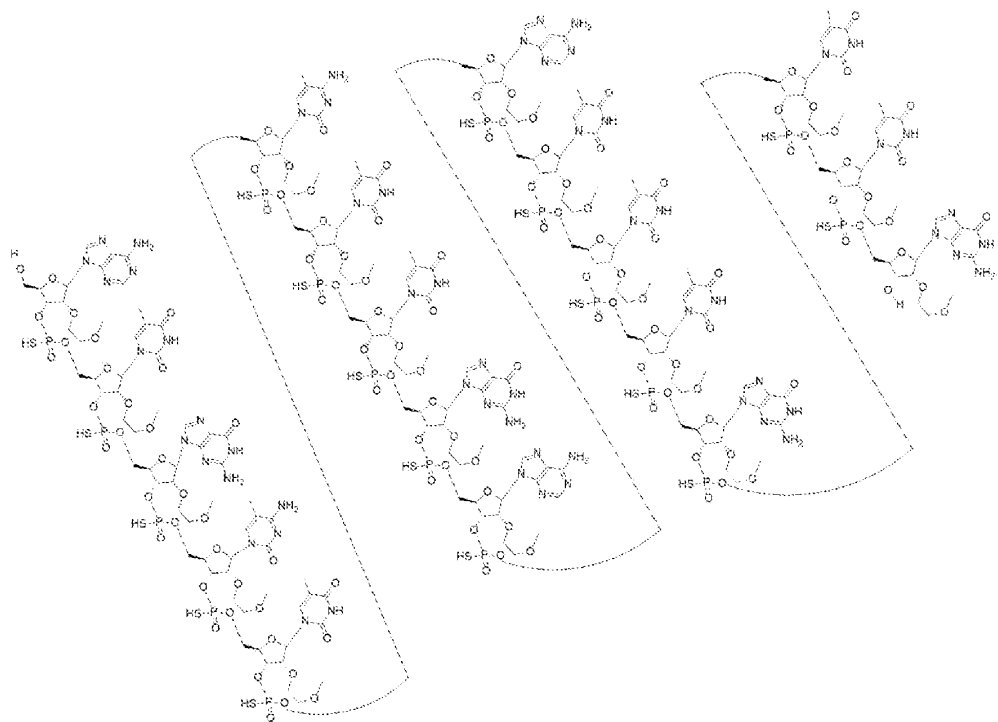
[00854] Embodiment 99. The method of embodiment 98, wherein the additional therapeutic agent comprises a small molecule.

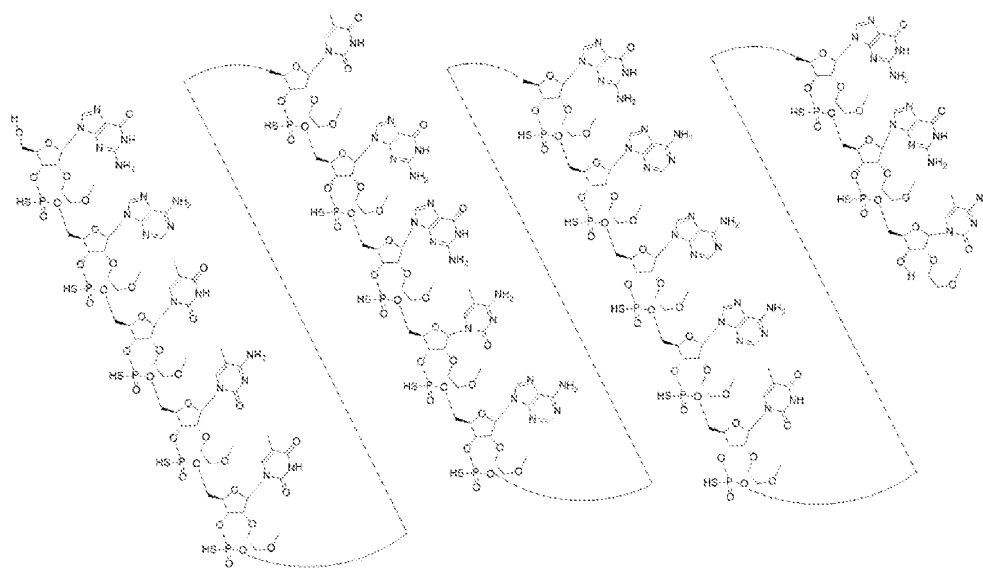
[00855] Embodiment 100. The method of embodiment 98, wherein the additional therapeutic agent comprises an antisense oligomer.

[00856] Embodiment 101. The method of embodiment 98, wherein the additional therapeutic agent comprises an ophthalmologic drug.

[00857] Embodiment 102. An antisense oligomer that is a compound according to any one of the following structures:

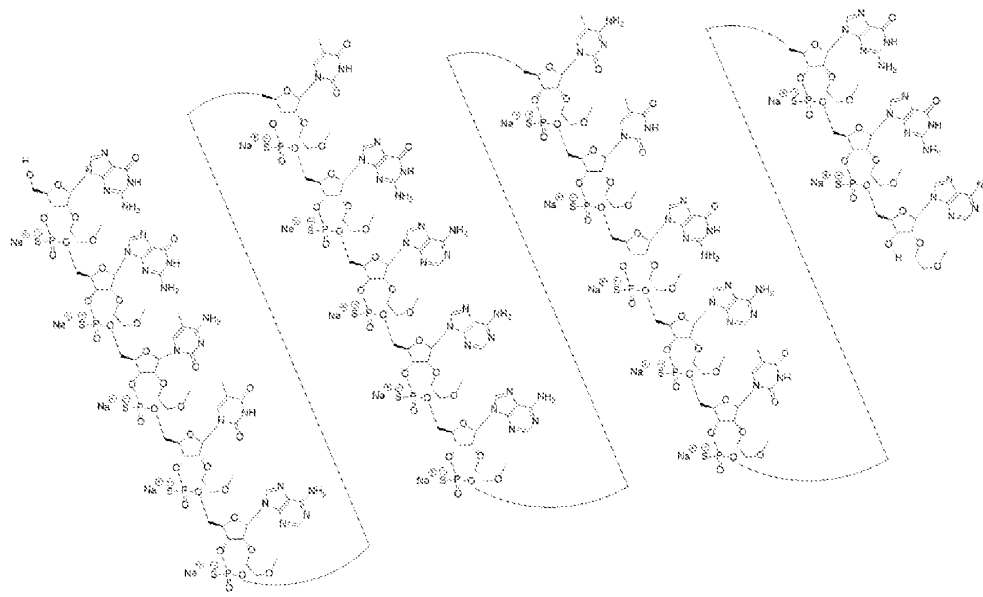


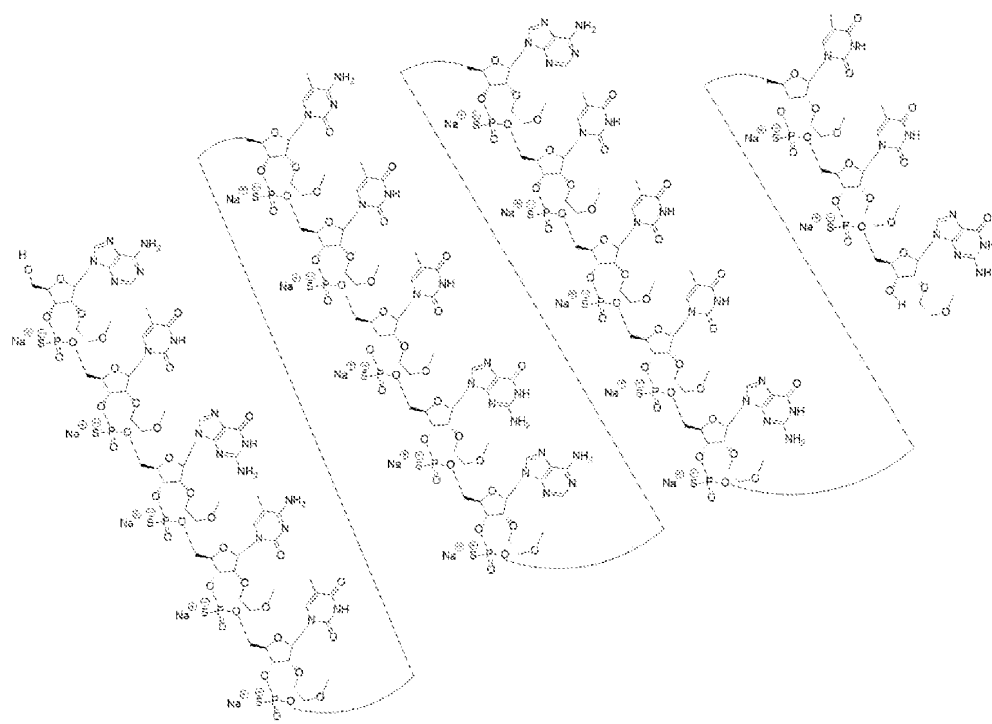
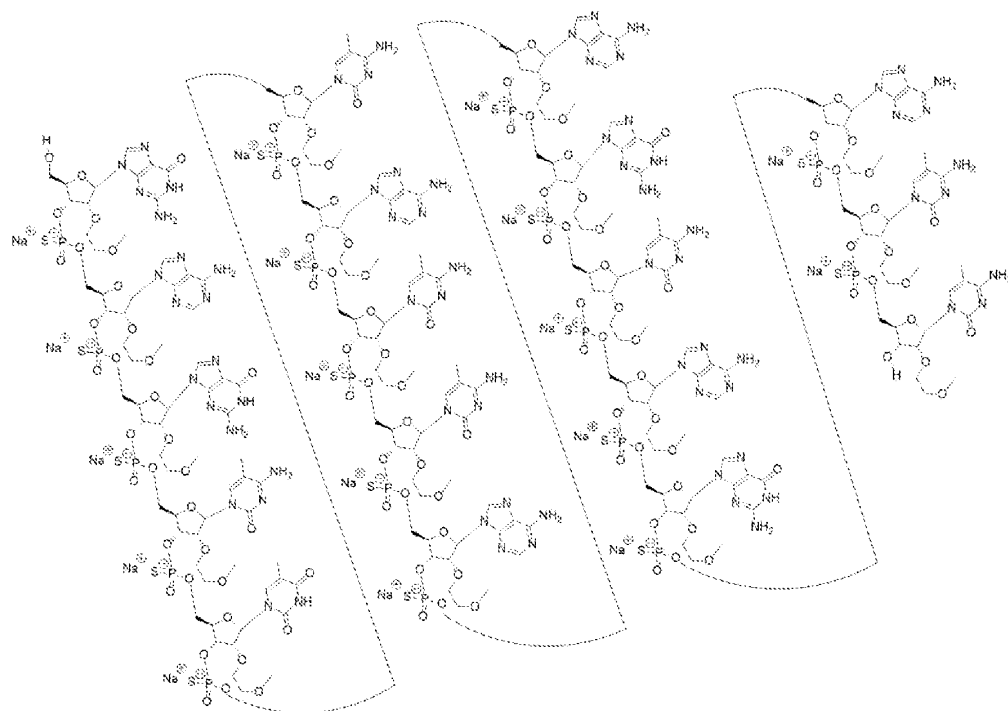


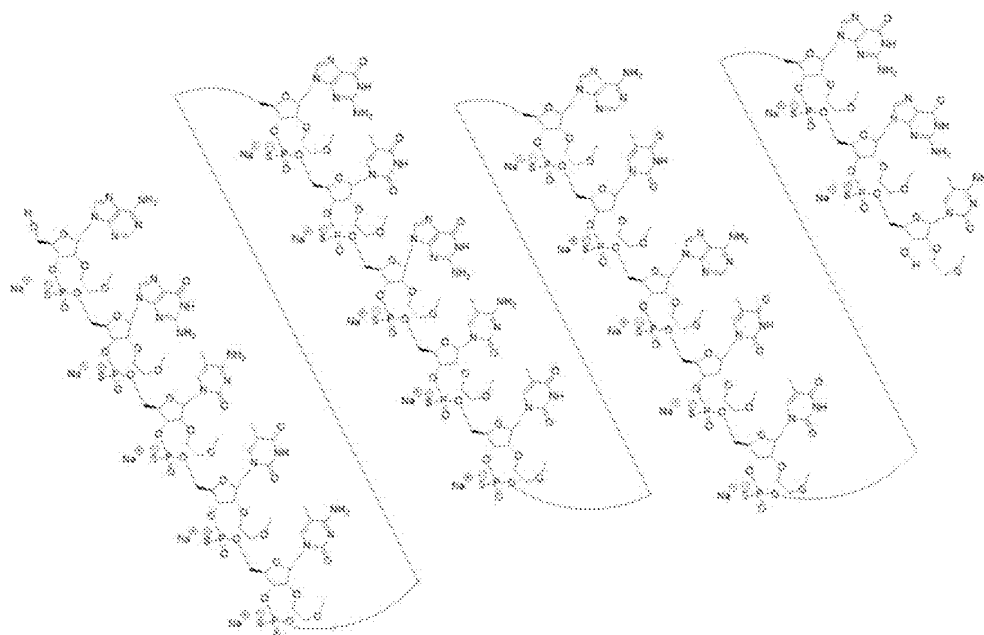


or a pharmaceutically acceptable salt thereof.

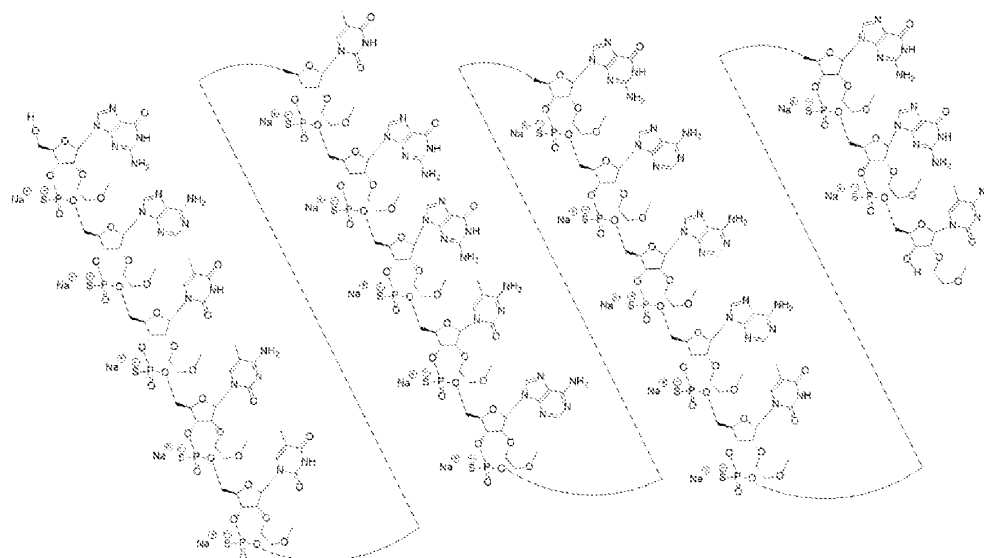
[00858] Embodiment 103. An antisense oligomer having any of the following structures:







; or



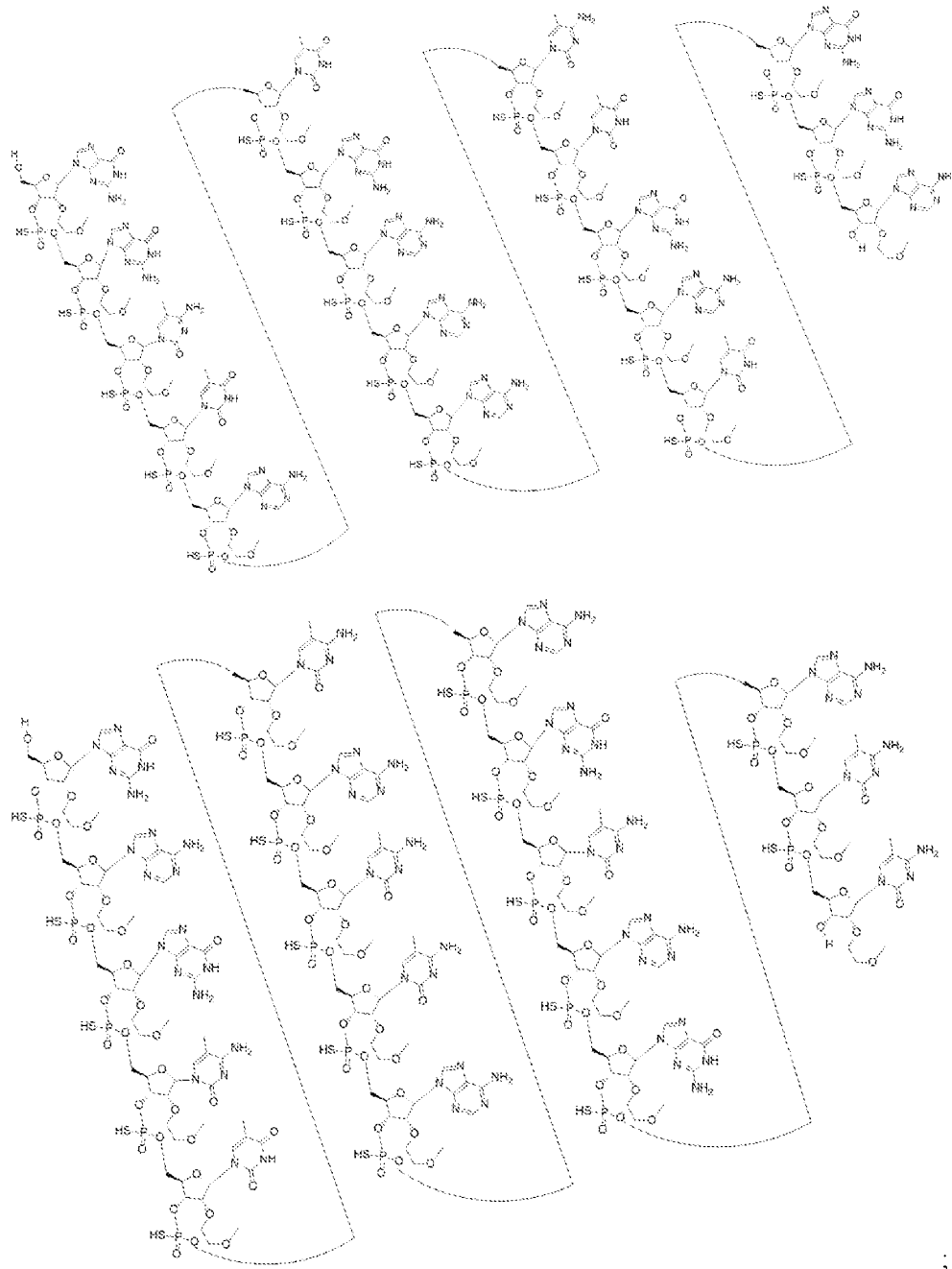
[00859] Embodiment 104. A pharmaceutical formulation comprising:

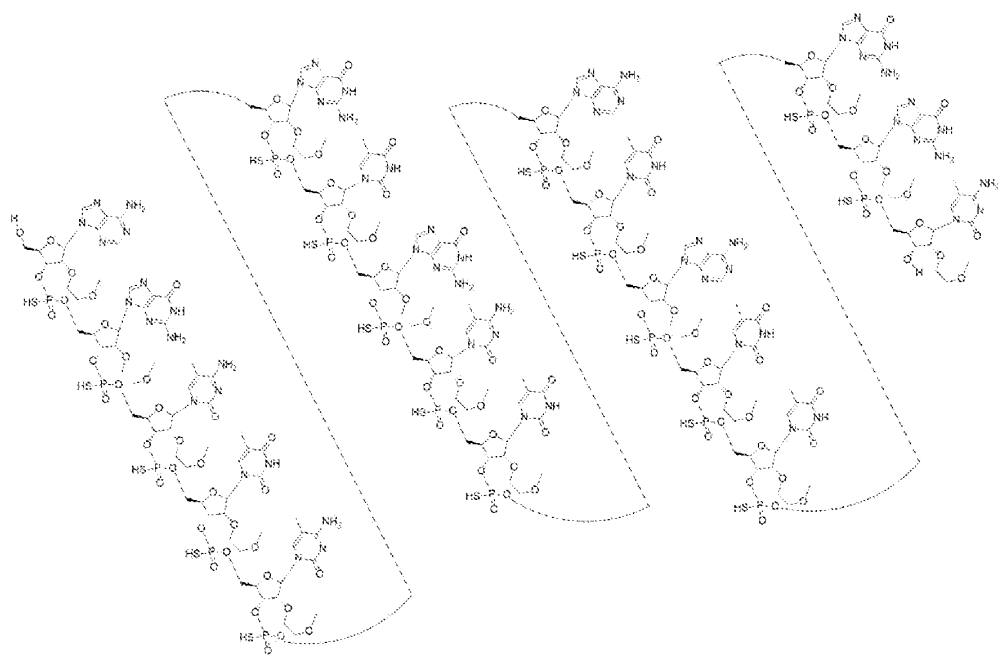
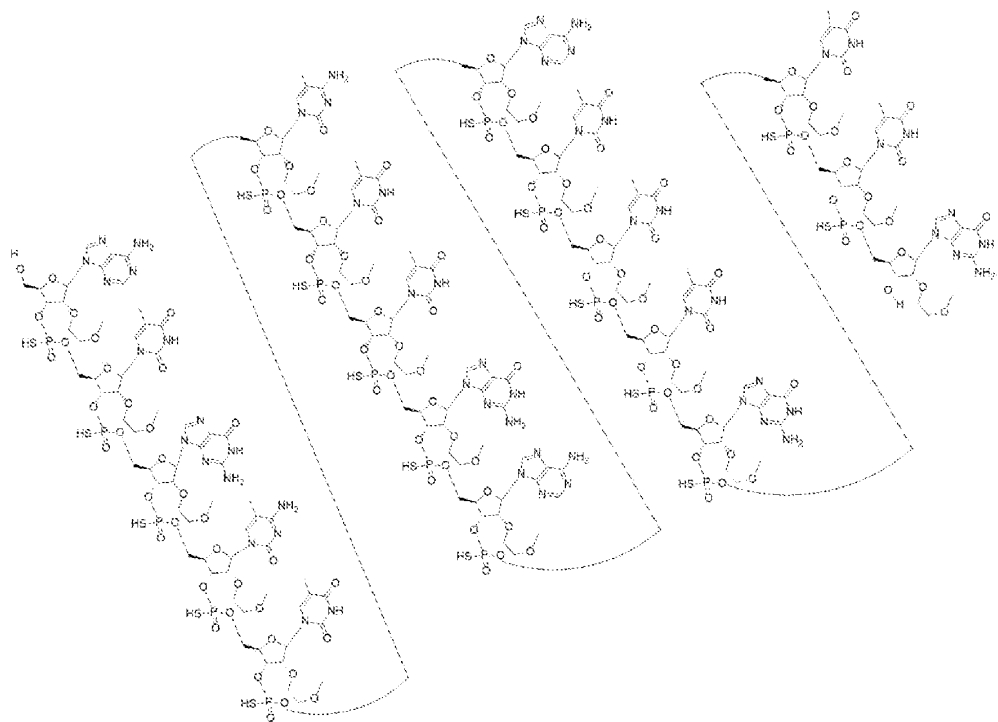
- (a) an antisense oligomer, wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and
 - (b) a pharmaceutically acceptable diluent;
- wherein the antisense oligomer is dissolved or suspended in a solution at a concentration of about 1 mg/mL to about 200 mg/mL.

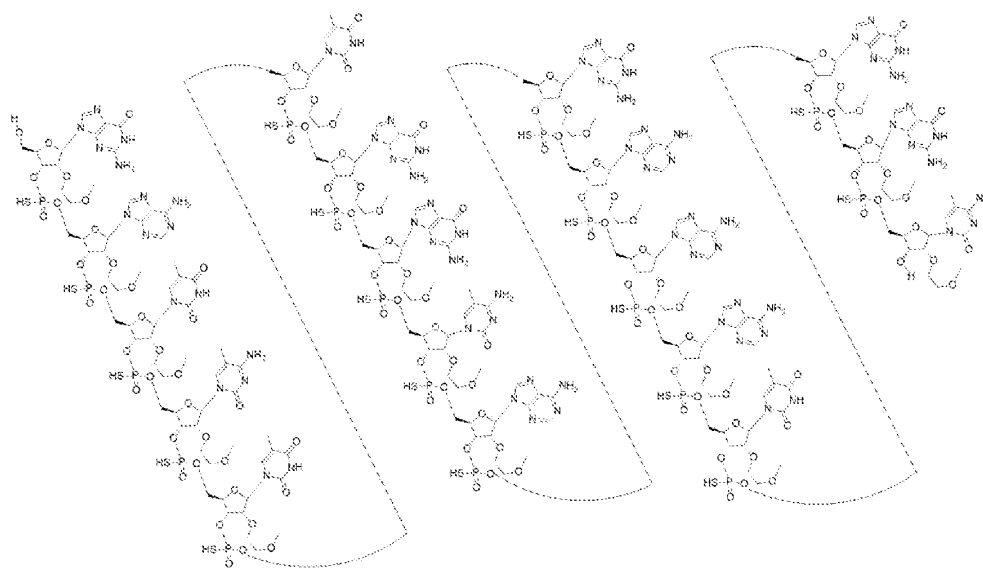
[00860] Embodiment 105. A pharmaceutical formulation comprising:

- (a) an antisense oligomer; and
 - (b) a pharmaceutically acceptable diluent;
- wherein the antisense oligomer is dissolved or suspended in a solution, and

wherein the antisense oligomer is a compound according to any one of the following chemical structures:







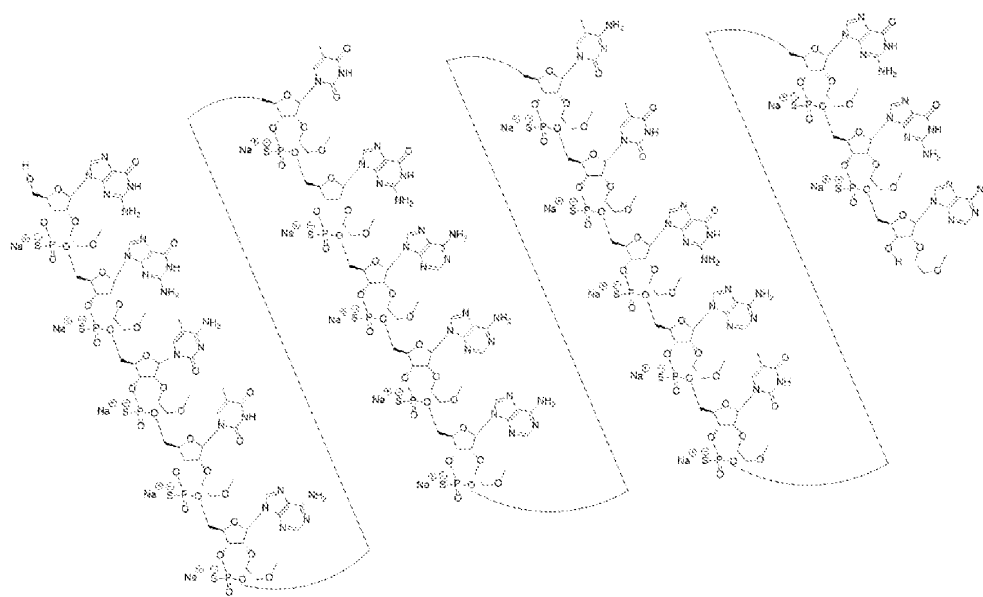
or a pharmaceutically acceptable salt thereof.

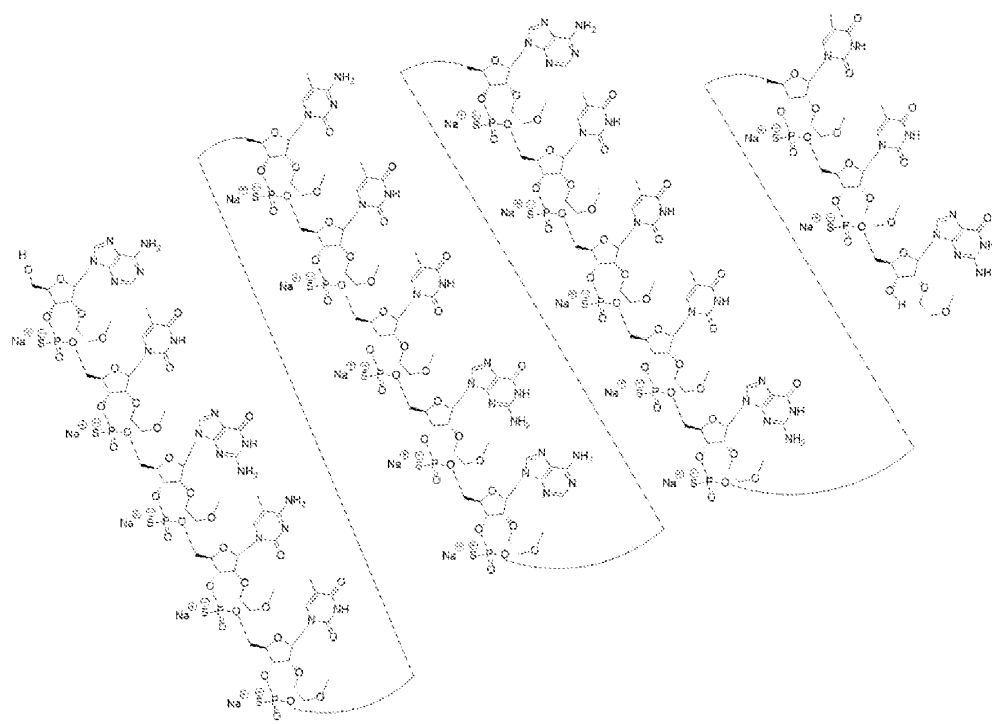
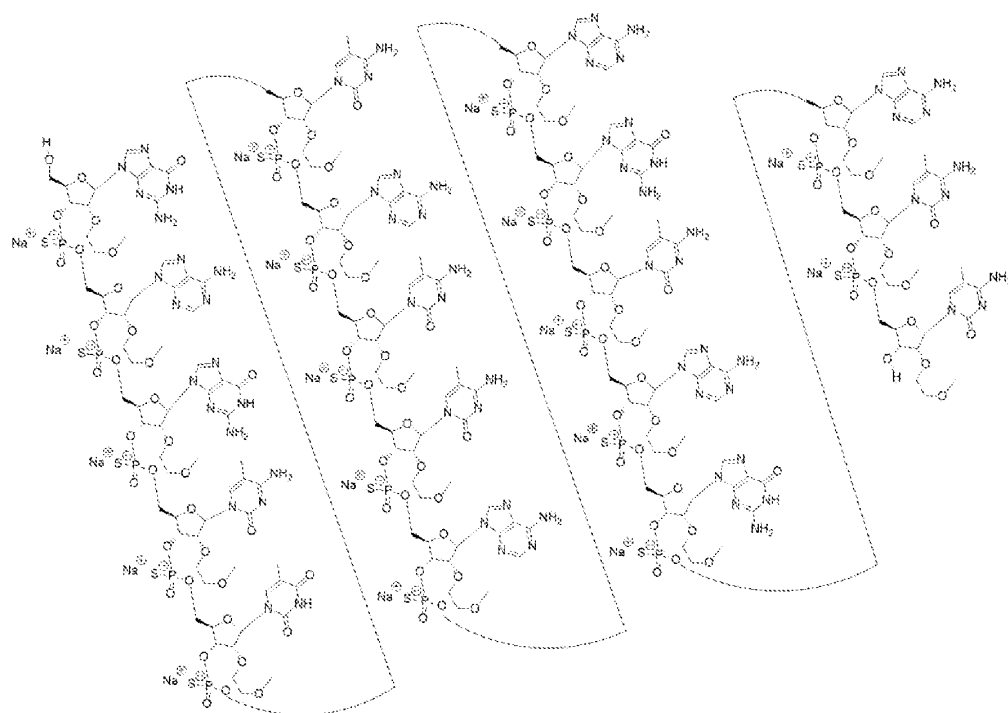
[00861] Embodiment 106. A pharmaceutical formulation comprising:

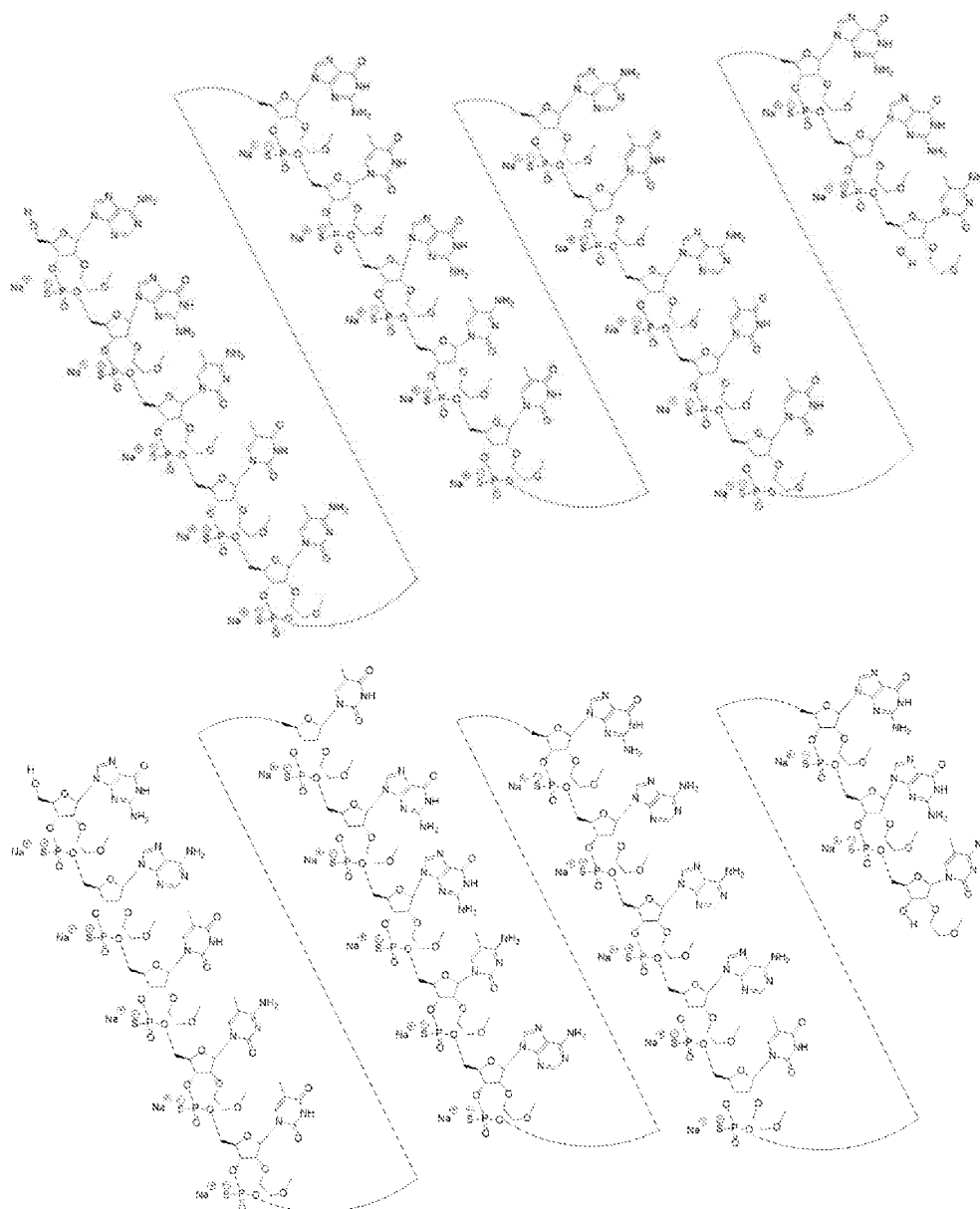
- (a) an antisense oligomer; and
- (b) a pharmaceutically acceptable diluent;

wherein the antisense oligomer is dissolved or suspended in a solution, and

wherein the antisense oligomer has any one of the following chemical structures:







[00862] Embodiment 107. The pharmaceutical formulation of embodiment 105 or 106, wherein the antisense oligomer is present in the solution at a concentration of about 1 mg/mL to about 200 mg/mL.

[00863] Embodiment 108. The pharmaceutical formulation of embodiment 104 or 107, wherein the antisense oligomer is present in the solution at a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL,

about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

[00864] Embodiment 109. The pharmaceutical formulation of embodiment 104, wherein the antisense oligomer is present in the solution at a concentration of about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00865] Embodiment 110. The pharmaceutical formulation of any one of embodiments 104-109, wherein the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer.

[00866] Embodiment 111. The pharmaceutical formulation of embodiment 110, wherein the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about

200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00867] Embodiment 112. The pharmaceutical composition of embodiment 110, wherein the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00868] Embodiment 113. The pharmaceutical formulation of any one of embodiments 104-112, wherein the concentrate is phosphate buffered.

[00869] Embodiment 114. The pharmaceutical formulation of any one of embodiments 104-113, wherein the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

[00870] Embodiment 115. The pharmaceutical formulation of any one of embodiments 104-113, wherein the antisense oligomer is solubilized or diluted in a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

[00871] Embodiment 116. The pharmaceutical formulation of any one of embodiments 104-113, wherein the antisense oligomer is solubilized or diluted in an isotonic solution.

[00872] Embodiment 117. The pharmaceutical formulation of any one of embodiments 104-116, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8.

[00873] Embodiment 118. The pharmaceutical formulation of any one of embodiments 104-116, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution.

[00874] Embodiment 119. The pharmaceutical formulation of any one of embodiments 104-118, wherein the pharmaceutical formulation does not comprise a preservative.

[00875] Embodiment 120. The pharmaceutical formulation of any one of embodiments 104-119, wherein the pharmaceutical formulation is suitable for an intravitreal injection.

[00876] Embodiment 121. The pharmaceutical formulation of any one of embodiments 104-120, wherein the pharmaceutical formulation is packaged in a single-use vial.

[00877] Embodiment 122. A kit comprising:

- (i) a concentrate comprising an antisense oligomer (ASO), wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and
- (ii) a diluent, wherein the concentrate is miscible with the diluent; and
- (iii) instructions for diluting the concentrate with the diluent.

[00878] Embodiment 123. The kit of embodiment 122, wherein the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

[00879] Embodiment 124. The kit of embodiment 122, wherein the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

[00880] Embodiment 125. The kit of any one of embodiments 122-124, wherein the concentrate is phosphate buffered.

[00881] Embodiment 126. The kit of any one of embodiments 122-125, wherein the diluent is a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

[00882] Embodiment 127. The kit of any one of embodiments 122-125, wherein the diluent is a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

[00883] Embodiment 128. The kit of any one of embodiments 122-127, wherein the diluent comprises an isotonic solution.

[00884] Embodiment 129. The kit of any one of embodiments 122-128, wherein the diluent comprises a phosphate-buffered solution with at least pH 5.8.

[00885] Embodiment 130. The kit of any one of embodiments 122-128, wherein the diluent comprises a phosphate-buffered (pH 6.6 - 7.6) solution.

[00886] Embodiment 131. The kit of any one of embodiments 122-130, wherein the concentrate or the diluent does not comprise a preservative.

[00887] Embodiment 132. The kit of any one of embodiments 122-131, wherein the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 2 mg/mL to 200 mg/mL in the diluent.

[00888] Embodiment 133. The kit of any one of embodiments 122-132, wherein the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80

mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL in the diluent.

[00889] Embodiment 134. The kit of any one of embodiments 122-132, wherein the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the antisense oligomer to a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL in the diluent.

[00890] Embodiment 135. The pharmaceutical composition or kit of any one of embodiments 104 or 108-134, wherein the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 or 280-299.

[00891] Embodiment 136. The pharmaceutical composition or kit of any one of embodiments 104 or 108-134, wherein the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

[00892] Embodiment 137. The pharmaceutical composition or kit of any one of embodiments 104 or 108-136, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00893] Embodiment 138. The pharmaceutical composition or kit of any one of embodiments 104 or 108-137, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety.

[00894] Embodiment 139. The pharmaceutical composition or kit of any one of embodiments 104 or 108-138, wherein the antisense oligomer comprises at least one modified sugar moiety.

[00895] Embodiment 140. The pharmaceutical composition or kit of embodiment 139, wherein each sugar moiety is a modified sugar moiety.

[00896] Embodiment 141. The pharmaceutical composition or kit of any one of embodiments 104 or 108-140, wherein the antisense oligomer comprises a 5'-methylcytosine (5'-MeC).

[00897] Embodiment 142. The pharmaceutical composition or kit of any one of embodiments 104 or 108-141, wherein each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC).

[00898] Embodiment 143. The pharmaceutical composition or kit of any one of embodiments 104 or 108-142, wherein the antisense oligomer comprises a 5'-methyluracil (5'-MeU).

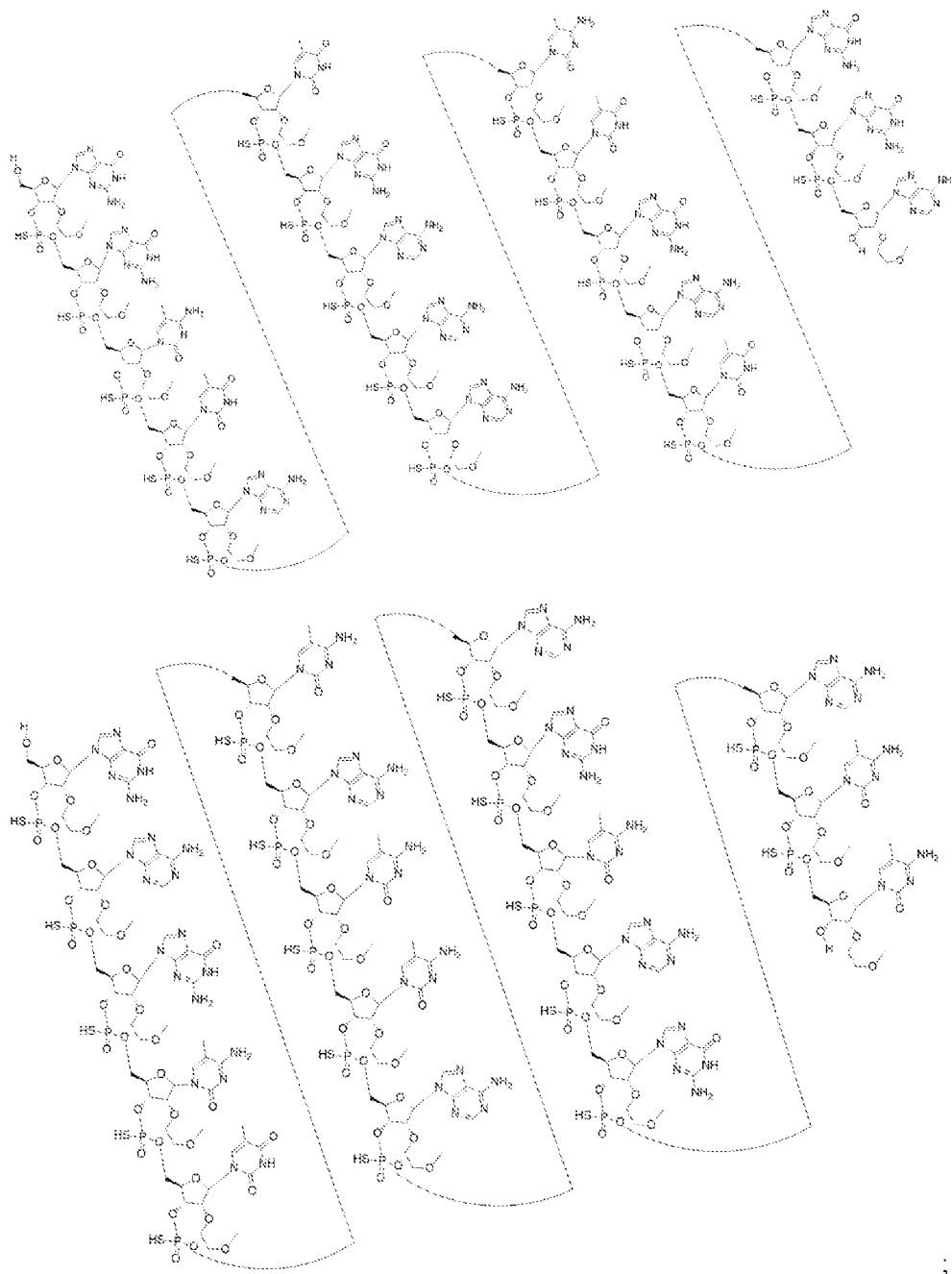
[00899] Embodiment 144. The pharmaceutical composition or kit of any one of embodiments 104 or 108-143, wherein the antisense oligomer comprises a phosphorothioate linkage.

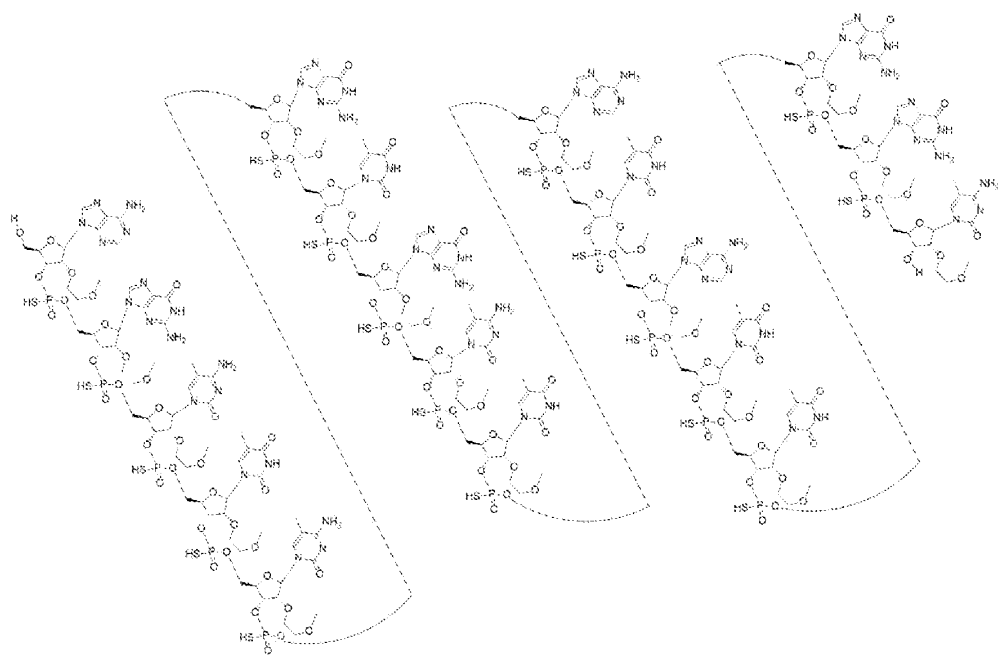
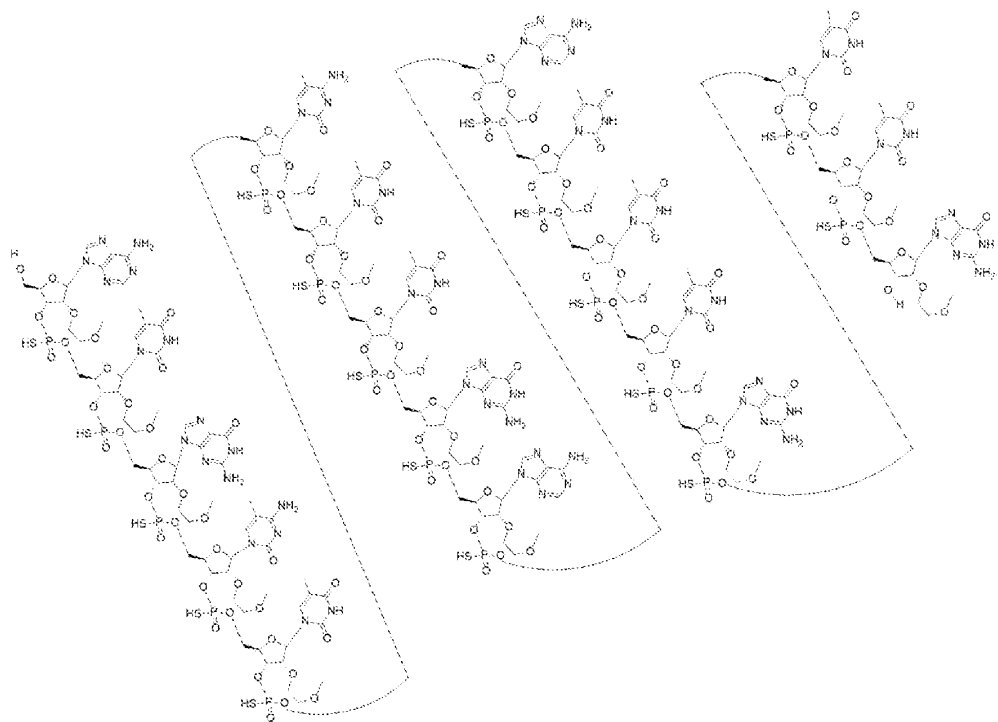
[00900] Embodiment 145. The pharmaceutical composition or kit of any one of embodiments 104 or 108-144, wherein each internucleoside linkage of the ASO is a phosphorothioate linkage.

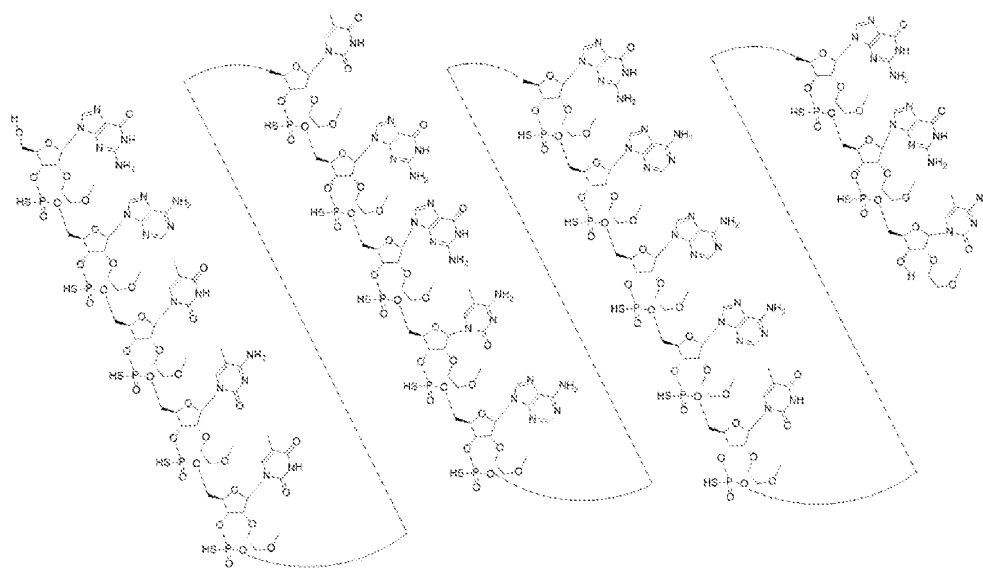
[00901] Embodiment 146. The pharmaceutical composition or kit of any one of embodiments 104 or 108-145, wherein the antisense oligomer comprises a locked nucleic acid (LNA).

[00902] Embodiment 147. The pharmaceutical composition or kit of any one of embodiments 104 or 108-146, wherein the antisense oligomer is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases in length.

[00903] Embodiment 148. The pharmaceutical composition or kit of any one of embodiments 104 or 108-134, wherein the antisense oligomer is a compound according to any one of the following chemical structures:

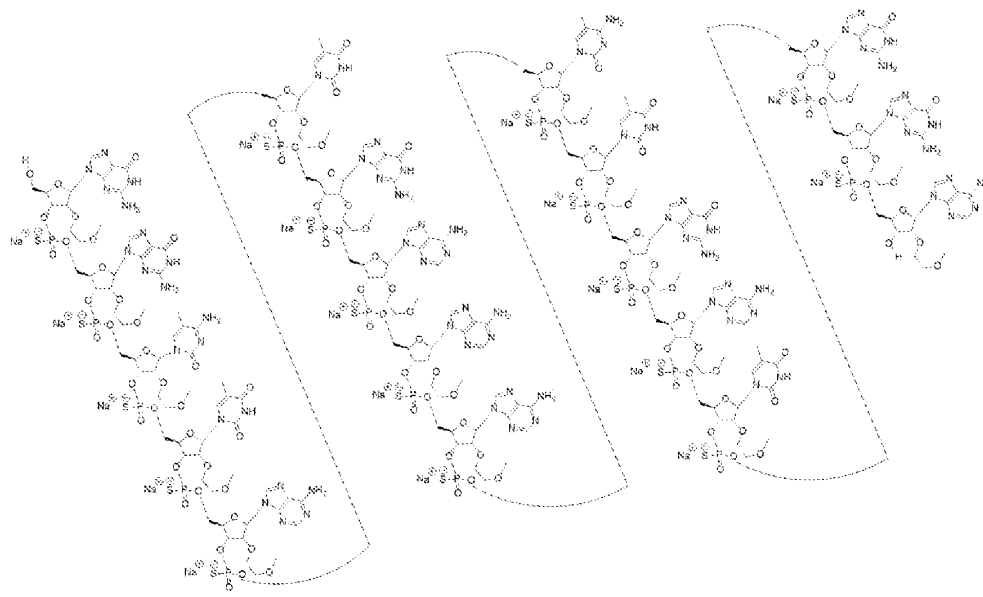


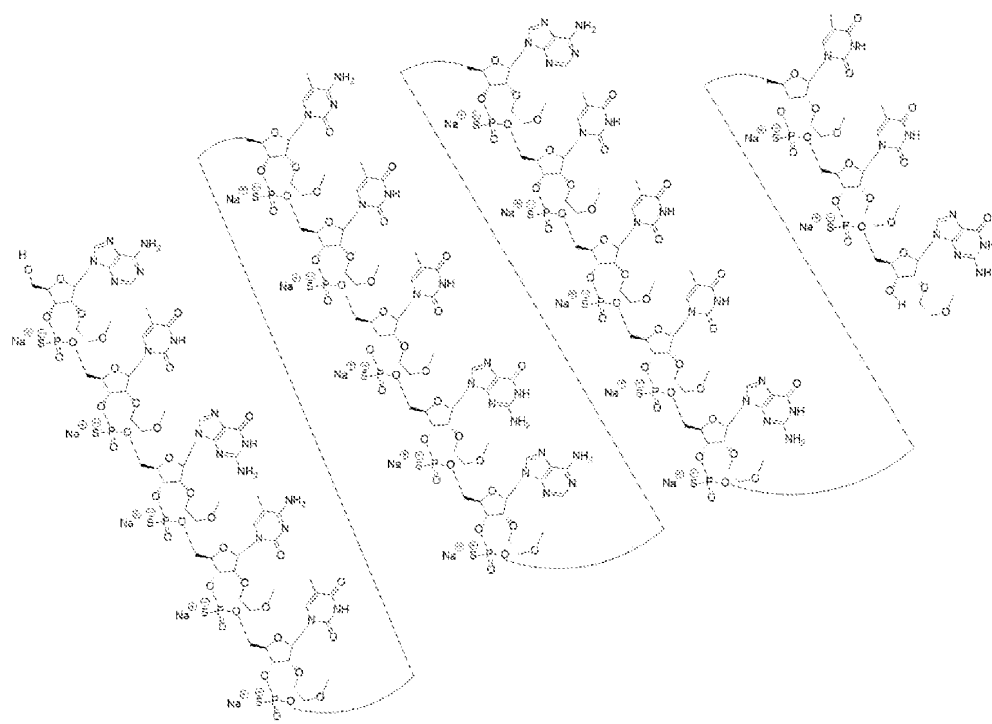
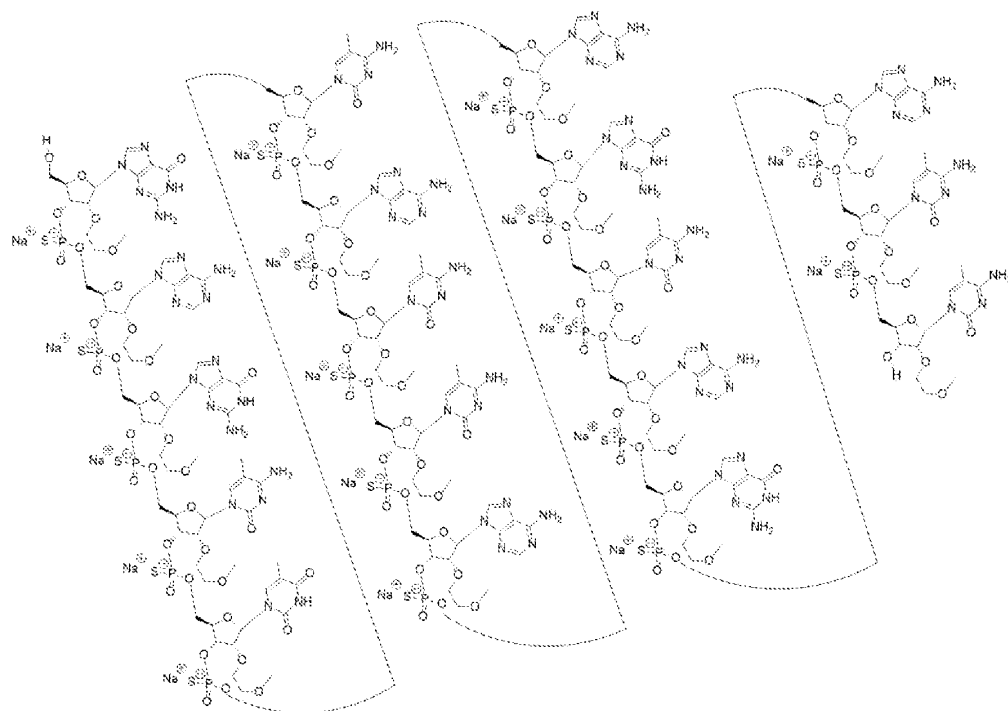


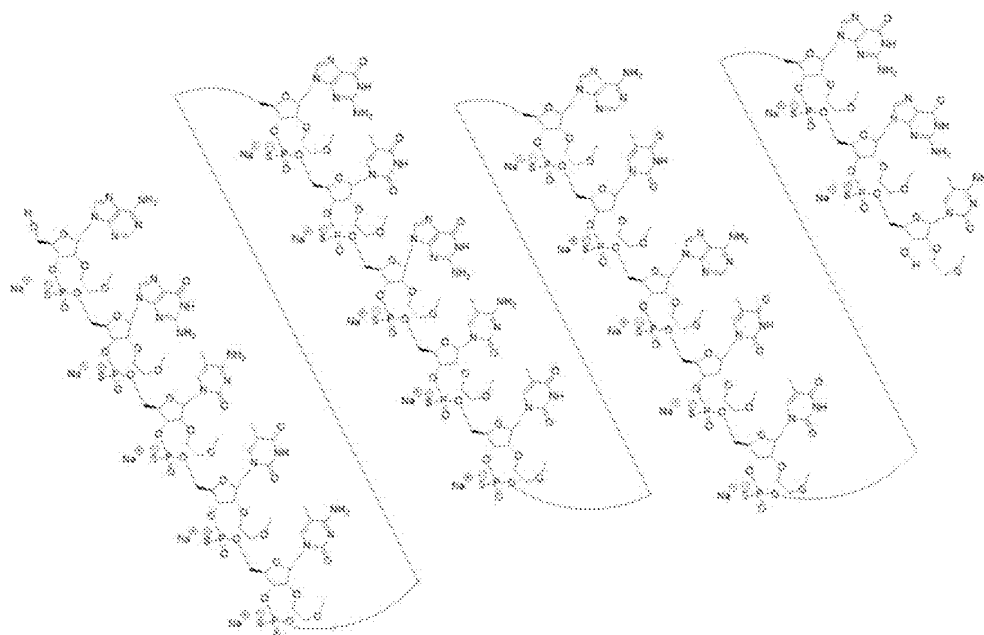


or a pharmaceutically acceptable salt thereof.

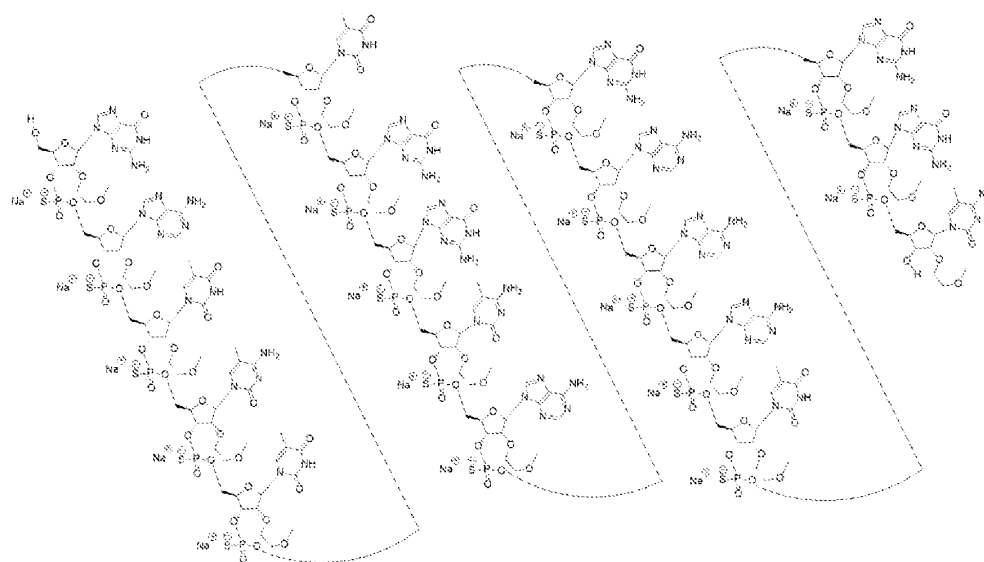
[00904] Embodiment 149. The pharmaceutical composition or kit of any one of embodiments 104 or 108-134, wherein the antisense oligomer has any one of the following chemical structures:







; or



[00905] Embodiment 150. Use of an antisense oligomer for the manufacture of a medicament for treating a disease or condition characterized by a reduced expression or function of OPA1 protein in a human subject in need thereof, or reducing likelihood of the subject developing the disease or condition, wherein the medicament is administered to one eye of the subject at a dose of about 0.005 mg to about 20 mg, and wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299.

[00906] Embodiment 151. The use of embodiment 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg

to about 15 mg, about 0.01 mg to about 10 mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg, about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

[00907] Embodiment 152. The use of embodiment 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

[00908] Embodiment 153. The use of embodiment 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

[00909] Embodiment 154. The use of embodiment 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg,

about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer, preferably about 0.1 mg to about 1.2 mg of the antisense oligomer.

EXAMPLES

[00910] The present disclosure will be more specifically illustrated by the following Examples. However, it should be understood that the present disclosure is not limited by these examples in any manner.

Example 1: Identification of NMD-Inducing Exon Inclusion Events in Transcripts by RNAseq using Next Generation Sequencing.

[00911] Exemplary genes and intron sequences are summarized in **Table 1** and **Table 2** (SEQ ID NOs indicate the corresponding nucleotide sequences represented by the Gene ID Nos). The sequence for each intron is summarized in **Table 3** and **Table 4**. **Table 5** lists sequences of OPA1 antisense oligomers of this disclosure.

Table 1. List of exemplary target gene sequences.

Gene Symbol	Gene ID No.	SEQ ID NO:	Disease	OMIM	Genetics	Introns
<i>OPA1</i>	4976	1	Optic atrophy type 1; Autosomal dominant optic atrophy (ADOA)	165500	Haploinsufficient	ENST00000361908.7:6 ENST00000361908.7:28

Table 2. List of exemplary target gene sequences.

Gene Symbol	Gene ID No.	SEQ ID NO:	Disease	OMIM	Genetics	Introns
<i>OPA1</i>	4976	1	Optic atrophy type 1; Autosomal dominant optic atrophy (ADOA)	165500	Haploinsufficient	GRCh38/ hg38: chr3 193626203 193631611 GRCh38/ hg38: chr3 193593374 193614710

Table 3. Sequences of exemplary target introns in pre-mRNA transcripts.

Gene	SEQ ID NO:	Intron
<i>OPA1</i>	2	<u>Intron 6:</u> gtgatggatggttaaggggctaccgatacattcacactaatcagccatttctgccaagatcatgtcacctcaatctgt tcatggactccaaatacaagaaattaatttgacaaagtgaataataaaagatgcatcatataaatatgtaacttttctgg agtggttagtataggtaaagccaaaagaacaaattcaagcagaggaatttggtttctgaaaattaggtgtctgtag ggtcctgtatttatacttagaacaataattaggaatttctgttatgtgtccagtattgagtcaccctaagtttaggca tcttacctacactgtctcccaagttttatttctaaaatgaaaagcattgctgtagatgaccagttacactaaagaata

Gene	SEQ ID NO:	Intron
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Gene	SEQ ID NO:	Intron
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Table 4. Exemplary target gene intron sequences

SEQ ID NO: 4 Gene: <i>OPA1</i> Intron: GRCh38/ hg38:chr3 193626203 to 193631611 Intron Sequence: gtgatggatgggttaaggggctaccgataattcacactaatcagccatttctccaagatcatgtcacctcaatctgttcattggactcca aatacaagaaattaatttgacaaagtgaaaatataaaagatgcatcatataaatatgaactttctggagtgggtagtagtaagcca aaagaaacaaattcaagcagaggaattttggttctgaaaattaggtgtctgtagggtccctgtatttatactagaacaaaattaggaattt ctgtttatgtgtccagttattgagtcaccctaagttgtaggcatcttacctacttgcctcccaagttttatttctaaaatgaaaagcat tgctgtagatgaccagtttacactaaagaataacatttattttgttttagctaaagtatatggacaggggaacattcatattctgtagaaga aaattttttgacttttgggcaaaagcatgtagttcttatacactttgacaaactcattgcgtacattttcacattaatcaaagtcagcacaat aaattttcaccttgaccacggagggtttgaacactggaaattgatataattctggttgctaaagaacaagttctaataaaagcttaagtgt ataccaatatgtgctgttggtgcaatcagcagggtccgtaaaaaatgatgttaattggttaggtaatccacaacggagatcccaaagttc atgtttggaagagacttttgggtcaaaagtgaatcagtgtaataaattataactctgagatctgaaatcagctaattatgttacatct tattagctcagaaaagtttgaagtatacaaatgctagtcaggaaaaaagattcagtcataattctgtacattctactatttaaatcaa ccaatattatagattatgatttagtcagtaattctgctggctaaccttatctcatttgggtggttagtacttcagagtactccatagtttc atttatgtttcagcatcacttctggtttttctcaattccatggctgtggaatcaattcatatgtatatttagcttcgggtgagcaaaaacatagct agaaaaagaaaagaagtgagtttctacctggttaataaagtcgatgtgtaagccaaggaggactctttgaaatggtactttaacaat ccctgttctgtactgtgaatatacatttaaatagcctaataaattggatgcttaggctgagccacctatacttttagtttggtaagaga aggagagaggagcaagtatgttcttatgttacttagaataagaatgtagctgtatgacacattgttctaagtttttctgaagacaactt gaaatgagtgccataggcctgctatttaacattctaagatatgacttaaggtaatatgatgagctttgaaatctgacaattcaagagatatccat aatgaatactgattcattttctacattgctgaaagctaattgttcaatttaagcctacttttagtagcctttatttgggcttagagatgttattcctctt ctgatatttattgggttatctgtttaacccttttatctcccttcccgatttgtaaattagagactggcaagacttttaccctgagtagagcac caaacatggctgtttctgcccacactgtagttaccttgagggaagtaaatgggactttaaaagcaatttatgctcttttatagtgaattat ccctcttactatccgaaagactgttaccttacaatatcctccactccttccccctgtagttactatagagatgacttttcggttcttactgc cataatgatcaaaatcctaattcatgagattttatcattccaggcatgtgaggttactgtatgcataaaaccgcaagacttttgtgttttt

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SEQ ID NO: 5

Gene: *OPA1*

Intron: GRCh38/ hg38:chr3 193593374 to 193614710

Intron Sequence:

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SEQ ID NO: 276

Gene: *OPA1*

Intron: GRCh38/ hg38:chr3 193618937 to 193626091

Intron Sequence:

gtaagtgtaaaagagaattgtcatgtaggtagcttgaaagatttttaaagttttacttcttggagatttttaaataataacatctgagaa
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SEQ ID NO: 277

Gene: *OPAI***Exon:** GRCh38/ hg38:chr3 193626092 to 193626202**Exon Sequence:**

GGTCTGCTTGGTGAGCTCATTCTCTTACAACAACAAATTCAAGAGCATGAAGAGG
 AAGCGCGCAGAGCCGCTGGCCAATATAGCACGAGCTATGCCCAACAGAAGCGCA
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SEQ ID NO: 278**Gene:** *OPAI***Intron:** GRCh38/ hg38:chr3 193626203 to 193631611**Intron Sequence:**

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SEQ ID NO: 279**Gene:** *OPA1***NMD Exon:** GRCh38/ hg38:chr3 193628509 to 193628616

NMD Exon Sequence:

CTTTAGCTTGTTTCTGCGGAGGATTCCGCTCTTTCTCCATCAGTTTCATAGCCCTG
GAATTGTAGAAAAGCTCTGGTTTCAAGACCATTGATATCCATTTCTGTCAGG

Example 2: *OPA1* Nonproductive Splicing Event Identification and Validation.

[00912] A novel nonsense-mediated decay (NMD) exon inclusion event (Exon X) was identified in the *OPA1* gene which leads to the introduction of a premature termination codon (PTC) resulting in a nonproductive mRNA transcript degraded by NMD (**FIG. 1A**). As NMD is a translation-dependent process, the protein synthesis inhibitor cycloheximide (CHX) was used to evaluate the true abundance of the event. **FIG. 1B** shows an increase in *OPA1* transcripts containing the NMD exon in HEK293 cells with increasing cycloheximide dose. Treatment of HEK293 cells with cycloheximide for 3 hours increases nonproductive mRNA transcripts. Other ocular cell lines were also validated for the presence of the NMD exon (ARPE-19, Y79).

Example 3: *OPA1* NMD Event is Conserved in Primate Eyes.

[00913] **FIG. 1C** shows reverse transcription PCR data from the macular and peripheral regions of both eyes of a human donor. OD: oculus dexter (right eye), OS: oculus sinister (left eye). The nonproductive transcript was also detected in human retinal tissue. The abundance of the transcript in the tissue is potentially higher than what is detected by RT-PCR given that NMD is presumed active, and therefore, that the transcript is being continuously degraded *in vivo*.

Example 4: ASO-1 Decreases Nonproductive *OPA1* mRNA and Increases Total *OPA1* mRNA and *OPA1* Protein Expression in a Dose-Dependent Manner *In Vitro*.

[00914] HEK293 cells were transfected with different doses of ASO-1 (vehicle, or 1, 5, or 20 nM) or non-targeting (NT) ASO. RNA was isolated 24 hours after transfection and analyzed for impact on nonproductive *OPA1* mRNA transcript abundance by RT-PCR (**FIG. 2A**) and total *OPA1* mRNA expression by qPCR (**FIG. 2B**) similarly to in Example 11. For nonproductive transcript analysis, cells were treated with CHX-containing media (50 µg/ml final concentration) for the 3 hours prior to RNA isolation. The RT-PCR gel image of **FIG. 2A** is from one replicate while plotted data represent mean ± SD of three biological replicates. *OPA1* qPCR data in **FIG. 2B** is normalized to the house-keeping gene *RPL32*. For protein analysis, cells were lysed with RIPA buffer 48 hours after transfection and Western Blots were probed with antibodies targeting *OPA1* or β-actin, as shown in **FIG. 2C**. In **FIG. 2E**, cells were lysed with RIPA buffer 72 hours after transfection and Western Blots were probed with antibodies targeting *OPA1* or β-tubulin. Multiple bands in the Western Blots correspond to different isoforms of *OPA1*. Data in **FIG. 2B** and **FIG. 2C** are plotted relative to the Vehicle group. Protein data in **FIG. 2C** represent mean ± SD of three biological replicates and **FIG. 2E** represent mean ± SD of six biological replicates.

*P<0.05, ***P<0.001, ****P<0.0001 by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test. The Non-targeting ASO (NT ASO) targets an unrelated gene.

[00915] Transfection of HEK293 cells with ASO-1 showed dose-dependent reduction in nonproductive transcripts by RT-PCR (**FIG. 2A**). ASO-1 treatment results in a dose-dependent increase in both total *OPA1* mRNA (**FIG. 2B**) and OPA1 protein (**FIG. 2C**) in HEK293 cells. Western Blot analyses showed an increase in multiple OPA1 protein isoforms (**FIG. 2C**). A nontargeting (NT) ASO control directed against an unrelated gene did not have any effect on any of these three parameters. These results indicate that ASO-1 specifically reduces this nonproductive splicing event and increases total *OPA1* mRNA and protein expression in HEK293 cells in a dose-dependent manner.

[00916] To calculate the half maximal effective concentration (EC₅₀) in HEK293 cells, ASO-1 was delivered by gymnotic (free) uptake and levels of the nonproductive transcript were detected by a primer-probe qPCR assay and normalized to house-keeping gene *RPL32*. A dose-dependent reduction in nonproductive transcripts was seen by gymnotic (free) uptake and the EC₅₀ for ASO-1 was determined as 5.63 μ M (**FIG. 2D**). No effects on cell viability were observed at any of these doses with ASO-1 treatment.

Example 5: ASO Microwalk Evaluated by RT-qPCR.

[00917] In one experiment, a microwalk was conducted to test ASOs that have sequences listed in **Table 7**. Briefly, about 30,000 HEK293 cells per well were treated gymnotically with 20 μ M of one of the 20 exemplary ASOs (gymnotic (free) uptake) listed in **Table 7** for 72 hours. After the treatment, the cells were harvested for analysis. RT-PCR reactions were conducted for products corresponding to Exon 7 or Exon 7x inclusion, and full-length *OPA1* mRNA.

Table 5. Exemplary OPA1 ASO sequences

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
6	AGGCCATTCTGAAATTCT	193628406	193628423
7	CATTGAGGCCATTCTGAA	193628411	193628428
8	TAAGGCATTGAGGCCATT	193628416	193628433
9	CCTATTAAGGCATTGAGG	193628421	193628438
10	TTCTTCCTATTAAGGCAT	193628426	193628443
11	AGTATTTCTTCCTATTAA	193628431	193628448
12	TTTCAAGTATTTCTTCCT	193628436	193628453
13	AAAAATTTCAAGTATTC	193628441	193628458
14	AATTTAAAAATTTCAAGT	193628446	193628463
15	GCCCTAATTTAAAAATTT	193628451	193628468

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
16	ACCAAGCCCTAATTTAAA	193628456	193628473
17	ACAAAACCAAGCCCTAAT	193628461	193628478
18	TCCTCACAAAACCAAGCC	193628466	193628483
19	CTAGCTCCTCACAAAACC	193628471	193628488
20	CTTTACTAGCTCCTCACA	193628476	193628493
21	AAAACCTTTACTAGCTCC	193628481	193628498
22	AGAGAAAAACCTTTACTA	193628486	193628503
23	CTGAAAGAGAAAAACCTT	193628491	193628508
24	AAGCTGAAAGAGAAAAAC	193628494	193628511
25	CTAAAGCTGAAAGAGAAA	193628497	193628514
26	AAGCTAAAGCTGAAAGAG	193628500	193628517
27	AACAAGCTAAAGCTGAAA	193628503	193628520
28	AGAAACAAGCTAAAGCTG	193628506	193628523
29	CGCAGAAACAAGCTAAAG	193628509	193628526
30	TCCTCCGCAGAAACAAGC	193628514	193628531
31	CGGAATCCTCCGCAGAAA	193628519	193628536
32	AAGAGCGGAATCCTCCGC	193628524	193628541
33	GGAGAAAGAGCGGAATCC	193628529	193628546
34	CTGATGGAGAAAGAGCGG	193628534	193628551
35	TGAAACTGATGGAGAAAG	193628539	193628556
36	GGCTATGAAACTGATGGA	193628544	193628561
37	TCCAGGGCTATGAAACTG	193628549	193628566
38	ACAATTCCAGGGCTATGA	193628554	193628571
39	TTTCTACAATTCCAGGGC	193628559	193628576
40	GAGCTTTTCTACAATTCC	193628564	193628581
41	AACCAGAGCTTTTCTACA	193628569	193628586
42	CTTGAAACCAGAGCTTTT	193628574	193628591
43	ATGGTCTTGAAACCAGAG	193628579	193628596
44	TATCAATGGTCTTGAAAC	193628584	193628601
45	ATGGATATCAATGGTCTT	193628589	193628606
46	CAGAAATGGATATCAATG	193628594	193628611
47	CCTGACAGAAATGGATAT	193628599	193628616
48	CACCCTGACAGAAATGGA	193628602	193628619
49	ACTCACCTGACAGAAAT	193628605	193628622
50	AAAACCTCACCTGACAGA	193628608	193628625

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
51	TTTAAAACTCACCTGAC	193628611	193628628
52	AAATTTAAAACTCACCT	193628614	193628631
53	AATAAATTTAAAACTCAC	193628617	193628634
54	CATGAAATAAATTTAAAA	193628622	193628639
55	TGCATCATGAAATAAATT	193628627	193628644
56	TTGTTTGCATCATGAAAT	193628632	193628649
57	ATATATTGTTTGCATCAT	193628637	193628654
58	GTTCAATATATTGTTTGC	193628642	193628659
59	CTGTTGTTCAATATATTG	193628647	193628664
60	ATGTCCTGTTGTTCAATA	193628652	193628669
61	AGTTCATGTCCTGTTGTT	193628657	193628674
62	GAACAAGTTCATGTCCTG	193628662	193628679
63	AACAAGAACAAGTTCATG	193628667	193628684
64	CTTACAACAAGAACAAGT	193628672	193628689
65	AGCCACTTACAACAAGAA	193628677	193628694
66	AATTCAGCCACTTACAAC	193628682	193628699
67	GATAAAATTTCAGCCACTT	193628687	193628704
68	TTACTGATAAAATTCAGC	193628692	193628709
69	GTGCTTTACTGATAAAAT	193628697	193628714
70	TTGATGTGCTTTACTGAT	193628702	193628719
71	TGGAGAAAGAGCGGAATC	193628530	193628547
72	ATGGAGAAAGAGCGGAAT	193628531	193628548
73	GATGGAGAAAGAGCGGAA	193628532	193628549
74	TGATGGAGAAAGAGCGGA	193628533	193628550
75	ACTGATGGAGAAAGAGCG	193628535	193628552
76	AACTGATGGAGAAAGAGC	193628536	193628553
77	AACTGATGGAGAAAGAG	193628537	193628554
78	GAAACTGATGGAGAAAGA	193628538	193628555
79	ATGAAACTGATGGAGAAA	193628540	193628557
80	TATGAAACTGATGGAGAA	193628541	193628558
81	CTATGAAACTGATGGAGA	193628542	193628559
82	GCTATGAAACTGATGGAG	193628543	193628560
83	GGGCTATGAAACTGATGG	193628545	193628562
84	AGGGCTATGAAACTGATG	193628546	193628563
85	CAGGGCTATGAAACTGAT	193628547	193628564

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
86	CCAGGGCTATGAAACTGA	193628548	193628565
87	CTGATGGAGAAAGAGCGGAATC	193628530	193628551
88	CTGATGGAGAAAGAGCGGAA	193628532	193628551
89	AACTGATGGAGAAAGAGCGGAA	193628532	193628553
90	AACTGATGGAGAAAGAGCGG	193628534	193628553
91	GAAACTGATGGAGAAAGAGCGG	193628534	193628555
92	GGCTATGAAACTGATGGAGAAA	193628540	193628561
93	GGCTATGAAACTGATGGAGA	193628542	193628561
94	AGGGCTATGAAACTGATGGAGA	193628542	193628563
95	AGGGCTATGAAACTGATGGA	193628544	193628563
96	CCAGGGCTATGAAACTGATGGA	193628544	193628565
97	TTCTTACCCATTTAATTA	193655041	193655059
98	TGCTTCTTACCCATTTAA	193655044	193655062
99	TAATGCTTCTTACCCATT	193655047	193655065
100	AGATAATGCTTCTTACCC	193655050	193655068
101	CAGATAATGCTTCTTACC	193655051	193655069
102	CCCTTCAGATAATGCTTC	193655056	193655074
103	CTACTCCCTTCAGATAAT	193655061	193655079
104	AGCTCCTACTCCCTTCAG	193655066	193655084
105	TTCACAGCTCCTACTCCC	193655071	193655089
106	TAAAATTCACAGCTCCTA	193655076	193655094
107	AAATCTAAAATTCACAGC	193655081	193655099
108	GAATAAAATCTAAAATTC	193655086	193655104
109	GATGGGAATAAAATCTAA	193655091	193655109
110	GCTGTGATGGGAATAAAA	193655096	193655114
111	TAGAGGCTGTGATGGGAA	193655101	193655119
112	AAAGATAGAGGCTGTGAT	193655106	193655124
113	AAAAGAAAGATAGAGGCT	193655111	193655129
114	GACCTAAAAGAAAGATAG	193655116	193655134
115	ATAAAGACCTAAAAGAAA	193655121	193655139
116	GAGATATAAAGACCTAAA	193655126	193655144
117	GGCTGTGATGGGAATAAA	193655097	193655115
118	AGGCTGTGATGGGAATAA	193655098	193655116
119	GAGGCTGTGATGGGAATA	193655099	193655117
120	AGAGGCTGTGATGGGAAT	193655100	193655118

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
121	ATAGAGGCTGTGATGGGA	193655102	193655120
122	GATAGAGGCTGTGATGGG	193655103	193655121
123	AGATAGAGGCTGTGATGG	193655104	193655122
124	AAGATAGAGGCTGTGATG	193655105	193655123
125	TAGAGGCTGTGATGGGAATAAA	193655097	193655119
126	ATAGAGGCTGTGATGGGAATAA	193655098	193655120
127	GATAGAGGCTGTGATGGGAATA	193655099	193655121
128	AGATAGAGGCTGTGATGGGAAT	193655100	193655122
129	AAGATAGAGGCTGTGATGGGAA	193655101	193655123
130	GAGGCTGTGATGGGAATAAA	193655097	193655117
131	AGAGGCTGTGATGGGAATAA	193655098	193655118
132	TAGAGGCTGTGATGGGAATA	193655099	193655119
133	ATAGAGGCTGTGATGGGAAT	193655100	193655120
134	GATAGAGGCTGTGATGGGAA	193655101	193655121
135	AGATAGAGGCTGTGATGGGA	193655102	193655122
136	AAGATAGAGGCTGTGATGGG	193655103	193655123
137	CTGTGATGGGAATAAA	193655097	193655113
138	GCTGTGATGGGAATAA	193655098	193655114
139	GGCTGTGATGGGAATA	193655099	193655115
140	AGGCTGTGATGGGAAT	193655100	193655116
141	GAGGCTGTGATGGGAA	193655101	193655117
142	AGAGGCTGTGATGGGA	193655102	193655118
143	TAGAGGCTGTGATGGG	193655103	193655119
144	ATAGAGGCTGTGATGG	193655104	193655120
145	GATAGAGGCTGTGATG	193655105	193655121
146	AGATAGAGGCTGTGAT	193655106	193655122
147	AAGATAGAGGCTGTGA	193655107	193655123
148	AGGCTGTGATGTGAATAA	193655099	193655116
149	AGAGGCTGTGATGTGAAT	193655101	193655118
150	TAGAGGCTGTGATGTGAA	193655102	193655119
151	GATAGAGGCTGTGATTGG	193655104	193655121
152	GGCTGTGATGTGAATA	193655100	193655115
153	GAGGCTGTGATGTGAA	193655102	193655117
154	TAGAGGCTGTGATTGG	193655104	193655119
155	ATGAAACTGATGGAGA	193628542	193628557

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
156	CTATGAAACTGATGGA	193628544	193628559
157	GGCTATGAAACTGATG	193628546	193628561
158	GAAACTGATGGAGA	193628542	193628555
159	ATGAAACTGATGGA	193628544	193628557
160	CTATGAAACTGATG	193628546	193628559
161	GGCTATGAAACTGA	193628548	193628561
162	TAGAGGCTGTGATGGGAATAAAAT	193655096	193655119
163	ATAGAGGCTGTGATGGGAATAAAA	193655097	193655120
164	ATAGAGGCTGTGATGGGAATAAAAT	193655096	193655120
165	AAAGATAGAGGCTGTGATGGGAATA	193655100	193655124
166	GGCTATGAAACTGATGGAGAA	193628541	193628561
167	GGCTATGAAACTGATGGAGAAAGA	193628538	193628561
168	AGGGCTATGAAACTGATGGAGAAAG	193628539	193628563
169	CATTTAATTAAATTATAT	193655033	193655051
170	CCATTTAATTAAATTATA	193655034	193655052
171	CCCATTTAATTAAATTAT	193655035	193655053
172	ACCCATTTAATTAAATTA	193655036	193655054
173	TACCCATTTAATTAAATT	193655037	193655055
174	TTACCCATTTAATTAAAT	193655038	193655056
175	CTTACCCATTTAATTAAA	193655039	193655057
176	TCTTACCCATTTAATTAA	193655040	193655058
177	GATAGAGGCTGTGATGG	193655104	193655122
178	GGCTGTGAAACTGATGGA	89481662	89481679
179	GGCTGTGAAACTGATGGAGA	89481660	89481679
180	GCTATGAAACTGATGG	193628545	193628560
181	TATGAAACTGATGG	193628545	193628558
182	GCTATGAAACTGAT	193628547	193628560
183	GCTGTGAAACTGATGGAGAA	89481659	89481678
184	GGGCTGTGAAACTGATGGAG	89481661	89481680
185	TGTGAAACTGATGGAGAA	89481659	89481676
186	CTGTGAAACTGATGGAGA	89481660	89481677
187	GCTGTGAAACTGATGGAG	89481661	89481678
188	GGGCTGTGAAACTGATGG	89481663	89481680
189	TGAAACTGATGGAGAA	89481659	89481674
190	GTGAAACTGATGGAGA	89481660	89481675

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
191	TGTGAAACTGATGGAG	89481661	89481676
192	CTGTGAAACTGATGGA	89481662	89481677
193	GCTGTGAAACTGATGG	89481663	89481678
194	GGCTGTGAAACTGATG	89481664	89481679
195	GGGCTGTGAAACTGAT	89481665	89481680
196	CGGTCCAGGAATGAC	193593285	193593303
197	CCGGTCCAGGAATGA	193593286	193593304
198	CCCGGTCCAGGAATG	193593287	193593305
199	TCCCGGTCCAGGAAT	193593288	193593306
200	CTCCCGGTCCAGGAA	193593289	193593307
201	GCTCCCGGTCCAGGA	193593290	193593308
202	GGCTCCCGGTCCAGG	193593291	193593309
203	CGGGAGCCCCCGTGT	193593318	193593336
204	GCGGGAGCCCCCGTG	193593319	193593337
205	CGCGGGAGCCCCCGT	193593320	193593338
206	ACGCGGGAGCCCCCG	193593321	193593339
207	CACGCGGGAGCCCCC	193593322	193593340
208	CCACGCGGGAGCCCC	193593323	193593341
209	GCCACGCGGGAGCCC	193593324	193593342
210	GGCCACGCGGGAGCC	193593325	193593343
211	CGGCCACGCGGGAGC	193593326	193593344
212	ACGGCCACGCGGGAG	193593327	193593345
213	GACGGCCACGCGGGA	193593328	193593346
214	AGACGGCCACGCGGG	193593329	193593347
215	GCTAGGGAGGGATGGTTA	193625988	193626006
216	TGTAAGCTAGGGAGGGAT	193625993	193626011
217	ACAGATGTAAGCTAGGGA	193625998	193626016
218	AAGGAACAGATGTAAGCT	193626003	193626021
219	CAACAAAGGAACAGATGT	193626008	193626026
220	GGGTGCAACAAAGGAACA	193626013	193626031
221	ACCAAGGGTGCAACAAAG	193626018	193626036
222	GTAAACCAAGGGTGCAA	193626023	193626041
223	ATAATGTTAAACCAAGGG	193626028	193626046
224	GGAGAATAATGTTAAACC	193626033	193626051
225	GGGGAGGAGAATAATGTT	193626038	193626056

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
226	AAATTGGGGAGGAGAATA	193626043	193626061
227	AGAGGAAATTGGGGAGGA	193626048	193626066
228	GGAGAAGAGGAAATTGGG	193626053	193626071
229	AATGAGGAGAAGAGGAAA	193626058	193626076
230	TTCACAATGAGGAGAAGA	193626063	193626081
231	ACGAGTTCACAATGAGGA	193626068	193626086
232	CTGCCACGAGTTCACAAT	193626073	193626091
233	AGACCCTGCCACGAGTTC	193626078	193626096
234	CAAGCAGACCCTGCCACG	193626083	193626101
235	CTCACCAGCAGACCCTG	193626088	193626106
236	GAGCTACCAAGCAGACC	193626091	193626109
237	AGAATGAGCTACCAAGC	193626096	193626114
238	GTAAGAGAATGAGCTCAC	193626101	193626119
239	TTGTTGTAAGAGAATGAG	193626106	193626124
240	ATTTGTTGTTGTAAGAGA	193626111	193626129
241	CTTGAATTTGTTGTTGTA	193626116	193626134
242	ATGCTCTTGAATTTGTTG	193626121	193626139
243	TCTTCATGCTCTTGAATT	193626126	193626144
244	CTTCCTCTTCATGCTCTT	193626131	193626149
245	GCGCGCTTCCTCTTCATG	193626136	193626154
246	GCTCTGCGCGCTTCCTCT	193626141	193626159
247	GGCCAGCGGCTCTGCGCG	193626149	193626167
248	ATATTGGCCAGCGGCTCT	193626154	193626172
249	GTGCTATATTGGCCAGCG	193626159	193626177
250	AGCTCGTGCTATATTGGC	193626164	193626182
251	GGCATAGCTCGTGCTATA	193626169	193626187
252	TGTTGGGCATAGCTCGTG	193626174	193626192
253	GCTTCTGTTGGGCATAGC	193626179	193626197
254	CTTGCGCTTCTGTTGGGC	193626184	193626202
255	CACCTTGCGCTTCTGTTG	193626187	193626205
256	TCCATCACCTTGCGCTTC	193626192	193626210
257	AACCATCCATCACCTTGC	193626197	193626215
258	CCTTAAACCATCCATCAC	193626202	193626220
259	AGCCCCCTTAAACCATCC	193626207	193626225
260	TCGGTAGCCCCCTTAAAC	193626212	193626230

SEQ ID NO:	Sequence (5'-3')	Coordinates GRCh38/hg38: chr3	
		Oligo Start	Oligo End
261	ATGTATCGGTAGCCCCCT	193626217	193626235
262	TGTGAATGTATCGGTAGC	193626222	193626240
263	ATTAGTGTGAATGTATCG	193626227	193626245
264	GGCTGATTAGTGTGAATG	193626232	193626250
265	GAAATGGCTGATTAGTGT	193626237	193626255
266	TGGCAGAAATGGCTGATT	193626242	193626260
267	GATCTTGGCAGAAATGGC	193626247	193626265
268	GACATGATCTTGGCAGAA	193626252	193626270
269	GAGGTGACATGATCTTGG	193626257	193626275
270	AGATTGAGGTGACATGAT	193626262	193626280
271	TGAACAGATTGAGGTGAC	193626267	193626285
272	GTCCATGAACAGATTGAG	193626272	193626290
273	TTGGAGTCCATGAACAGA	193626277	193626295
274	TGTATTTGGAGTCCATGA	193626282	193626300
275	TTTCTTGTATTTGGAGTC	193626287	193626305

Table 6. Exemplary OPA1 ASO sequences

SEQ ID NO:	Region	Sequence (5'-3')	Coordinates: GRCh38/hg38: chr3	
			Oligo Start	Oligo End
215	OPA1-IVS6-86	GCTAGGGAGGGATGGTTA	193625988	193626006
216	OPA1-IVS6-81	TGTAAGCTAGGGAGGGAT	193625993	193626011
217	OPA1-IVS6-76	ACAGATGTAAGCTAGGGA	193625998	193626016
218	OPA1-IVS6-71	AAGGAACAGATGTAAGCT	193626003	193626021
227	OPA1-IVS6-26	AGAGGAAATTGGGGAGGA	193626048	193626066
228	OPA1-IVS6-21	GGAGAAGAGGAAATTGGG	193626053	193626071
229	OPA1-IVS6-16	AATGAGGAGAAGAGGAAA	193626058	193626076
230	OPA1-IVS6-11	TTCACAATGAGGAGAAGA	193626063	193626081
231	OPA1-IVS6-6	ACGAGTTCACAATGAGGA	193626068	193626086
232	OPA1-IVS6-1	CTGCCACGAGTTCACAAT	193626073	193626091
233	OPA1-IVS6-EX7+5	AGACCCTGCCACGAGTTC	193626078	193626096
234	OPA1-IVS6-EX7+10	CAAGCAGACCCTGCCACG	193626083	193626101
235	OPA1-IVS6-EX7+15	CTCACCAAGCAGACCCTG	193626088	193626106
236	OPA1-EX7+1	GAGCTCACCAAGCAGACC	193626091	193626109
237	OPA1-EX7+6	AGAATGAGCTCACCAAGC	193626096	193626114
238	OPA1-EX7+11	GTAAGAGAATGAGCTCAC	193626101	193626119

SEQ ID NO:	Region	Sequence (5'-3')	Coordinates: GRCh38/hg38: chr3	
			Oligo Start	Oligo End
239	OPA1-EX7+16	TTGTTGTAAGAGAATGAG	193626106	193626124
240	OPA1-EX7+21	ATTTGTTGTTGTAAGAGA	193626111	193626129
241	OPA1-EX7+26	CTTGAATTTGTTGTTGTA	193626116	193626134
242	OPA1-EX7+31	ATGCTCTTGAATTTGTTG	193626121	193626139
250	OPA1-EX7-21	AGCTCGTGCTATATTGGC	193626164	193626182
267	OPA1-IVS7+46	GATCTTGGCAGAAATGGC	193626247	193626265

Table 7. Exemplary OPA1 ASO sequences

SEQ ID NO:	Region	Sequence (5'-3')	Coordinates: GRCh38/hg38: chr3	
			Oligo Start	Oligo End
280	OPA1-EX7+27	TCTTGAATTTGTTGTTGT	193626117	193626135
281	OPA1-EX7+28	CTCTTGAATTTGTTGTTG	193626118	193626136
282	OPA1-EX7+29	GCTCTTGAATTTGTTGTT	193626119	193626137
283	OPA1-EX7+30	TGCTCTTGAATTTGTTGT	193626120	193626138
284	OPA1-EX7+32	CATGCTCTTGAATTTGTT	193626122	193626140
285	OPA1-EX7+33	TCATGCTCTTGAATTTGT	193626123	193626141
286	OPA1-EX7+34	TTCATGCTCTTGAATTTG	193626124	193626142
287	OPA1-EX7+35	CTTCATGCTCTTGAATTT	193626125	193626143
288	OPA1-EX7+26	TGAATTTGTTGTTGTA	193626116	193626132
289	OPA1-EX7+27	TTGAATTTGTTGTTGT	193626117	193626133
290	OPA1-EX7+28	CTTGAATTTGTTGTTG	193626118	193626134
291	OPA1-EX7+29	TCTTGAATTTGTTGTT	193626119	193626135
292	OPA1-EX7+30	CTCTTGAATTTGTTGT	193626120	193626136
293	OPA1-EX7+31	GCTCTTGAATTTGTTG	193626121	193626137
294	OPA1-EX7+32	TGCTCTTGAATTTGTT	193626122	193626138
295	OPA1-EX7+33	ATGCTCTTGAATTTGT	193626123	193626139
296	OPA1-EX7+34	CATGCTCTTGAATTTG	193626124	193626140
297	OPA1-EX7+35	TCATGCTCTTGAATTT	193626125	193626141
298	OPA1-EX7+36	TTCATGCTCTTGAATT	193626126	193626142
299	OPA1-EX7+37	CTTCATGCTCTTGAAT	193626127	193626143

Example 6: ASO-1 Increases OPA1 Protein and Total Cellular ATP Levels in *OPA1*^{+/-} HEK293 Cells

[00918] Since most patients with ADOA show haploinsufficiency of OPA1, a cellular model of OPA1 haploinsufficiency was engineered in HEK293 cells by using CRISPR-Cas9 gene

editing to insert one nucleotide in one *OPAI* allele. The process yielded an *OPAI*^{+/-} HEK293 cell line that showed reduced OPA1 expression compared to an isogenic control cell line (*OPAI*^{+/+} HEK293) that underwent the gene editing process but did not develop any mutations (**FIG. 3D** and **FIG. 3E**, compare Vehicle groups of *OPAI*^{+/+} and *OPAI*^{+/-} cells). *OPAI*^{+/+} or *OPAI*^{+/-} HEK293 cells were treated with Vehicle or 10 μM ASO-1 by gymnotic uptake and assessed for effect OPA1 protein expression 72 hours after treatment. Protein data is normalized to β-actin and assessed for expression relative to the Vehicle group in *OPAI*^{+/+} cells. The mean ± SD of three biological replicates is shown for each cell and treatment. Western Blot analyses show reduced OPA1 protein levels in *OPAI*^{+/-} HEK293 cells compared to isogenic *OPAI*^{+/+} HEK293 cells (**FIG. 3A** and **FIG. 3D**). **FIG. 3E** shows the ATP or total protein levels in *OPAI*^{+/+} and *OPAI*^{+/-} cells 72 hours after treatment with either vehicle or ASO-1. Total protein levels were about the same when *OPAI*^{+/+} cells are treated with either vehicle or ASO-1. When *OPAI*^{+/-} cells were treated with vehicle, total protein levels were about 0.8 of the baseline, whereas treatment with ASO-1 resulted in total protein levels at about 0.9 of baseline.

[00919] *OPAI*^{+/+} or *OPAI*^{+/-} HEK293 cells were treated with Vehicle or 10 μM ASO-1 by gymnotic uptake for 72 hours and assessed for effect on *OPAI* mRNA expression (**FIGS. 4A-4B**) and OPA1 protein (**FIGS. 4C-4D**) 72 hours post-treatment. Data are plotted relative to the Vehicle group separately for both *OPAI*^{+/+} and *OPAI*^{+/-} cells. Representative Western Blots for data in (**FIGS. 4C-4D**) can be found in **FIG. 4E**. *P<0.05, **P<0.01 by unpaired t-test.

[00920] ASO-1 increased *OPAI* mRNA and OPA1 protein in both *OPAI*^{+/+} and *OPAI*^{+/-} HEK293 cell lines by gymnotic (free) uptake (**FIGS. 4A-4D**). The presence of only one *OPAI* allele in *OPAI*^{+/-} cells did not change the abundance of the nonproductive transcript (**FIG. 3F-3H**), suggesting that the increase in OPA1 protein mediated by reducing nonproductive splicing may be similar in both *OPAI* WT and haploinsufficient systems.

[00921] Mitochondria are primary centers of ATP production in the cell through oxidative phosphorylation. Given the critical role of OPA1 in the maintenance of mitochondria bioenergetics, *OPAI*^{+/-} HEK293 cells were examined to determine whether total cellular ATP levels were reduced.

[00922] *OPAI*^{+/+} or *OPAI*^{+/-} HEK293 cells were treated with Vehicle or 10 μM ASO-1 by gymnotic (free) uptake for 96 hours and total cellular ATP levels were measured and normalized to total protein levels (**FIG. 3B**). All values were plotted relative to the mean ATP level of WT *OPAI*^{+/+} HEK293 cells. The mean ± standard deviation of at least three independent experiments (each with 3 replicates) conducted on separate days are shown. *P<0.05, ****P<0.0001 by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test.

[00923] Total cellular ATP levels were found to be significantly lower in *OPAI*^{+/-} HEK293 cells compared to isogenic WT control cells (FIG. 3B). ASO-1 treatment increased ATP levels in *OPAI*^{+/-} HEK293 cells but did not modulate ATP levels in *OPAI*^{+/+} cells (FIG. 3B). These results support the concept that ASO-1 can positively modulate the reduced ATP phenotype found in *OPAI*^{+/-} cells.

Example 7: ASO-1 Reduces Nonproductive Exon Inclusion and Increases OPA1 Expression in ADOA Patient Fibroblast Cells in a Variant-Independent Manner

[00924] Previous studies have used primary dermal fibroblasts derived from patients with ADOA *OPAI* mutations as an *in vitro* model of disease pathophysiology. To test whether ASO-1 would increase OPA1 expression and potentially modify disease phenotype in ADOA, dermal fibroblasts from three unrelated ADOA patients (designated as F34, F35 and F36) with different *OPAI* pathogenic variants were evaluated (FIG. 5A).

[00925] WT (ATCC fibroblasts) and all three ADOA patient fibroblast cells were treated with 40 nM ASO-1 by transfection and harvested after 24 hours for analysis of nonproductive splicing (FIG. 5B) and *OPAI* mRNA levels (FIG. 5C), both normalized to *RPL32*. OPA1 protein levels (FIG. 5D) were determined by Western Blot 72 hours after transfection and normalized to β -actin (Western Blot images in FIG. 5I). qPCR data show mean $\pm$ SD of three biological replicates, while protein data show mean $\pm$ SD of nine biological replicates. All data are plotted relative to the WT Vehicle group. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 by unpaired t-test.

[00926] All three patients' fibroblast cells showed reduced *OPAI* mRNA and OPA1 levels compared to those of three different WT fibroblast cells (FIGS. 5E-5G). Patient fibroblast cells had *OPAI* mRNA levels ranging from 58-68% and OPA1 protein levels ranging from 36-47% of levels measured in WT fibroblasts (FIGS. 5C and 5D, compare Vehicle bars across the different genotypes). As seen previously with the *OPAI*^{+/-} HEK293 cells, the presence of one WT OPA1 allele did not reduce the abundance of the nonproductive transcript in any of the three patient fibroblast cells compared to all three WT fibroblasts (FIG. 5H). Treatment with ASO-1 reduced nonproductive splicing and increased *OPAI* mRNA and protein in WT fibroblasts as well as in all three patients' cells (FIGS. 3F-3H, 5B-5D, and 5I), supporting the mutation-independent nature of the TANGO approach.

Example 8: ASO-1 Enhances the Ability of ADOA Patient Fibroblast Cells to Respond to Mitochondrial Stress and Increases Mitochondrial Function in ADOA Patient Fibroblast Cells

[00927] The respiratory function of the three patient fibroblast cells (F34, F35, and F36) was compared to all three WT fibroblast cells by measuring the oxygen consumption rate (OCR;

pmol/min/cell) of live cells under basal and stress conditions and upon treatment with different electron transport chain (ETC) inhibitors using the Seahorse extracellular flux analyzer. F34, F35 and F36 patient fibroblast cells were treated with 20, 40 or 60 nM ASO-1 or Vehicle by transfection and their oxygen consumption rate was measured on a Seahorse XFe96 extracellular flux analyzer under basal and stress conditions and normalized to cell count. Specifically, basal respiration (**FIG. 6A** and **FIG. 6E**), ATP-linked respiration (**FIG. 6B** and **FIG. 6F**), maximal respiration (**FIG. 6C** and **FIG. 6G**), and spare (reserve) mitochondrial respiratory capacity were measured (**FIG. 6D** and **FIG. 6H**).

[00928] **FIGS. 6A-6H** show histograms demonstrating the reduction of mitochondrial function in ADOA patient-derived fibroblast cells oxygen consumption rate (OCR; pmol/min/cell) under both basal and stress conditions was measured by a Seahorse XFe96 extracellular flux analyzer. Data are normalized to mean WT values for each parameter. Data show mean $\pm$ SD of three biological replicates and plotted relative to the Vehicle group for each cell type. Vehicle group bars are not shown as it is 1.0 for each cell type. Each data point represents an average of OCR values from 8-12 wells. Biological replicates were pooled from separate experiments conducted on two different days. Statistical analysis was by ordinary one-way ANOVA followed by Dunnett's multiple comparison test against Vehicle for each cell type; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and **** $P < 0.0001$.

[00929] All three ADOA patient cells showed a significant reduction in these four parameters indicating a deficit in mitochondrial respiratory function under basal and stress-mediated conditions (**FIGS. 6A-6H**). All three ADOA patient cells showed a deficit in basal and ATP-linked respiration compared to the three WT cells. Maximal respiration and spare respiratory capacity were reduced in the three patient cells compared to WT cells from ATCC, while the other two WT cells showed more variability in these two parameters. This variability among the WT fibroblast cells could be attributed to differences in age, sex, and genetic backgrounds of the three donors and therefore a baseline for mitochondrial bioenergetics in *OPAI*^{+/+} fibroblasts could not be established.

[00930] The ability of ASO-1 treatment in enhancing the parameters of mitochondrial bioenergetics in the three ADOA patient cells was evaluated (**FIG. 7A-7D**). **FIGS. 7A-7D** show histograms of F34, F35 and F36 patient fibroblast cells that were treated with 20, 40 or 60 nM ASO-1 or Vehicle by transfection; their oxygen consumption rate (OCR; pmol/min/cell) was measured on a XFe96 extracellular flux analyzer under basal and stress conditions. Basal respiration (**FIG. 7A**), ATP-linked respiration (**FIG. 7B**), maximal respiration (**FIG. 7C**), and spare respiratory capacity (**FIG. 7D**) were reported. **FIGS. 7E-7H** show the effect of various concentrations of a nontargeting ASO (NT ASO) on the mitochondrial bioenergetics in ADOA

patient F35's fibroblast cells. Oxygen consumption rate measurements were taken from the patient fibroblast cells treated with 20, 40 or 60 nM of NT ASO). Data are normalized relative to the Vehicle group for each cell type. Statistical analysis was by ordinary ANOVA followed by Dunnett's multiple comparison test against Vehicle; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and **** $P < 0.0001$.

[00931] Treatment with ASO-1 increased the four OCR parameters in F35 and F36 fibroblast cells in a dose-dependent manner (**FIG. 7A-7D**). The non-targeting ASO tested in F35 cells resulted in little to no change in any of the parameters was observed (**FIG. 7E-7H**), suggesting that the increases observed in mitochondrial respiratory parameters were specific to ASO-1. In F34 fibroblast cells, ASO-1 treatment resulted in a dose-dependent increase in maximal respiration and spare-respiratory capacity, while increases in basal or ATP-linked respiration were not observed (**FIGS. 7A-7D**). Maximal respiration is measured upon the addition of the uncoupler FCCP, which mimics a physiological energy demand by stimulating the respiratory chain to operate at maximum capacity. The difference between maximal and basal respiration is the spare respiratory capacity, a measure of mitochondrial reserve. The increase in maximal respiration and spare respiratory capacity in all three patient cells with different pathogenic variants shows that ASO-1 enhances the ability of *OPA1* haploinsufficient cells to respond to conditions that induce mitochondrial stress (**FIG. 7D**).

Example 9: Surrogate ASO ST-1102 Produces Lasting Reduction in Nonproductive Splicing and Increases OPA1 Protein in Wild-Type Rabbit Retinal Tissue Following Single Intravitreal (IVT) Injection

[00932] Sequence alignments of the nonproductive exon suggested that it is well conserved in rabbit, pig, dog, and several nonhuman primate species (chimpanzee, rhesus monkey, green monkey). Interestingly, neither mouse nor rat have the nonproductive exon due to lack of conservation at the 3' splice site and therefore, rodents could not be used as models for *in vivo* proof-of-mechanism studies. While the event is conserved in rabbits, the ASO-1 target region is not perfectly conserved between humans and rabbits. Therefore, a rabbit surrogate ASO, ST-1102, with identical sequence complementarity to the rabbit *OPA1* target region was created for proof-of-mechanism studies. A single, bilateral intravitreal (IVT) injection of PBS or ST-1102 at 0.04, 0.12 or 0.23 mg/eye was administered to female adult New Zealand White (NZW) rabbits and retinas were harvested 7-, 14-, and 28-days post-injection for pharmacological assessments of nonproductive splicing, *OPA1* protein, and ASO exposure (**FIG. 8A**).

[00933] Nonproductive splicing was measured by RT-PCR using rabbit retinal RNA by quantifying the relative abundance of nonproductive *OPA1* mRNA transcripts to total *OPA1* transcripts (**FIG. 8B**). Data are plotted relative to the PBS injected group. Statistical significance

($P < 0.0001$ vs. PBS injected group) was observed at all time-points for all dose groups. RT-PCR gel images are included in **FIG. 9**. OPA1 protein was quantitated in retinal lysates by Western Blot and normalized to actin ($***P < 0.001$, $***P < 0.0001$ vs. PBS treated group) (**FIG. 8C**). Data are plotted relative to the PBS injected group. Western Blot images are included in **FIG. 10**. ST-1102 was detected in retinal tissue on Day 29 by HELISA at all three dose levels (**FIG. 8D**). Statistical analysis by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test.

[00934] A significant dose-related decrease in the nonproductive transcript was observed in rabbit retinal tissue as early as 7 days post-injection by RT-PCR. The levels of nonproductive transcripts further dropped at both 14- and 28-days post-injection (**FIG. 8B** and **FIG. 9**). No changes in retinal OPA1 protein levels were observed 7 days post-injection however significant dose-related upregulation of OPA1 protein levels were seen 14 days post-injection that remained significantly elevated at 28 days post-injection (**FIG. 8C** and **FIG. 10**). Retinal exposure of ST-1102 was measured only 28 days post-injection. ST-1102 retinal levels increased with dose but were not strictly dose-proportionate (**FIG. 8D**). Taken together, these data indicate that a single IVT administration of ST-1102 significantly reduced the levels of nonproductive transcripts and increased OPA1 protein in the rabbit retina for at least four weeks.

Example 10: ASO-1 Produces Lasting Reduction in NMD Exon Inclusion, Increase in OPA1 Protein, and Exposure in Cynomolgus Monkey Retinal Tissue

[00935] The presence of the nonproductive transcript was confirmed in cynomolgus monkey retinal tissue (**FIG. 11D**). The retinal tissues from both the left (OS) and right (OD) eyes of cynomolgus monkeys were dissected and evaluated for NMD exon inclusion after intravitreal injection of ASO-1. The qPCR gel images of three cynomolgus monkeys' retinal tissue show that the *OPA1* mRNA transcripts are skewed toward NMD exon exclusion after treatment with ASO-1 (*i.e.*, reduction in NMD exon inclusion) as can be visualized by the darker bands corresponding to *OPA1* mRNA transcripts without the NMD exon.

[00936] The ASO-1 target sequence is also perfectly conserved between cynomolgus monkeys and humans, and therefore, this species was used to evaluate pharmacology, exposure, and tolerability of ASO-1. A single, bilateral IVT injection of PBS or ASO-1 was administered to cynomolgus monkeys.

[00937] Intravitreal administration of ASO-1 induces dose-related reduction in NMD exon inclusion and increased OPA1 protein with sustained tissue exposure in the retina of cynomolgus monkeys. Pharmacology was assessed in the Vehicle (PBS-injected), and 0.1, 0.3, and 1.0 mg/eye ASO-1 treatment groups. Retinas were collected from indicated treatment groups at 4 or 8 weeks (**FIG. 11A**). Presence of nonproductive exon-containing RNA transcripts in retinal tissue was

assessed by qPCR and expressed relative to total *OPA1* transcripts. At 4 weeks after a single injection of ASO-1, detectable nonproductive RNA transcripts in the retinal samples were reduced by approximately 56% (0.1 mg/eye), 76% (0.3 mg/eye), and 94% (1.0 mg/eye) when compared to PBS-injected eyes (**FIG. 11A**).

[00938] **FIG. 11B** shows histograms OPA1 protein quantitated in retinal lysates by ELISA and normalized to total protein in the sample. The 0.3 and 1.0 mg/eye treatment groups were also evaluated at 8 weeks and found to maintain similar reductions. A corresponding increase in retinal OPA1 protein quantified by ELISA was observed. At week 4, the level of OPA1 protein increase was 31%, 47%, and 44% in the retinal tissue at ASO-1 doses of 0.1, 0.3, and 1.0 mg/eye, respectively (**FIG. 11B**). The 0.3 and 1.0 mg dose levels were also assessed in an additional cohort at week 8, and elevated OPA1 protein levels were maintained. Percent increase could not be determined since there were no control animals at week 8; however, the OPA1 level measured by ELISA was comparable and slightly higher than the respective week 4 dose group levels (**FIG. 11B**). These data indicate that a single IVT administration of ASO-1 was effective for at least 8 weeks in significantly reducing the levels of nonproductive mRNA transcripts and increasing OPA1 protein in the cynomolgus monkey retina.

[00939] Retinal tissue levels of ASO-1 were quantitated by HELISA at weeks 4 and 8. Samples were normalized by tissue weight. The exposure was dose-dependent but not strictly dose proportional in retinal tissue (**FIG. 11C**). At week 8 compared to week 4, mean ASO-1 concentration in the retina decreased by 34% in the 0.3 mg/eye group and remained at a similar level for 1 mg/eye dose (increased by 6%) (**FIG. 11C**). This sustained retention through week 8 suggests slow clearance and long tissue half-life of ASO-1 in retina.

[00940] Data in **FIGS. 11A-11C** are presented as mean $\pm$ standard deviation. Each data point represents one retina. Statistics were not performed for week 8 where N=2. Statistical analysis by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test. *P<0.05; **P<0.01; ***P<0.001 vs. Vehicle (PBS-treated) group.

Example 11: ASO-1 and OPA1 Protein Are Detected in Cynomolgus Monkey Retinal Ganglion Cells (RGCs) After IVT Administration

[00941] *ASO-1 Biodistribution in NHP Retina Following IVT Administration*

[00942] The loss of visual function in ADOA is due to the dysfunction and loss of RGCs, thus therapeutic targeting of this cell type can lead to successful treatment of ADOA. The biodistribution of ASO-1 in the non-human primate (NHP) retina was assessed following administration by intravitreal (IVT) administration into the eyes of cynomolgus monkeys. A 50- μ L aliquot of either PBS or ASO-1 was intravitreally injected into the eyes of cynomolgus monkeys and ocular tissues were collected four weeks after injection. The retinas of the right eyes

of the cynomolgus monkeys were then fixed for *in situ* hybridization (ISH) and immunohistochemical (IHC) staining (**FIG. 12E**). Layers of ocular tissue comprising retinal ganglion cells (RGC), inner nuclear layer (INL), outer nuclear layer (ONL), photoreceptors (PR), and retinal pigment epithelial cells (RPE) were examined for their staining pattern. Cell nuclei were stained blue with hematoxylin, and in PBS-only eyes, only blue staining for the nuclei was present (**FIG. 12C**). In ASO-1-treated eyes, ocular cells could be located by the blue staining of the nuclei, and ASO-1's presence could be observed by the red staining throughout the layers of ocular tissue comprising the RGC, INL, ONL, and RPE (**FIG. 12D**). The ASO-1 signal was strong and persistent in RGCs after IVT injection. ASO-1 was present in all retinal layers up to four weeks in nonhuman primates following IVT injection.

[00943] *ASO-1 and OPA1 Signals Increase in NHP Retinal Ganglion Cells with Increasing IVT Dose*

[00944] NHP Retinal Ganglion Cells were then evaluated for ASO-1 and OPA1 signals with increasing IVT dose. Eyes of cynomolgus monkeys were injected with PBS, or 0.1 mg ASO-1 per eye (low dose), 0.3 mg ASO-1 per eye (mid dose), or 0.5 mg ASO-1 per eye (high dose). Sections of Modified Davidson's-fixed paraffin-embedded retina from the Vehicle and 0.1, 0.3, and 1.0 mg/eye ASO-1 treatment groups were evaluated by miRNAscope™ *in situ* hybridization to detect ASO-1. **FIGS. 12A-12B** are exemplary images labeled with treatment type and ASO-1 dose per eye. PBS-injected eyes did not demonstrate any detectable signal, indicating that the probe was highly specific for ASO-1 (**FIGS. 12C**).

[00945] **FIG. 12A and 12D** are exemplary images of miRNAscope™ staining with a specific probe to ASO-1 performed on MDF-fixed, paraffin-embedded cynomolgus monkey eyes to demonstrate cellular location of ASO-1 (red) in RGCs. Increased ASO-1 signal is correlated with increased dose (*i.e.*, the higher the ASO-1 dosage, the more red staining for ASO-1 that was present and the more OPA1 protein staining that was shown by immunofluorescent staining) (**FIG. 12A**). Nuclei were counterstained with hematoxylin (blue).

[00946] **FIGS. 13A-13D** show histograms of the quantitation of mRNA copies (dots) per cell and percent positive RGCs in foveal (**FIGS. 13A-13B**) and peripheral retina (**FIGS. 13C-13D**) showed a dose-related increase in ASO-1 detected in retinal ganglion cells (RGCs) after intravitreal (IVT) administration to cynomolgus monkeys. Statistics by one-way ANOVA with Dunnett's multiple comparison test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to Vehicle (PBS-treated).

[00947] By visual inspection (**FIG. 12A**) as well as quantitative histological assessment (**FIGS. 13A-13D**), a dose-related increase was observed in ASO-1 signal in the RGC layer at week 4.

[00948] **FIG. 12B** is an exemplary image of immunofluorescence staining with OPA1 antibody, which shows apparent dose-related protein increase (red) at 0.3 and 1.0 mg in the RGCs in/near the fovea. Nuclei were stained with DAPI (blue), and the magnification was 40X.

[00949] By visual inspection after antibody staining, OPA1 protein immunofluorescence was observed in the RGC layer of the retina. The intensity of immunofluorescence appeared to increase with ASO-1 dose in the RGC cell layer, including RGCs in the foveal region (**FIG. 12B**) at 4 weeks after ASO-1 treatment. However, results of attempted quantitation of the immunofluorescence intensity showed an increasing trend but were not statistically significantly different from Vehicle (**FIGS. 14A-14B**). A substantial signal for OPA1 protein was apparent in the RGCs at baseline (**FIG. 12B, PBS**) as expected and consistent with published literature (Wang A., *et al.* (2006). OPA1 expression in the human retina and optic nerve. *Exp Eye Res* 83(5):1171-1178).

[00950] *Evaluation of ASO-1 Pharmacokinetics (PK) and Pharmacodynamics (PD) in Non-human Primates (NHPs) Following Intravitreal (IVT) Injection*

[00951] Either PBS (no ASO) or five doses of ASO-1 (0.05, 0.1, 0.2, 0.5, 1 mg/eye) were intravitreally injected into the eyes of cynomolgus monkeys (**Table 13**). Cynomolgus monkeys' ocular tissues were collected, and their retinas dissected eight weeks after the intravitreal injections. Retinas were dissected into four quadrants (**FIG. 15C**). Quadrant 1 (Q1) comprised the quadrant between the superior and temporal regions of the eye and was used to evaluate OPA1 protein expression. Quadrant 2 (Q2) comprised the quadrant between the superior and nasal regions of the eye and was used to evaluate ASO-1 exposure. Quadrant 3 (Q3) comprised the quadrant between the inferior and temporal regions of the eye and was used to evaluate *OPA1* mRNA expression. Quadrant 4 (Q4) comprised the quadrant between the inferior and nasal regions of the eye and was also used to evaluate ASO-1 exposure. ASO-1 quantification was evaluated by hybridization ELISA (hELISA), mRNA was measured by qPCR, and OPA1 protein measured by ELISA.

[00952] *ASO-1 Achieves Exposure in Retina, Demonstrates Target Engagement, and Increases OPA1 Protein Expression in NHPs*

[00953] The concentration of ASO-1 in retina tissue was assessed by hybridization ELISA (hELISA) and observed to increase with higher concentrations of ASO-1 in the retinas of cynomolgus monkeys, suggesting that ASO-1 exposure in the retina was dose-dependent (**FIG. 15D**). Dose 1 (0.05 mg ASO per eye) resulted in about 40 units/g of ASO-1 in the retina. Dose 2 (0.10 mg ASO per eye) resulted in about 50 units/g of ASO-1 in the retina. Dose 3 (0.20 mg ASO per eye) resulted in about 130 units/g of ASO-1 in the retina. Dose 4 (0.5 mg ASO per eye) resulted

in about 280 units/g of ASO-1 in the retina. Dose 5 (1 mg ASO per eye) resulted in about 360 units/g of ASO-1 in the retina.

[00954] NMD exon inclusion was evaluated by qPCR and demonstrates dose-dependent reduction of NMD exon inclusion by ASO-1 (**FIG. 15E**). Relative *OPAI* NMD exon inclusion for ASO-1 dose conditions were normalized to a PBS (no ASO) control. Dose 1 (0.05 mg ASO per eye) resulted in a reduction of NMD exon inclusion to about 0.4 of baseline NMD exon inclusion in the retina. Dose 2 (0.10 mg ASO per eye) resulted in a reduction of NMD exon inclusion to about 0.25 of baseline NMD exon inclusion in the retina. Dose 3 (0.20 mg ASO per eye) resulted in a reduction of NMD exon inclusion to about 0.2 of baseline NMD exon inclusion in the retina. Dose 4 (0.5 mg ASO per eye) resulted in a reduction of NMD exon inclusion to about 0.1 of baseline NMD exon inclusion in the retina. Dose 5 (1 mg ASO per eye) resulted in a reduction of NMD exon inclusion to about 0.05 of baseline NMD exon inclusion in the retina.

[00955] NHP retinal OPA1 protein expression levels were measured by ELISA. OPA1 protein levels appear to be upregulated with ASO-1 treatment (**FIG. 15F**). Relative OPA1 protein expression levels for ASO-1 dose conditions were normalized to a PBS (no ASO) control. Dose 1 (0.05 mg ASO per eye) resulted in an increase of OPA1 protein to about 1.2 units/mg of total protein in the retina. Dose 2 (0.10 mg ASO per eye) resulted in an increase of OPA1 protein to about 1.7 units/mg of total protein in the retina. Dose 3 (0.20 mg ASO per eye) resulted in an increase of OPA1 protein to about 2.5 units/mg of total protein in the retina. Dose 4 (0.5 mg ASO per eye) resulted in an increase of OPA1 protein to about 1.8 units/mg of total protein in the retina. Dose 5 (1 mg ASO per eye) resulted in an increase of OPA1 protein to about 1.2 units/mg of total protein in the retina. OPA1 protein levels increased up to 2-fold ($p=0.0002$).

[00956] The foregoing preclinical data support the TANGO disease-modifying approach in ADOA. As demonstrated by the data, the exemplary antisense oligomer, ASO-1, reduced nonproductive exon inclusion, increased total *OPAI* mRNA and protein expression in all three patient fibroblast cell lines; increased ASO-1 dose increased mitochondrial respiration in two fibroblast cell lines. The data further suggest that the ASO-mediated increase in OPA1 protein expression is disease-modifying in ADOA in a mutation-independent manner.

Example 12: Material and Methods

[00957] The materials and methods described below pertain to Examples 2-11.

Animal Care

[00958] In-life phase of the rabbit and monkey studies were carried out by external contract research organizations (CROs) (Absorption Systems, USA [rabbit] and Alta Biosciences, USA [monkey]). All animal experiments were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals under protocols approved by the

Institutional Animal Care and Use Committee (IACUC) of Stoke Therapeutics and/or contracted facility.

Human Tissue

[00959] Human retinal sample was obtained from the Lions Eye Institute for Transplant & Research (Tampa FL), which complies with regulations from the FDA, the Eye Bank Association of America (EBAA), as well as the State of Florida.

Bioinformatic Analysis

[00960] Bioinformatic RNA-sequencing analysis of the Sequence Read archive with the identifiers SRP026048 (12 samples), SRP107937 (31 samples), SRP174668 (36 samples) and ERP003613 (4 samples) was performed by using the previously described bioinformatic tools, transcript database, and quality control of samples to identify NMD splicing events, exon exclusion and skipping, and alternative splice junctions and intron events (Lim 2020).

[00961] Conservation of the NMD exon inclusion event on the *OPAI* gene across 100 different species was performed by using the human PhastCons 100-way conservation scores database, as previously described (Lim 2020).

Cells and Cell Cultures

[00962] All cells used in the study were cultured at 37°C in a humidified incubator with 5% CO₂. HEK293 cells were obtained from ATCC and cultured in DMEM high glucose with 10% FBS. HEK293 *OPAI*^{+/-} cells were generated as a cellular model of haploinsufficiency with CRISPR technology at GenScript. Briefly, the *OPAI* gene was targeted and mutated by transient co-transfection of plasmids carrying gRNA and Cas9. Confirmation of the knockout was performed with Sanger sequencing followed by full length sequencing of the *OPAI* gene. HEK293 cells that underwent the CRISPR process but maintained the *OPAI* WT sequence in both alleles were called *OPAI*^{+/+} HEK293 cells.

[00963] Primary human dermal fibroblasts: WT primary dermal fibroblasts were purchased from ATCC (PCS-201-012) and the Coriell Institute (GM02036 and GM03234). The three human primary dermal fibroblasts from ADOA patients were obtained from the National Eye Institute. Fibroblast cells were grown in an enriched medium (1:1 basic fibroblast media and AmnioMax™-II Complete medium) at 37°C in 5% CO₂. The composition of the basic fibroblast media was as follows: DMEM high glucose with 10% heat inactivated FBS, glutamine (2 mM final), sodium pyruvate (1 mM final), and antibiotics.

Treatment with Cycloheximide (CHX)

[00964] For any *in vitro* analysis of nonproductive transcript levels, cells were treated with their respective culture medium containing cycloheximide (50 µg/ml) for the 3 hours immediately

before harvesting. Control cells received culture medium containing an equivalent volume of DMSO.

RNA extraction

[00965] For cultured cells, total RNA was extracted using the Qiagen RNeasy Kit (Qiagen, 74106) or RNeasy 96 Kit (Qiagen 74181) following manufacturer's instructions. RNA isolation from tissue was performed using the QIAzol reagent as described previously (Lim 2020).

cDNA Synthesis

[00966] cDNA synthesis was conducted using SuperScript™ IV Reverse Transcriptase (Thermo Fisher, 18090010) following manufacturer's instructions.

ASO Delivery: Transfection and Gymnotic (Free) Uptake

[00967] For transfection, diluted Lipofectamine RNAiMAX reagent (0.9 µl in 100 µl OptiMem) was mixed with ASO at indicated concentration in Eppendorf tubes for 30 min and placed in 24-well plate. Cells (100,000 cells, suspended in 400 µl media) were added to 24-well plates and incubated for 5-6 hours at 37°C in 5% CO₂. Media was then either replaced entirely or an additional 500 µl fresh media was added to wells and plates were returned to incubator until harvesting.

[00968] For gymnotic (free) uptake, HEK293 cells (25,000-30,000 cells suspended in 450-490 µl culture media) were added to 24-well plates containing concentrated ASO-media (10-50 µl) to obtain final desired ASO concentration. Cells were incubated for 72-96 hours.

RT-PCR and qPCR Analysis

[00969] RT-PCR was performed as previously described (Lim 2020) with the primers and cycle conditions listed in **Tables 8-9**. The intensities of the nonproductive and productive *OPAI* mRNA transcripts from the RT-PCR gel images were calculated using the Multi Gauge software (Fujifilm V2.3). Relative nonproductive transcript abundance was calculated as the intensity of the (nonproductive transcript / [the nonproductive + productive transcript]) and expressed as a percentage.

[00970] qPCR was also performed as previously described (Lim 2020) with primer-probe assay information and cycle conditions listed in **Tables 10-12**. The 2^{-ΔΔCt} method was used to calculate relative expression levels. Total *OPAI* mRNA assessments in cell-based studies were normalized to house-keeping gene RPL32. Similarly, for EC50 evaluation, levels of nonproductive transcript were also normalized to RPL32. For rabbit and cynomolgus monkey studies, levels of nonproductive transcript were calculated relative to total *OPAI* levels.

[00971] For all *in vitro* RT-PCR, qPCR, and protein studies, one biological replicate is defined as data obtained from an individual well of cells that received a specific treatment, as

indicated in the experiment. For qPCR analysis, every biological replicate was run at least in duplicate, and these values were averaged to generate one data point.

ATP Measurement

[00972] *OPAI*^{+/+} or *OPAI*^{+/-} HEK293 cells were seeded at 3×10^5 cells in a T-25 flask in cell culture media containing 10 μ M ASO-1 or an equivalent volume of Vehicle and incubated for 96 hours. Every biological replicate in the data presented represents a separate flask of cells treated with ASO-1 or Vehicle. Cells were washed twice with cold PBS and harvested with 120 ml cold ATP assay buffer. Samples were centrifuged at 13,000 x g for 5 min at 4°C and the supernatants were collected and kept on ice. An aliquot of lysed sample was set aside to be quantified by BCA assay for total protein concentration. The remaining sample was deproteinized using the Deproteinizing TCA kit (Abcam, ab204708) and ATP levels were measured in the lysates using the ATP assay kit (Abcam, ab83355) according to the manufacturer's instructions.

***In vivo* Assessment of ST-1102**

[00973] Adult female NZW rabbits received a bilateral single IVT injection of 30 μ l containing PBS (N=3 per time point) or ST-1102 (0.04, 0.12, or 0.23 mg/eye). Clinical ophthalmic examinations were performed on days 8, 15 and 29. For nonproductive splicing and OPA1 protein analysis, each dose group included three animals for each time-point. Retinas were divided along the nasal temporal axes into two halves and collected in separate tubes, flash frozen and stored below -70°C until analysis. The nasal halves were used to isolate RNA while the temporal halves were used for protein analysis. ASO exposure was only measured on Day 29 and included three animals per dose group. Whole retinas were used for analysis.

***In vivo* Assessment of ASO-1**

[00974] 1.8-2.0-year-old male and female cynomolgus monkeys received a bilateral single 50- μ l IVT injection of PBS (N=4 animals per time point) or ASO-1 (0.1, 0.3, and 1.0 mg/eye; N=4 per dose) and followed for 4 weeks. Clinical ophthalmic exams were performed at baseline, and on study days 3, 15, 28 (cohorts 1 and 2), and 56 (cohort 2 only). A separate cohort at 0.3 and 1.0 mg/eye (N=2 per dose) were followed an additional 4 weeks. Ocular tissues were harvested at weeks 4 or 8. One eye (OS) was dissected, and retinal tissue was snap frozen, and the fellow eye (OD) was fixed intact in modified Davidson's fixative, processed, and embedded in paraffin.

Protein Extraction and OPA1 Protein Assessment by Western Blot

[00975] Protein was extracted from rabbit retinal tissues in Red RINO tubes containing zirconium beads by adding RIPA buffer containing protease and phosphatase inhibitors (10 μ l per mg tissue) followed by homogenization with a Bullet Blender Storm 24. Samples were then passed through the QIAshredder column by centrifugation. For protein extraction from cells, cell pellets

were lysed in RIPA buffer and passed through the QIAshredder columns. Protein concentration in the flow through was measured by the BCA assay.

[00976] Immunoblotting was carried out as previously described (Lim 2020) with the following modifications. The following primary antibodies used were as follows: α -OPA1 protein (Abcam 119685), primary monoclonal antibody against α - β -actin (Abcam ab8226), α - β -tubulin (Bio-Rad VMA00222). The secondary antibody used was the IRDye® 800CW Donkey anti-mouse IgG secondary antibody (Licor, 926-32212). For detection, membranes were scanned on an Odyssey CLx Imaging System and ImageJ or Multi Gauge software (Fujifilm V2.3) was used to quantify all OPA1 isoforms and actin or β -tubulin in each sample lane. OPA1 protein levels were normalized to β -actin or β -tubulin as indicated and expressed relative to the average OPA1 expression levels of the Vehicle group.

OPA1 Protein Quantitation by ELISA

[00977] Cynomolgus monkey tissue homogenate was prepared by adding retina to pre-filled tubes with zirconium beads (SPEX SamplePrep) at a 20 mg/ml concentration using 1X Lysis buffer (Ray Biotech) plus 100X protease and phosphatase Inhibitor cocktail (Thermo Fisher). Sample tubes were added to a pre-cooled cryoblock and lysed in a SPEX 1600 Mini G homogenizer at 1425 rpm for 5 min. After homogenization, samples were centrifuged at 10,000 x g for 5 min at 4°C, and supernatant was collected and transferred to a new polypropylene tube for analysis. The relative quantity of OPA1 protein in monkey retinal tissues was measured by an ELISA. Briefly, Nunc plates (Thermo Fisher) were coated with the capture mouse monoclonal antibody against OPA1 protein 500 ng/ml final concentration (BD Transduction Labs, 612007) overnight at 4°C. After the plates were washed, blocked with SuperBlock T20, (300 μ l, Thermo Fisher) for 60 min at room temperature on a plate shaker and rinsed, the test samples, OPA1 calibration curve (Origene, 311417) and Vehicle (100 μ l) were added to the plates and incubated for 2 hours at room temperature on a plate shaker. After plates were washed, the detection antibody HRP-rabbit monoclonal anti-OPA1 antibody, 1000 ng/ml final concentration, (Novus Biologicals) was added (100 μ l), and plates were incubated for 60 min at room temperature in the dark on a plate shaker. After plates were washed, the signal was developed with TMB substrate (Thermo Fisher) for 20 min and stopped in accordance with manufacturer's instructions. The plates were read at 450 nm by SpectraMax 5Me (Molecular Devices) and a four-parameter logistic model was used to generate the standard curve and the quantity of OPA1 in each test sample was extrapolated. OPA1 protein levels in each sample were normalized to the total protein content determined by the BCA assay.

Cell Viability Assessment

[00978] Cell viability was assessed using the RealTime-Glo™ MT Cell Viability Assay from Promega (G9711) following the manufacturer's instructions for the continuous-read method. Briefly, 5,000 HEK293 cells were plated in each well of a 96-well white, clear bottom plate in media containing ASO at the final desired concentration. Assay reagents were added at the same time and the plate was incubated at 37°C in a humidified incubator with 5% CO₂. Luminescence was measured at 30 min and 72 h post-plating. Luminescence readings at 30 min were subtracted from the 72 h readings to account for any initial differences in plating. Luminescence readings were normalized to Vehicle treated wells to calculate relative changes in viability.

Mitochondrial Respiratory Functions Measured By Seahorse XFe96 Analyzer

[00979] For characterization of mitochondrial bioenergetics in ADOA patient fibroblast cells, 10,000 fibroblast cells were plated in Seahorse XFe96 PDL plate for 24 hr. The Seahorse XF Cell Mito Stress Test Kit (Agilent 103015-100) was used to assess mitochondrial function following manufacturer's instructions. The final concentration of the ETC modulators was: oligomycin (1.5 µM), FCCP (1 µM), and rotenone/antimycin (0.5 µM). Cell counts for each well were captured by staining with Cyquant and imaged using an ImageXpress (Molecular Devices). The Wave software program (Agilent Technologies V2.6.1) was used to normalize the OCR to the cell count and reported as pmol/min/cell.

[00980] To study effect of ASO-1, fibroblast cells were treated with different doses of ASO-1 by transfection using Lipofectamine RNAiMAX for 72 hours. Cells were then trypsinized and re-plated on Seahorse XFe96 PDL plate for additional 24 hours and assay was performed as described above.

[00981] One biological replicate in an experiment represents an average of OCR values obtained from 8-12 wells of the XFe96 plate. The data plotted represent the mean ± SD of three biological replicates.

Quantification of ASO *In vivo* by Hybridization Enzyme Linked Immunosorbent Assay (HELISA)

[00982] Prior to the measuring of ASO, retinal tissues were processed in tubes containing zirconium beads by adding homogenate buffer containing proteinase K (0.25 mg/ml) followed by homogenization with Spex 1600 MiniG homogenizer for 2x1 min at 1200 rpm. Then the homogenate was incubated at 55°C for an hour for tissue lysis. The concentrations of ST-1102 in rabbit and ASO-1 in monkey retinal tissues were measured by using an established HELISA for quantifying ST-1102 or ASO-1, respectively with the following modifications of previously described methods (Burki 2015; Straarup 2010). Briefly, the complementary capture probe with biotin at 3' end was coated onto a NeutrAvidin ELISA plate (Pierce 15507). Separately, the 5'

digoxigenin-labeled detection probe was incubated with a standard curve of ST-1102 samples or test samples. After the complex was hybridized with the capture probe, an anti-digoxigenin antibody conjugated to alkaline phosphatase (Sigma, 11093274910) was added to detect the bound analyte, and ATToPhos® (Promega, S1000) was used as a substrate for fluorescent measurements with excitation wavelength of 435 nm and emission wavelength of 555 nm. The standard curves of ST-1102 and ASO-1 were calculated with a four-parameter logistic, and the quantity in each test sample was extrapolated.

***In Situ* Detection of ASO-1 by miRNAscope™**

[00983] Whole globes were harvested and fixed for 24 hours in Modified Davidson's fixative (MDF), then subjected to routine processing and paraffin embedding. A specific probe directed against the ASO-1 sequence was generated and used for detection by custom miRNAscope™ LS RED *in situ* hybridization (probe dilution 1:5000). RNA *in situ* hybridization was performed on automation platform using the miRNAscope™ Red Reagent Kit (Advanced Cell Diagnostics, Inc., Newark, CA) according to the manufacturer's instructions. Briefly, 5 µm MDF-fixed, paraffin-embedded tissue sections were pretreated with heat and protease prior to hybridization with the target oligo probes. Preamplifier, amplifier, and AP-labeled oligos were then hybridized sequentially, followed by chromogenic precipitate development. Each sample was quality controlled for RNA integrity with miRNAscope™ probe specific to PPIB RNA and for background with a probe specific to bacterial dapB RNA. Specific RNA staining signal was identified as red, punctate dots. Samples were counterstained with Gill's Hematoxylin. Regions of interest (ROI) included RGC layer in or near the fovea and RGC layer in peripheral retina. Visual scoring was performed by a qualified scientist who was blinded to treatment groups but not controls. miRNAscope™ quantification is based on number of dots per cell and not on intensity. Percentage of cells positive is scored visually based on number of cells with ≥1 dot/cell. Quantitative image analysis was performed using HALO® software on 1-mm lengths of each ROI.

Immunofluorescence Detection of OPA1 Protein

[00984] Eyes were fixed and embedded as described above. Immunostaining was performed on Leica automation platforms using the Bond™ Polymer Refine Detection Kit (Leica Biosystems). Briefly, 5 µm MDF fixed tissue sections were pretreated with heat and target retrieval solution prior to incubation with the target antibody (Novus Anti-OPA1 antibody NB110-55290 at 5 µg/ml), and polymer. Fluorescent whole slide images were acquired using 3DHistech Panoramic SCAN II digital slide scanner equipped with a 40x objective. Images were obtained from the regions of interest as described above. Quantitative image analysis was performed using HALO® software on 1-mm lengths of each ROI.

Biostatistical Analysis

[00985] Differences among groups were determined by ordinary one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test or by unpaired t-test, as indicated. All statistical comparisons were performed with GraphPad Prism. Levels of significance began at $P=0.05$.

[00986] A significant dose-related decrease in the nonproductive transcript was observed in rabbit retinal tissue as early as 7 days post-injection by RT-PCR. The levels of nonproductive transcripts further dropped at both 14- and 28-days post-injection (**FIG. 8B** and **FIG. 9**). No changes in retinal OPA1 protein levels were observed 7 days post-injection; however, significant dose-related upregulation of OPA1 protein levels were seen 14 days post-injection that remained significantly elevated at 28 days post-injection (**FIG. 8C** and **FIG. 10**). Retinal exposure of ST-1102 was measured only 28 days post-injection. ST-1102 retinal levels increased with dose but were not strictly dose-proportionate (**FIG. 8D**). Taken together, these data indicate that a single IVT administration of ST-1102 significantly reduced the levels of nonproductive transcripts and increased OPA1 protein in the rabbit retina for at least four weeks.

[00987] Treatment with ASO-1 significantly increased the four OCR parameters in F35 and F36 fibroblast cells in a dose-dependent manner. In F34 fibroblast cells, ASO-1 treatment resulted in a dose-dependent increase in maximal respiration and spare-respiratory capacity, while increases in basal or ATP-linked respiration were not observed (**FIG. 7A-7D**). Maximal respiration is measured upon the addition of the uncoupler FCCP, which mimics a physiological energy demand by stimulating the respiratory chain to operate at maximum capacity. The difference between maximal and basal respiration is the spare respiratory capacity, a measure of mitochondrial reserve. The increase in maximal respiration and spare respiratory capacity in all three patient cells with different pathogenic variants shows that ASO-1 enhances the ability of *OPA1* haploinsufficient cells to respond to conditions that induce mitochondrial stress (**FIG. 7D**).

Table 8. Exemplary RT-PCR primers

Primer	Sequence
Human <i>OPA1</i> forward primer	5'-GCCAATATAGCACGAGCTAT-3'
Human <i>OPA1</i> reverse primer	5'-CTGAGTGTGCAGAAGTTCTTCC-3'
Rabbit <i>OPA1</i> forward primer	5'-TGCTTGGTGAGCTCATTCTC-3'
Rabbit <i>OPA1</i> reverse primer	5'-GAAGTTCTTCTTGAAGTTGGTCA-3'

Table 9. Exemplary PCR Thermocycling Conditions

Temperature and time	Cycles
95°C for 9 min	1X
1. 95°C for 30 sec 2. 52°C (human), 54°C (rabbit) for 30 sec 3. 72°C for 30 sec	28X
72°C for 10 min	1X
4°C	hold

qPCR Assay Information

[00988] Probe-based qPCR assays were either purchased from Thermo Fisher or custom-designed by IDT. qPCR was performed with the following cycle conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles (95°C for 15 sec, 60°C for 1 min)

[00989] The commercially purchased assays were as follows: human *OPAI*: Hs01047013_m1, human *RPL32*: Hs00851655_g1.

[00990] **Tables 10-12** comprise exemplary sequences of the custom-designed qPCR assays.

Table 10. Primers and Probes Used in qPCR Assay for Detection of Human Nonproductive *OPAI* transcript.

Primer/Probe	Sequence (5' to 3')
Forward Primer	ATTCCGCTCTTTCTCCATCAG
Reverse Primer	CTCTTTGTCTGACACCCTGAC
Probe	/56-FAM/AG CTC TGG T/Zen/TTCAAGACCATTGATATCC A/3IABkFQ/

Table 11. Primers and Probes Used in qPCR Assay for Detection of Cyno Nonproductive *OPAI* Transcript.

Primer/Probe	Sequence
Forward Primer	5'-AGC TCT GGT TTC AAG ACC ATT-3'
Reverse primer	5'-GCA GAA GTT CTT CTT GAA GTT GG-3'
Probe	5'-/56-FAM/CC ATT TCT G/Zen/T CAG GGT GTC AGA CAA AGA /3IABkFQ/-3'

Table 12. Primers and Probes Used in qPCR Assay for Detection of Cyno Total *OPA1* Transcripts.

Primer/Probe	Sequence
Forward Primer	5'-GAA AGT ATT AAA CGA CAC AAG TGG A-3'
Reverse primer	5'-TGT TTA TCA GAT ATG GAT CGG TCT T-3'
Probe	5'-/56-FAM/AG GAC AGC T/Zen/T GAG GGT TAT TCA ACA CA/3IABkFQ/-3'

Table 13. ASO-1 Treatment Parameters.

Group	Treatment	Dose Level	Concentration (mg/eye)	Necropsy 8 weeks Male/Female
1	PBS	NA	0	2/2
2	ASO-1	Dose 1	0.05	2/2
3	ASO-1	Dose 2	0.1	2/2
4	ASO-1	Dose 3	0.2	2/2
5	ASO-1	Dose 4	0.5	2/2
6	ASO-1	Dose 5	1	2/2

Example 13: OSPREY: an Open-Label Study to Investigate Safety, Tolerability, and Exposure of the Antisense Oligonucleotide (ASO) ASO-1 in Patients with *OPA1* Autosomal Dominant Optic Atrophy (ADOA)

[00991] *OSPREY study objectives*

[00992] ASO-1 is an investigational antisense oligonucleotide (ASO) treatment designed to upregulate *OPA1* protein expression with the aim of stopping or slowing vision loss in ADOA. The OSPREY study described herein endeavors to achieve the primary objective of evaluating the safety, tolerability, and exposure (serum) of single ascending doses of ASO-1 in a human Phase 1 study, in addition to the secondary objective of evaluating changes in visual function, ocular structures, and quality of life after single doses of ASO-1.

[00993] *OSPREY recruitment criteria*

[00994] Key criteria used in the inclusion of study participants: those who (1) are clinically confirmed to have ADOA with the heterozygous *OPA1* variant predicted to cause haploinsufficiency; (2) are between the ages of about 6 and 55 years old (divided into two cohorts: cohort A comprising those between at least 18 years old and less than 55 years old, and cohort B comprising those at least 6 years old and less than 18 years old); and (3) have Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity letter scores of from at least 35 to 70 letters for each eye.

[00995] Key criteria used in the exclusion of study participants: those who have (1) ADOA plus or Behr syndrome; (2) gain-of-function or compound heterozygous *OPAI* variants; (3) conditions, medications, or surgical operations that could affect optic nerve function; and/or (4) high myopia (worse than -6 diopters (>6D)).

[00996] *OSPREY study design – dosages and assessments*

[00997] Intravitreal, 50- μ l doses of ASO-1 are administered to the *pars plana* in one eye (the eye determined to have the more severe disease condition) of each patient following a 3+3 ascending dose design over the course of 48 weeks. The study sample size *N* is 60 (48 participants in cohort A and 12 participants in cohort B). Cohort A comprises four dose levels (0.1, 0.3, 0.5, and 0.7 mg of ASO-1) with an option to add two dose levels, capped at a maximum total dosage per administration of 1.2 mg of ASO-1 according to the Safety Monitoring Committee (SMC).

[00998] Ophthalmic assessments include Best Corrected Visual Acuity (BCVA) tests, Minnesota low vision reading (MNREAD) acuity tests, slit lamp examinations, static automated perimetry (24-2), full-field electroretinograms (ffERG), photopic negative response (PhNR) measurements, and spectral-domain optical coherence tomography (SD-OCT).

[00999] Other assessments include quality-of-life (QOL) assessments, blood and/or urine for safety and exposure pharmacokinetics (POK) evaluation, biomarkers that may be present in the serum, plasma, or whole blood), and/or other adverse events.

[001000] *OSPREY study design – progression of dose administration in cohort A (≥ 18 to < 55 years old)*

[001001] The progression of dose administration in cohort A follows the general scheme of having an administration of a dosage of the ASO per eye followed by approval from the Safety Monitoring Committee (SMC), who determines whether the cohort should continue with the same dosage or escalate to the next dosage level, with the SMC evaluating after each administration.

[001002] Cohort A begins with 0.1 mg ASO-1 per eye at a sample size of *N*=1, followed by approval from the Safety Monitoring Committee (SMC), which provides a recommendation on whether to continue dosing, and then whether to recommend escalating to the next dosage level of 0.3 mg ASO-1 per eye. After dosage with 0.3 mg/eye, the Safety Monitoring Committee (SMC) provides a recommendation on whether to continue administration at the same dose and then whether to recommend escalating to the next dosage level of 0.5 mg ASO-1 per eye. After dosage with 0.5 mg/eye, the Safety Monitoring Committee (SMC) provides a recommendation on whether to continue administration at the same dose and then whether to recommend escalating to the next dosage level of 0.7 mg ASO-1 per eye. Safety follow-ups are performed 48 weeks after the first dosage.

[001003] Six or fewer patients may be added to any dosage level that is tolerated with fewer than two patients experiencing dose-limiting toxicities (DLT) during corresponding evaluation steps by the SMC (four-pointed stars). Three additional patients may be added for safety assessment and the SMC may recommend de-escalation to a lower dosage during corresponding evaluation steps.

[001004] *OSPREY study design – progression of dose administration in cohort (≥ 6 to < 18 years old)*

[001005] Cohort B begins with an ASO dosage recommended by the Safety Monitoring Committee (SMC) from cohort A (sample size $N = 1$, ≥ 12 to 18 years old), followed by review by the SMC, who provides a recommendation on whether to continue administration at the same dose. After confirmation by the SMC from the previous step, the sample size increases to $N = 2$, ≥ 6 to 18 years old, followed by another round of review by the SMC. Safety follow-ups are performed 48 weeks after the first dosage. Three patients may be added after the second evaluation by the SMC. The dosage may be escalated or de-escalated in a 3+3 fashion if necessary to find an optimal dose in pediatric participants. After the 48-week safety follow-up, six or fewer patients may be added if the dosage is tolerated well, or the highest dose level tested is well-tolerated, or confirm safety or continue to the second endpoint.

[001006] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

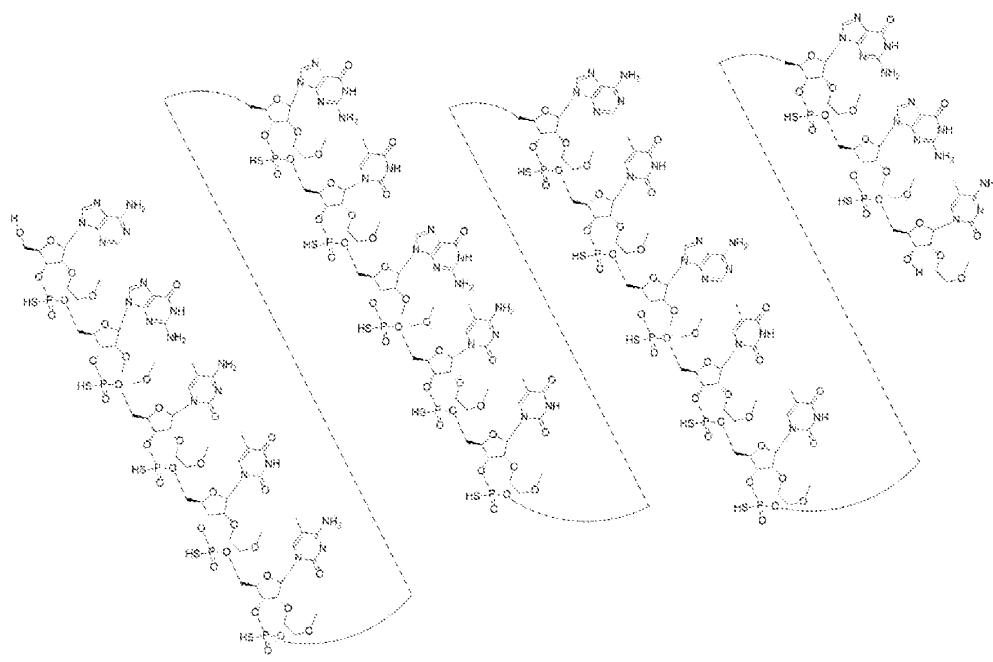
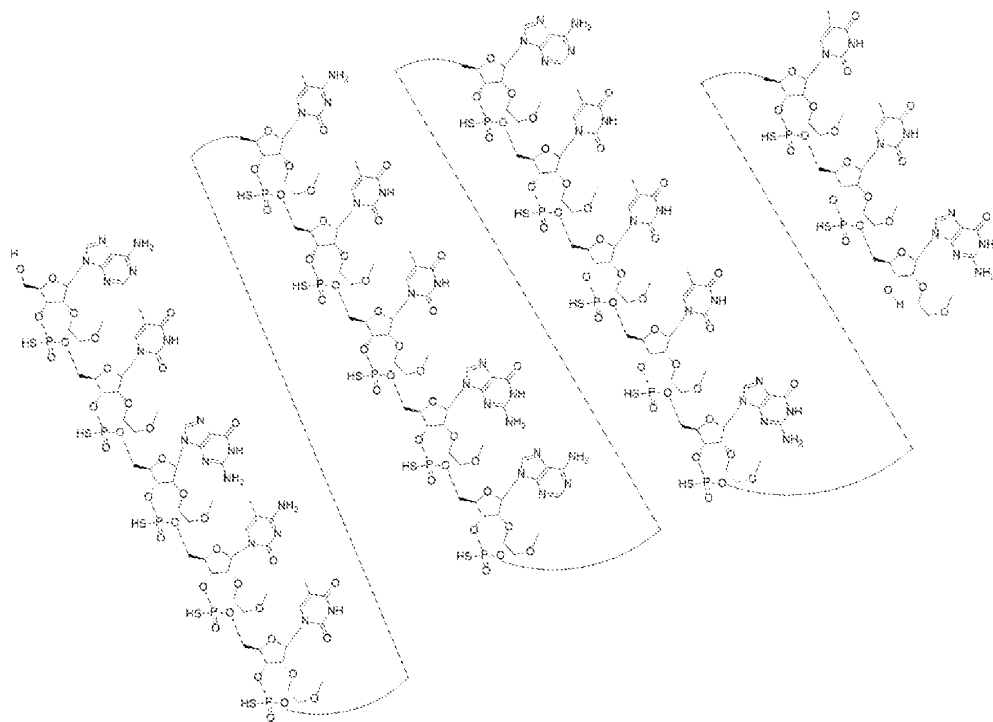
CLAIMS

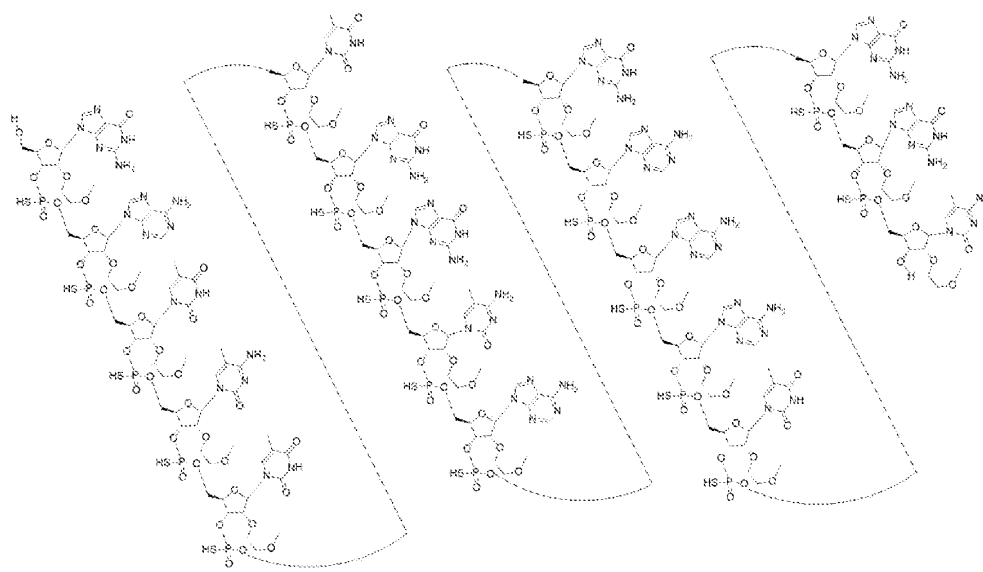
What is claimed is:

1. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,
wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299, and
wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.
2. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,
wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299,
wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and
wherein the subject is characterized by having:
 - (i) a heterozygous *OPA1* gene variant;
 - (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments;
 - (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity;
 - (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or
 - (v) any combination of (i)-(iv).
3. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer,
wherein the antisense oligomer comprises a nucleotide sequence having at least 80% sequence identity to the sequence set forth in any one of SEQ ID NO: 6-275 or 280-299,
wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA), and
wherein the subject is characterized by:
 - (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPA1* gene;
 - (2) not having only benign or likely benign variants in the *OPA1* gene;

- (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus);
 - (4) not having been diagnosed with Behr syndrome;
 - (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases;
 - (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy;
 - (7) not having or having a history of any ocular condition in either eye;
 - (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering;
 - (9) not having a history of retinal photocoagulation;
 - (10) not having a history or presence of retinal vein occlusion;
 - (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering;
 - (12) not having dry age-related macular degeneration in either eye;
 - (13) not having high myopia (>6 diopters);
 - (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated);
 - (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy;
 - (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery; or
 - (17) any combination of (1)-(16).
4. A method of treating a subject having a disease or condition or reducing likelihood of the subject developing the disease or condition, the method comprising administering to the subject a pharmaceutical composition comprising an antisense oligomer, wherein the antisense oligomer is a compound according to any one of the following chemical structures:







or a pharmaceutically acceptable salt thereof.

5. The method of any one of claims 2-4, wherein the method comprises administering to one eye of the subject the pharmaceutical composition at a dose of about 0.005 to about 20 mg of the antisense oligomer.

6. The method of any one of claims 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg to about 15 mg, about 0.01 mg to about 10 mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg, about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

7. The method of any one of claims 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

8. The method of any one of claims 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

9. The method of any one of claims 1-5, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer.

10. The method of any one of claims 1-9, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μ L to about 250 μ L, about 10 μ L to about 250 μ L, about 20 μ L to about 250 μ L, about 30 μ L to about 250 μ L, about 40 μ L to about 250 μ L, about 50 μ L to about 250 μ L, about 60 μ L to about 250 μ L, about 70 μ L to about 250 μ L, about 80 μ L to about 250 μ L, about 100 μ L to about 250 μ L, about 120 μ L to about 250 μ L, about 150 μ L to about 250 μ L, about 160 μ L to about 250 μ L, about 180 μ L to about 500 μ L, about 200 μ L to about 250 μ L, about 220 μ L to about 250 μ L, about 5 μ L to about 220 μ L, about 10 μ L to about 220 μ L, about 20 μ L to about 220 μ L, about 30 μ L to about 220 μ L, about 40 μ L to about 220 μ L, about 50 μ L to about 220 μ L, about 60 μ L to about 220 μ L, about 70 μ L to about 220 μ L, about 80 μ L to about 220 μ L, about 100 μ L to about 220 μ L, about 120 μ L to about 220 μ L, about 150 μ L to about 220 μ L, about 160 μ L to about 220 μ L, about 180 μ L to about 220 μ L, about 5 μ L to about 200 μ L, about 10 μ L to about 200 μ L, about 20 μ L to about 200 μ L, about 30 μ L to about 200 μ L, about 40 μ L to about 200 μ L, about 50 μ L to about 200 μ L, about 60 μ L to about 200 μ L, about 70 μ L to about 200 μ L, about 80 μ L to about 200 μ L, about 100 μ L to about 200 μ L, about 120 μ L to about 200 μ L, about 150 μ L to about 200 μ L, about 160

μL to about 200 μL, about 180 μL to about 200 μL, about 5 μL to about 180 μL, about 10 μL to about 180 μL, about 20 μL to about 180 μL, about 30 μL to about 180 μL, about 40 μL to about 180 μL, about 50 μL to about 180 μL, about 60 μL to about 180 μL, about 70 μL to about 180 μL, about 80 μL to about 180 μL, about 100 μL to about 180 μL, about 120 μL to about 180 μL, about 150 μL to about 180 μL, about 5 μL to about 150 μL, about 10 μL to about 150 μL, about 20 μL to about 150 μL, about 30 μL to about 150 μL, about 40 μL to about 150 μL, about 50 μL to about 150 μL, about 60 μL to about 150 μL, about 70 μL to about 150 μL, about 80 μL to about 150 μL, about 100 μL to about 150 μL, about 120 μL to about 150 μL, about 5 μL to about 100 μL, about 10 μL to about 100 μL, about 20 μL to about 100 μL, about 30 μL to about 100 μL, about 40 μL to about 100 μL, about 50 μL to about 100 μL, about 60 μL to about 100 μL, about 70 μL to about 100 μL, about 80 μL to about 100 μL, about 5 μL to about 80 μL, about 10 μL to about 80 μL, about 20 μL to about 80 μL, about 30 μL to about 80 μL, about 40 μL to about 80 μL, about 50 μL to about 80 μL, about 60 μL to about 80 μL, about 5 μL to about 60 μL, about 10 μL to about 60 μL, about 20 μL to about 60 μL, about 30 μL to about 60 μL, about 40 μL to about 60 μL, or about 50 μL to about 60 μL.

11. The method of any one of claims 1-9, wherein the method comprises administering to the one eye of the subject the pharmaceutical composition in a volume of about 5 μL, about 8 μL, about 10 μL, about 12 μL, about 15 μL, about 18 μL, about 20 μL, about 25 μL, about 28 μL, about 30 μL, about 35 μL, about 40 μL, about 45 μL, about 48 μL, about 50 μL, about 55 μL, about 60 μL, about 65 μL, about 70 μL, about 75 μL, about 80 μL, about 90 μL, about 100 μL, about 120 μL, about 150 μL, about 160 μL, about 180 μL, about 200 μL, about 220 μL, or about 250 μL.

12. The method of any one of claims 1-11, wherein the method comprises administering the pharmaceutical composition to both left eye and right eye of the subject.

13. The method of claim 12, wherein the method comprises administering the pharmaceutical composition at the same dose to both the left eye and the right eye of the subject.

14. The method of claim 12, wherein the method comprises administering the pharmaceutical composition at different doses to the left eye and the right eye of the subject.

15. The method of any one of claims 1-3 or 5-14, wherein the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to the sequence set forth in any one of SEQ ID NOs: 6-275 or 280-299.

16. The method of any one of claims 1-3 or 5-14, wherein the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to the sequence set forth in any one of SEQ ID NO: 36, 236, 242, 250, 280-283, 288, or 290-292.
17. The method of any one of claims 1-3 or 5-14, wherein the antisense oligomer modulates splicing of a non-sense mediated RNA decay-inducing exon (NMD exon) from a pre-mRNA in a cell of the subject, wherein the pre-mRNA encodes the OPA1 protein and comprises the NMD exon, thereby modulating a level of processed mRNA that is processed from the pre-mRNA, and modulating expression of the OPA1 protein in the cell.
18. The method of claim 17, wherein the antisense oligomer:
- (a) binds to a targeted portion of the pre-mRNA;
 - (b) modulates binding of a factor involved in splicing of the NMD exon; or
 - (c) a combination of (a) and (b).
19. The method of claim 18, wherein the targeted portion of the pre-mRNA is proximal to the NMD exon.
20. The method of claim 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of 5' end of the NMD exon.
21. The method of claim 18, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides upstream of 5' end of the NMD exon.
22. The method of claim 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of 3' end of the NMD exon.
23. The method of claim 18, wherein the targeted portion of the pre-mRNA is at least about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60

nucleotides, about 50 nucleotides, about 40 nucleotides, about 30 nucleotides, about 20 nucleotides, about 10 nucleotides, about 5 nucleotides, about 4 nucleotides, about 2 nucleotides, about 1 nucleotides downstream of 3' end of the NMD exon.

24. The method of claim 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

25. The method of claim 18, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides upstream of genomic site GRCh38/ hg38: chr3 193628509.

26. The method of claim 18, wherein the targeted portion of the pre-mRNA is at most about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

27. The method of claim 18, wherein the targeted portion of the pre-mRNA is about 1500 nucleotides, about 1000 nucleotides, about 800 nucleotides, about 700 nucleotides, about 600 nucleotides, about 500 nucleotides, about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, about 100 nucleotides, about 80 nucleotides, about 70 nucleotides, about 60 nucleotides, about 50 nucleotides downstream of genomic site GRCh38/ hg38: chr3 193628616.

28. The method of claim 18, wherein the targeted portion of the pre-mRNA is located in an intronic region between two canonical exonic regions of the pre-mRNA, and wherein the intronic region contains the NMD exon.

29. The method of claim 18, wherein the targeted portion of the pre-mRNA at least partially overlaps with the NMD exon.

30. The method of claim 18, wherein the targeted portion of the pre-mRNA at least partially overlaps with an intron upstream or downstream of the NMD exon.

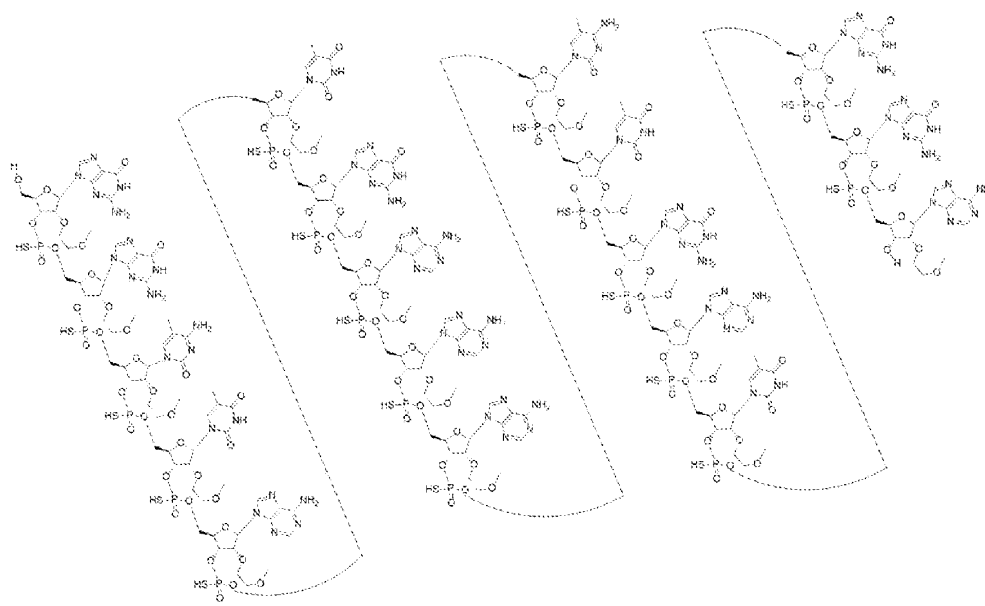
31. The method of claim 18, wherein the targeted portion of the pre-mRNA comprises 5' NMD exon-intron junction or 3' NMD exon-intron junction.

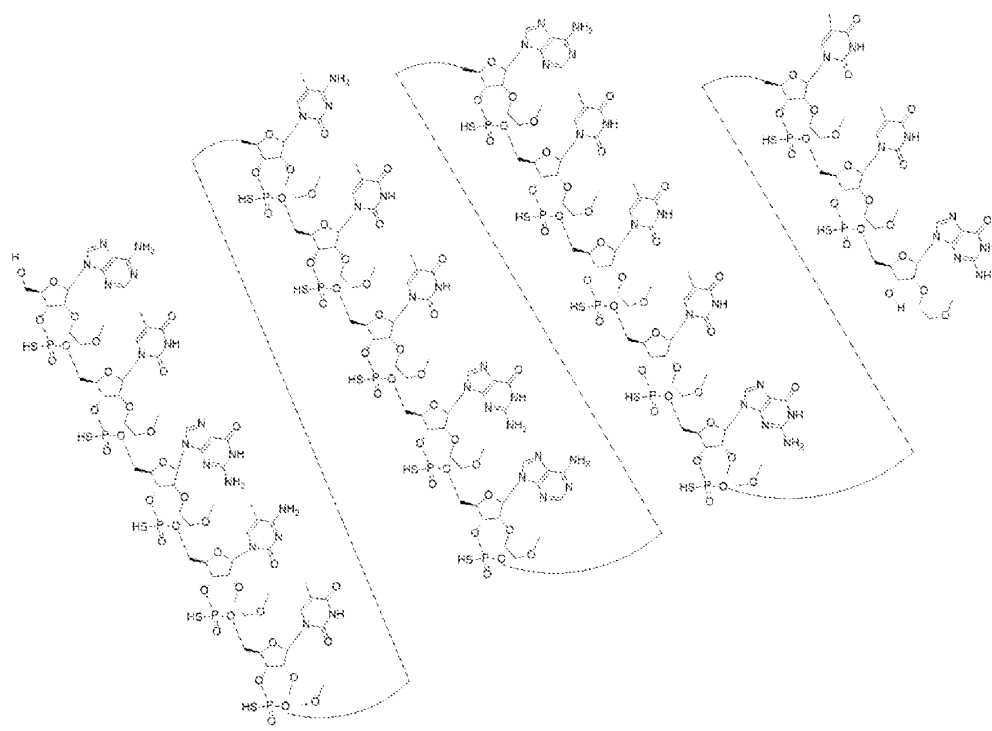
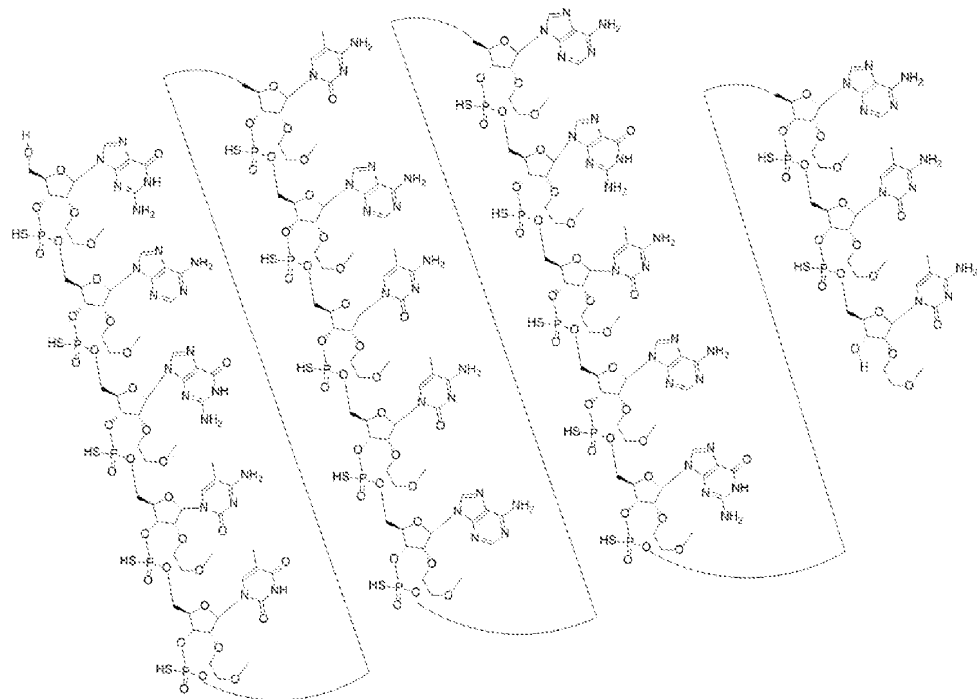
32. The method of claim 18, wherein the targeted portion of the pre-mRNA is within the NMD exon.

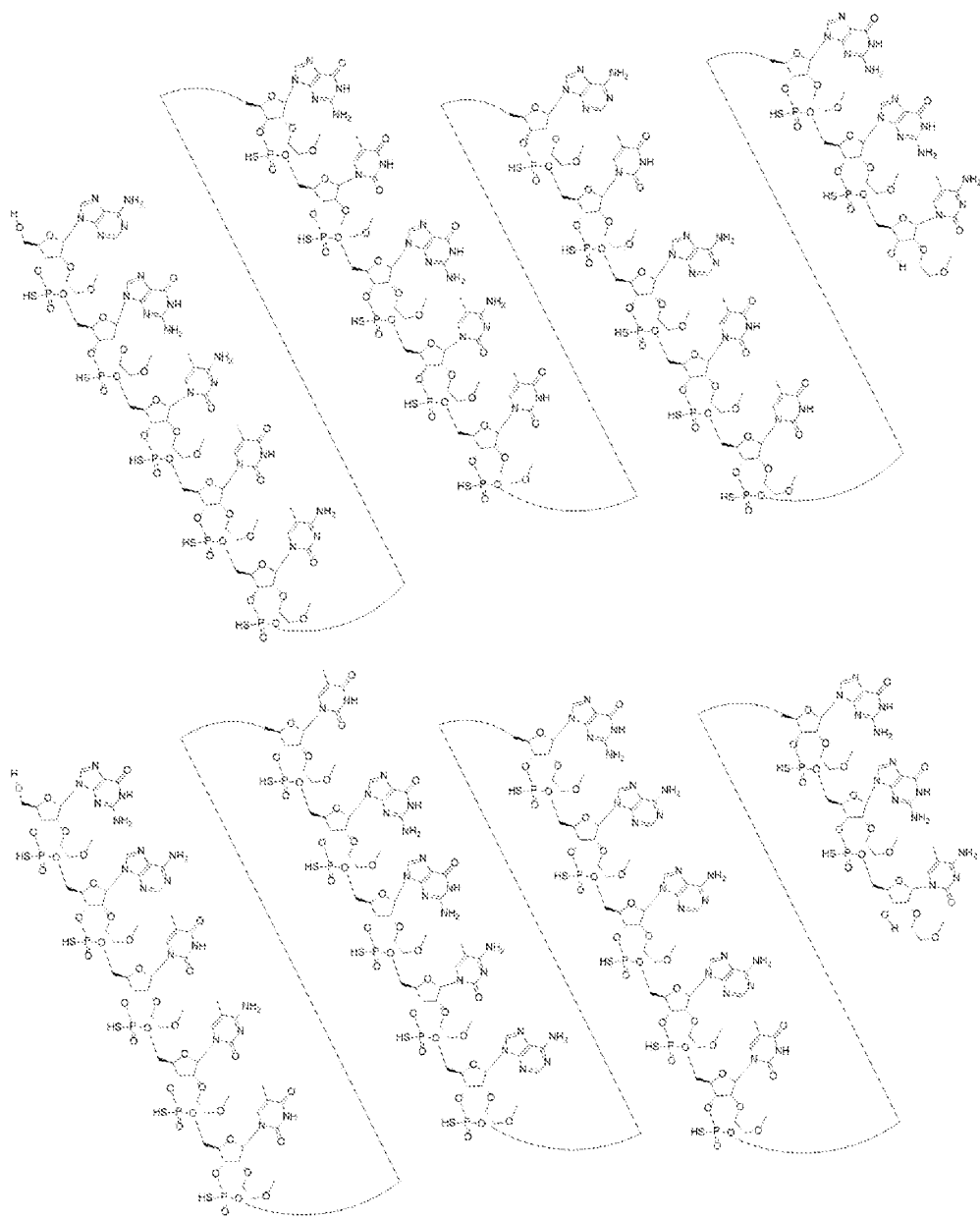
33. The method of claim 18, wherein the targeted portion of the pre-mRNA comprises about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotides of the NMD exon.
34. The method of any one of claims 17-33, wherein the NMD exon comprises a sequence with at least 80%, at least 90%, or 100% sequence identity to SEQ ID NO: 279.
35. The method of any one of claims 17-33, wherein the NMD exon comprises a sequence of SEQ ID NO: 279.
36. The method of claim 18, wherein the targeted portion of the pre-mRNA is within the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.
37. The method of claim 18, wherein the targeted portion of the pre-mRNA is upstream or downstream of the non-sense mediated RNA decay-inducing exon GRCh38/ hg38: chr3 193628509 to 193628616.
38. The method of claim 18, wherein the targeted portion of the pre-mRNA comprises an exon-intron junction of exon GRCh38/ hg38: chr3 193628509 to 193628616.
39. The method of any one of claims 17-38, wherein the OPA1 protein expressed from the processed mRNA is a full-length OPA1 protein or a wild-type OPA1 protein.
40. The method of any one of claims 17-38, wherein the OPA1 protein expressed from the processed mRNA is a functional OPA1 protein.
41. The method of any one of claims 17-38, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a wild-type OPA1 protein.
42. The method of any one of claims 17-38, wherein the OPA1 protein expressed from the processed mRNA is at least partially functional as compared to a full-length wild-type OPA1 protein.
43. The method of any one of claims 17-42, wherein the antisense oligomer promotes exclusion of the NMD exon from the pre-mRNA.
44. The method of claim 43, wherein the exclusion of the NMD exon from the pre-mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.

45. The method of any one of claims 17-44, wherein the method results in an increase in the level of the processed mRNA in the cell.
46. The method of claim 45, wherein the level of the processed mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.
47. The method of any one of claims 17-46, wherein the method results in an increase in the expression of the OPA1 protein in the cell.
48. The method of claim 47, wherein a level of the OPA1 protein expressed from the processed mRNA in the cell contacted with the antisense oligomer is increased by about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to in the absence of the antisense oligomer.
49. The method of any one of claims 1-3 or 5-48, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.
50. The method of any one of claims 1-3 or 5-48, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety.
51. The method of any one of claims 1-3 or 5-48, wherein the antisense oligomer comprises at least one modified sugar moiety.
52. The method of claim 51, wherein each sugar moiety is a modified sugar moiety.
53. The method of any one of claims 1-3 or 5-52, wherein the antisense oligomer comprises a 5'-methylcytosine (5'-MeC).

54. The method of any one of claims 1-3 or 5-53, wherein each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC).
55. The method of any one of claims 1-3 or 5-54, wherein the antisense oligomer comprises a 5'-methyluracil (5'-MeU).
56. The method of any one of claims 1-3 or 5-55, wherein the antisense oligomer comprises a phosphorothioate linkage.
57. The method of any one of claims 1-3 or 5-56, wherein each internucleoside linkage of the ASO is a phosphorothioate linkage.
58. The method of any one of claims 1-3 or 5-57, wherein the antisense oligomer comprises a locked nucleic acid (LNA).
59. The method of any one of claims 1-3 or 5-58, wherein the antisense oligomer is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases in length.
60. The method of any one of claims 1-3 or 5-59, wherein the antisense oligomer is a compound according to any one of the following chemical structures:

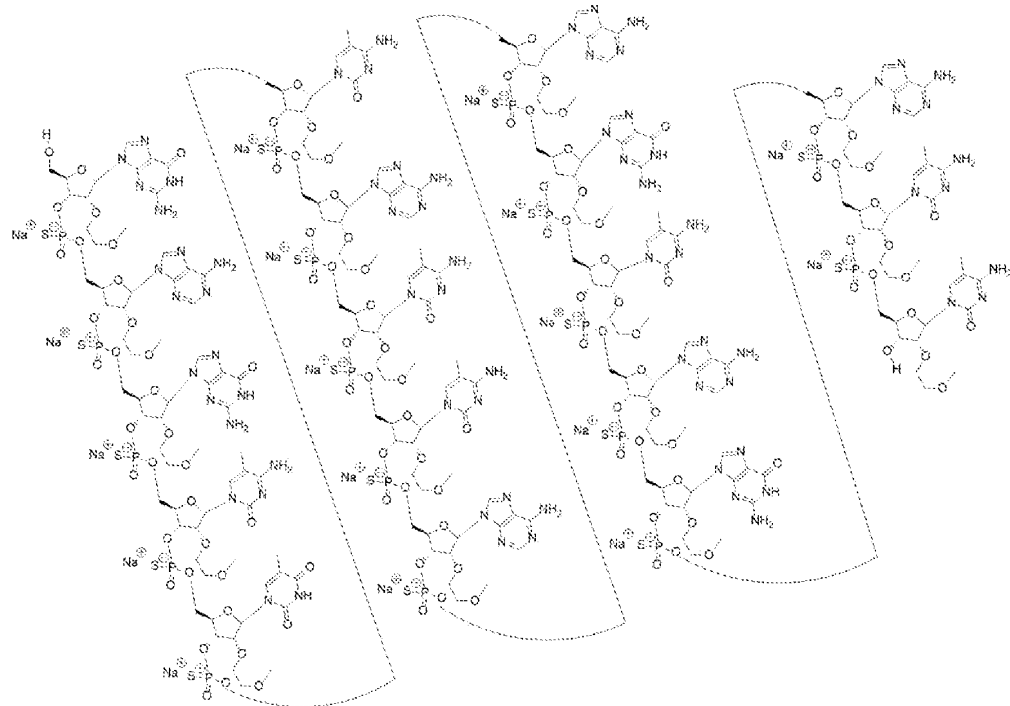
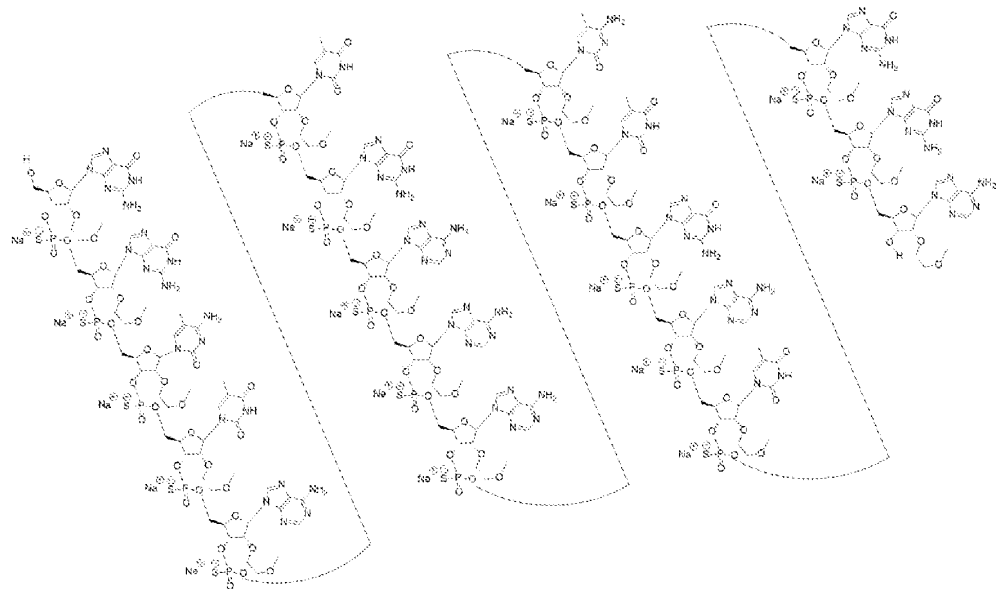


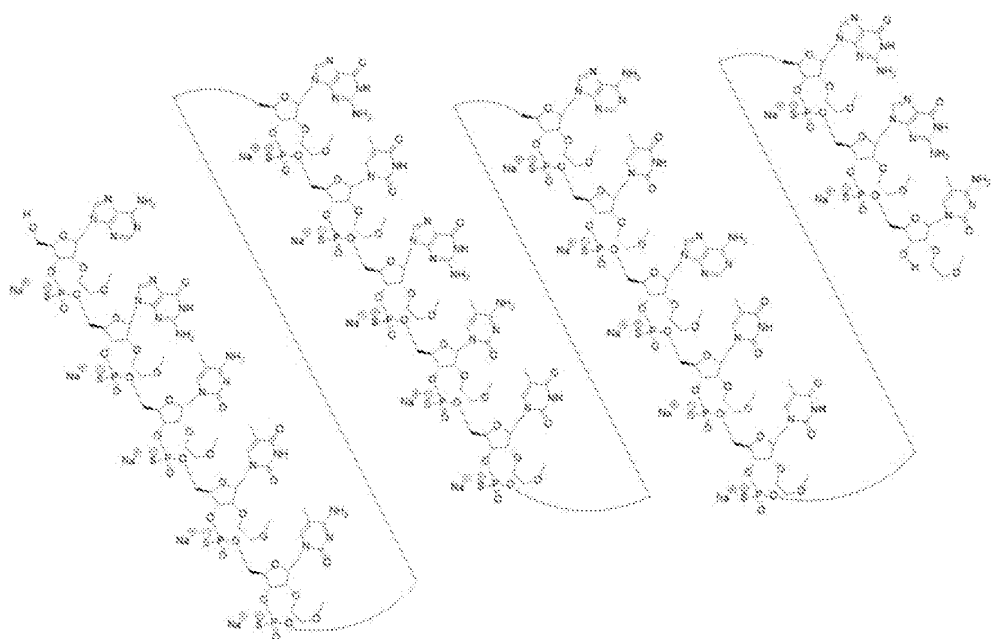
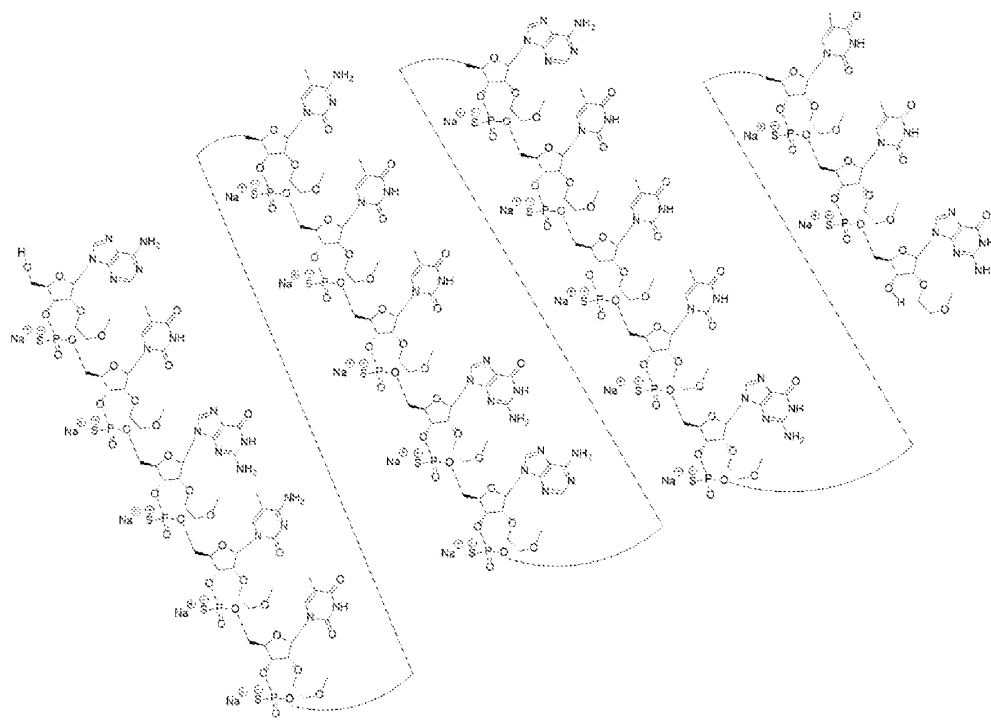




or a pharmaceutically acceptable salt thereof.

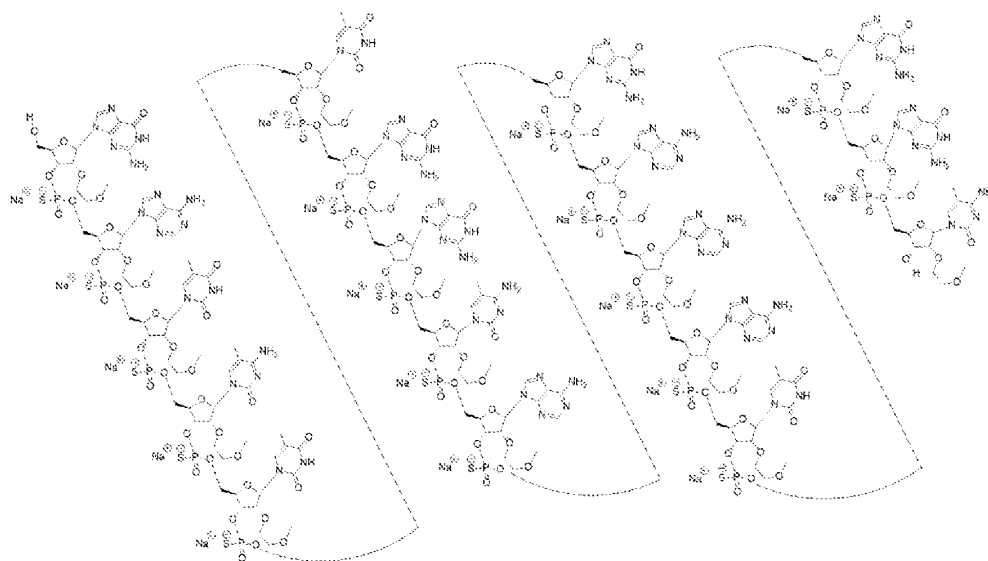
61. The method of claim 4 or 60, wherein the antisense oligomer is a compound according to any of the following structures:





;

; or



62. The method of any one of claims 1-61, wherein the pharmaceutical composition is a liquid composition.
63. The method of any one of claims 1-62, wherein the method comprises administering the pharmaceutical composition as a bolus injection.
64. The method of any one of claims 1-63, wherein the method comprises administering the pharmaceutical composition as a bolus injection over 1 to 60 minutes, 1 to 50 minutes, 1 to 40 minutes, 1 to 30 minutes, 1 to 20 minutes, 1 to 10 minutes, 1 to 5 minutes, or 1 to 3 minutes.
65. The method of any one of claims 1-64, wherein the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.
66. The method of any one of claims 1-64, wherein the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.
67. The method of any one of claims 1-66, wherein the antisense oligomer is solubilized or diluted in an isotonic solution.
68. The method of any one of claims 1-67, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8.
69. The method of any one of claims 1-67, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution.
70. The method of any one of claims 1-69, wherein the pharmaceutical formulation does not comprise a preservative.
71. The method of any one of claims 1-70, wherein the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL.

72. The method of any one of claims 1-70, wherein the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL to about 200 mg/mL, about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

73. The method of any one of claims 1-70, wherein the antisense oligomer is present in the pharmaceutical composition at a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

74. The method of any one of claims 1-73, wherein the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer.
75. The method of claim 74, wherein the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.
76. The method of claim 74, wherein the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.
77. The method of any one of claims 74-76, wherein the concentrate is a phosphate-buffered solution.
78. The method of any one of claims 1-77, wherein the subject is a human subject.
79. The method of any one of claims 1 or 4-78, wherein the disease or condition is associated with a deficient amount or activity of the OPA1 protein.
80. The method of any one of claims 1 or 4-79, wherein the disease or condition comprises an eye disease or condition.
81. The method of any one of claims 1 or 4-79, wherein the disease or condition comprises a cardiovascular disease or condition.
82. The method of any one of claims 1 or 4-79, wherein the disease or condition comprises a neurological disease or condition.
83. The method of any one of claims 1 or 4-79, wherein the disease or condition comprises ADOA-plus syndrome; a mitochondrial disorder; glaucoma; normal tension glaucoma; Charcot-Marie-Tooth disease; mitochondria dysfunction; diabetic retinopathy; age-related macular degeneration; retinal ganglion cell death; mitochondrial fission-mediated mitochondrial dysfunction; progressive external ophthalmoplegia; deafness; ataxia; motor neuropathy; sensory

neuropathy; myopathy; Behr syndrome; brain dysfunction; encephalopathy; peripheral neuropathy; fatal infantile mitochondrial encephalomyopathy; hypertrophic cardiomyopathy; spastic ataxic syndrome; sensory motor peripheral neuropathy; hypotonia; gastrointestinal dysmotility and dysphagia; optic atrophy; optic atrophy plus syndrome; Mitochondrial DNA depletion syndrome 14; late-onset cardiomyopathy; diabetic cardiomyopathy; Alzheimer's Disease; focal segmental glomerulosclerosis; kidney disease; Huntington's Disease; cognitive function decline in healthy aging; Prion diseases; late onset dementia and parkinsonism; mitochondrial myopathy; Leigh syndrome; Friedreich's ataxia; Parkinson's disease; MELAS (Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes); pyruvate dehydrogenase complex deficiency; chronic kidney disease; Leber's hereditary optic neuropathy; obesity; age-related systemic neurodegeneration; skeletal muscle atrophy; heart and brain ischemic damage; or massive liver apoptosis.

84. The method of any one of claims 1 or 4-79, wherein the disease or condition comprises Optic atrophy type 1.

85. The method of any one of claims 1 or 4-79, wherein the disease or condition comprises autosomal dominant optic atrophy (ADOA).

86. The method of claim 85, wherein the subject is characterized by having:

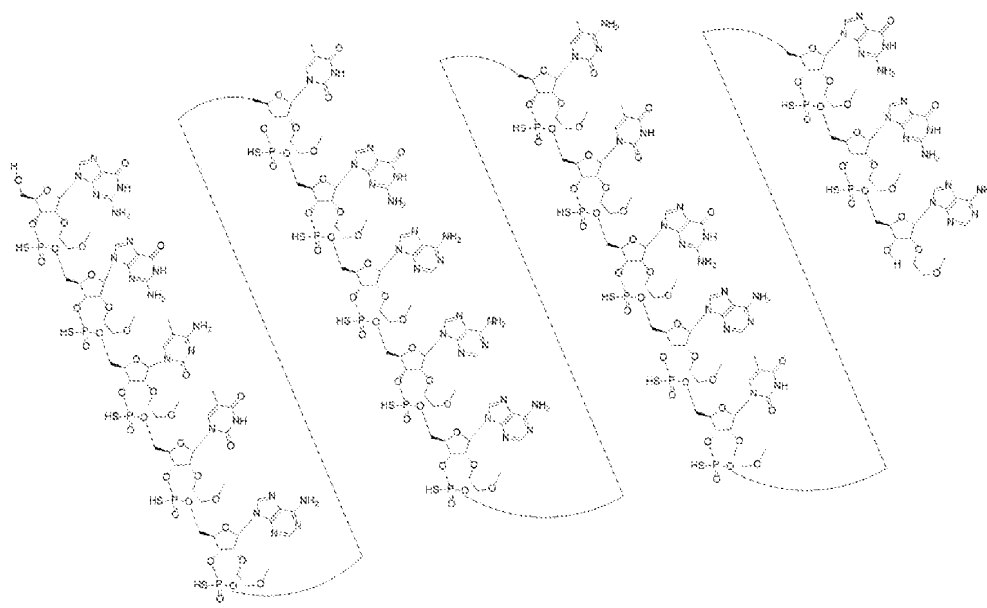
- (i) a heterozygous *OPA1* gene variant;
- (ii) clear ocular media to allow for adequate visualization of the vitreous and fundus and to achieve appropriate quality of all ophthalmic assessments;
- (iii) an AREDS Clinical Lens Standard of <1 for posterior subcapsular (PSC) opacity;
- (iv) a BCVA EDTRS letter score of ≥ 35 and ≤ 70 with each eye individually that is administered with the pharmaceutical composition; or
- (v) any combination of (i)-(iv).

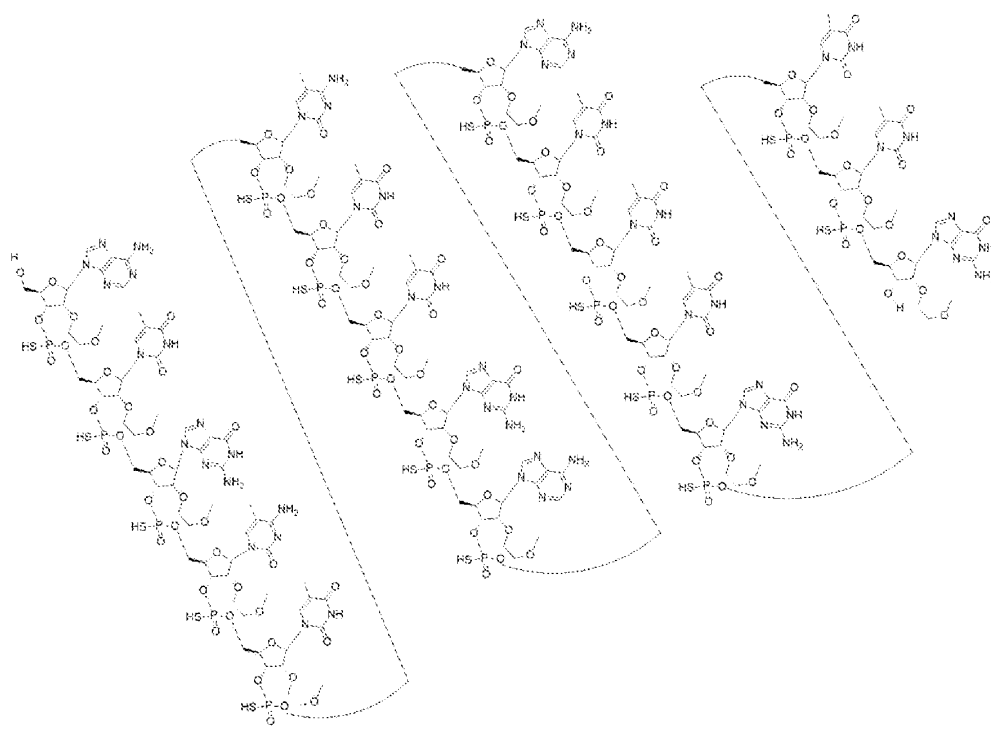
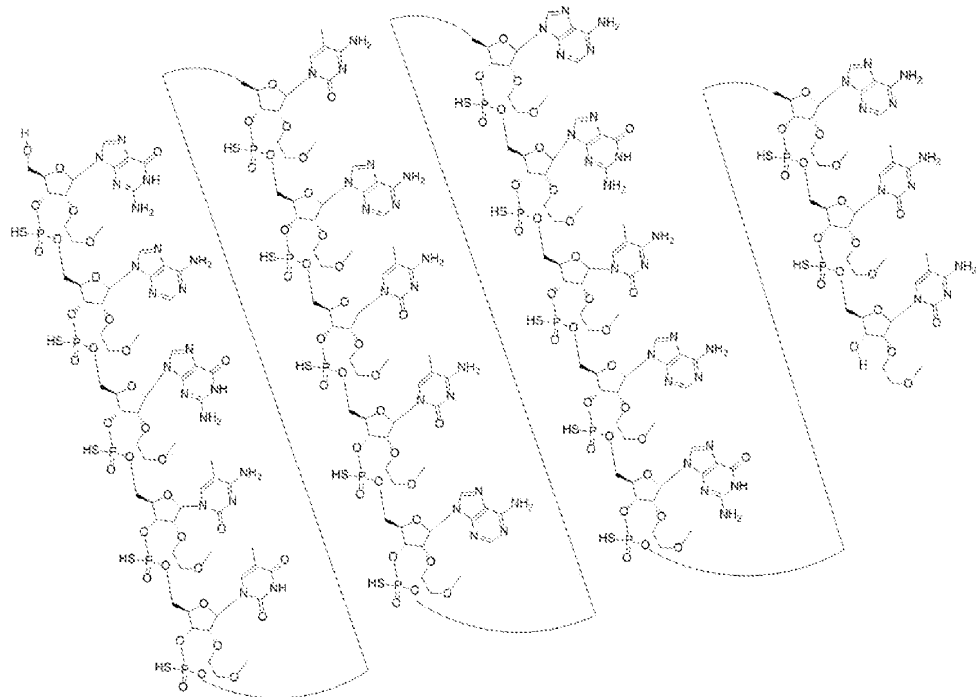
87. The method of claim 86, wherein the subject is additionally characterized by:

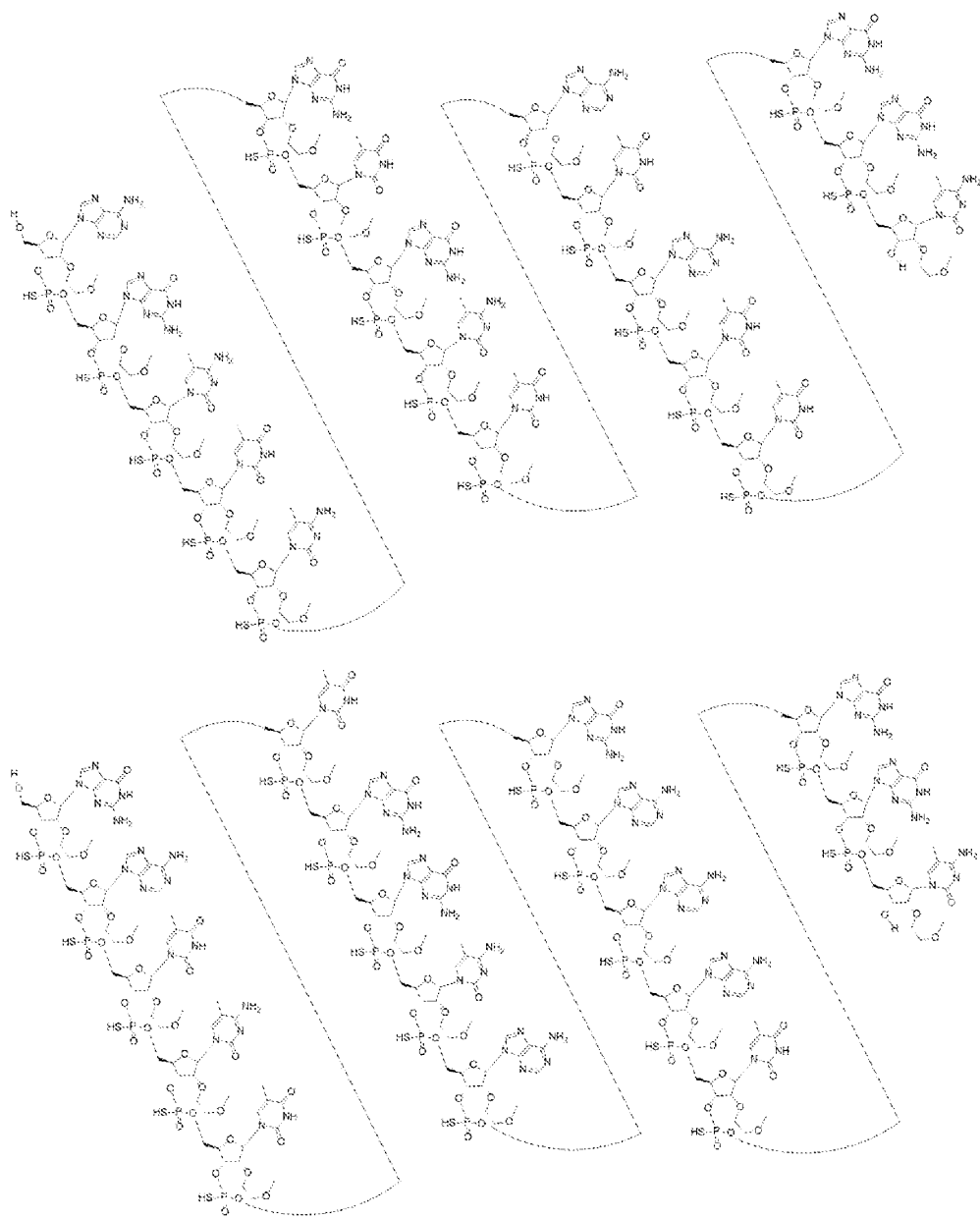
- (1) not having a gain-of-function variant, or compound heterozygous or homozygous pathogenic or likely pathogenic variant in *OPA1* gene;
- (2) not having only benign or likely benign variants in the *OPA1* gene;
- (3) not having extraocular phenotypic manifestations of (syndromic) ADOA (ADOA-plus);
- (4) not having been diagnosed with Behr syndrome;
- (5) not having a known pathogenic mutation in another gene implicated in optic atrophy or retinal diseases;
- (6) not having diabetic retinopathy with potential for development of proliferative diabetic retinopathy, diabetic macular edema, or optic neuropathy;
- (7) not having or having a history of any ocular condition in either eye;

- (8) not having a history of intraocular surgery or corneal surgery including refractive surgery in either eye within 12 weeks prior to the administering;
 - (9) not having a history of retinal photocoagulation;
 - (10) not having a history or presence of retinal vein occlusion;
 - (11) not being considered to be at risk for uveitis or ocular infection because of having any of the following: an active flare of non-infectious uveitis or an episode of infectious uveitis or other ocular infection in either eye within 12 months prior to the administering;
 - (12) not having dry age-related macular degeneration in either eye;
 - (13) not having high myopia (>6 diopters);
 - (14) not having a history of cancer (except a diagnosis of basal cell, squamous cell skin cancer, or carcinoma in situ of the cervix that has been successfully treated);
 - (15) not taking, or having taken at any time, any medication or treatment that can or might cause an optic neuropathy;
 - (16) not having any history of nutritional deficiency (including B12 and/or folate deficiencies), or not having a known deficiency in serum B12 or folate, not having had bariatric surgery;
 - (17) any combination of (1)-(16).
88. The method of any one of claims 1-87, wherein administering comprises administering multiple doses of the pharmaceutical composition to the human subject.
89. The method of claim 88, wherein administering comprises administering a first dose of the pharmaceutical composition to the human subject and a subsequent dose of the pharmaceutical composition to the human subject.
90. The method of claim 89, wherein the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is not tolerated.
91. The method of claim 89, wherein the subsequent dose is the same as the previous dose following an indication that administration of the previous dose is tolerated.
92. The method of claim 89, wherein the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is tolerated.
93. The method of claim 89, wherein the subsequent dose is the same as the previous dose following an indication that administration of the previous dose is effective.
94. The method of claim 89, wherein the subsequent dose is lower than the previous dose following an indication that administration of the previous dose is effective.
95. The method of claim 89, wherein the subsequent dose is higher than the previous dose following an indication that administration of the previous dose is not effective.

96. The method of any one of claims 1-95, wherein the pharmaceutical composition is administered via intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.
97. The method of any one of claims 1-87, wherein the pharmaceutical composition is administered via intravitreal injection.
98. The method of any one of claims 1-97, wherein the method further comprises administering an additional therapeutic agent.
99. The method of claim 98, wherein the additional therapeutic agent comprises a small molecule.
100. The method of claim 98, wherein the additional therapeutic agent comprises an antisense oligomer.
101. The method of claim 98, wherein the additional therapeutic agent comprises an ophthalmologic drug.
102. An antisense oligomer that is a compound according to any one of the following structures:

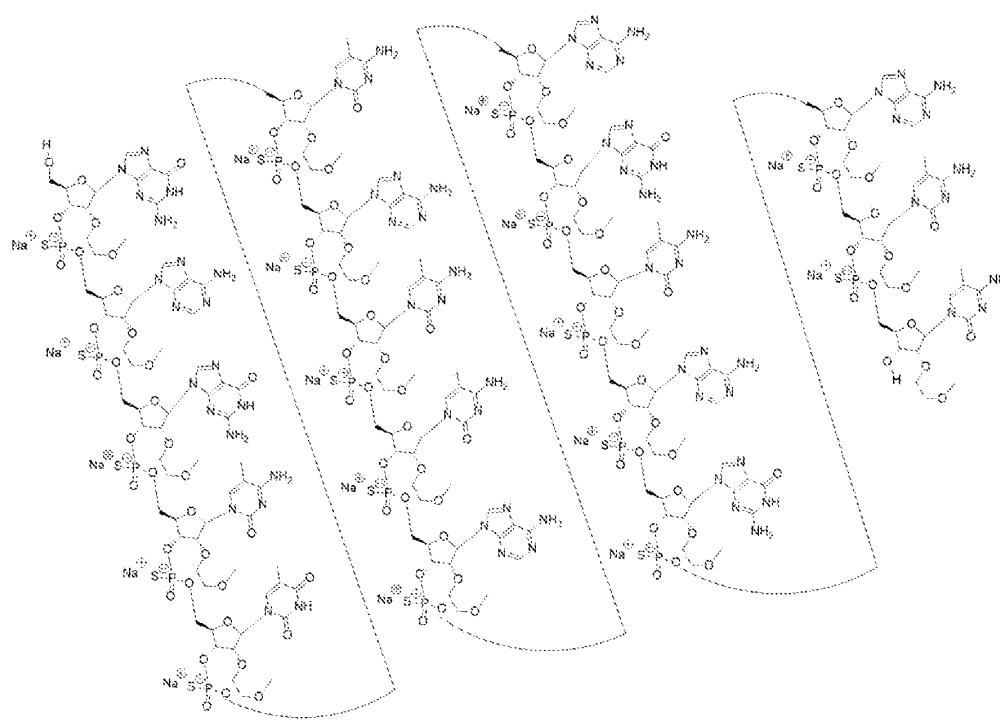


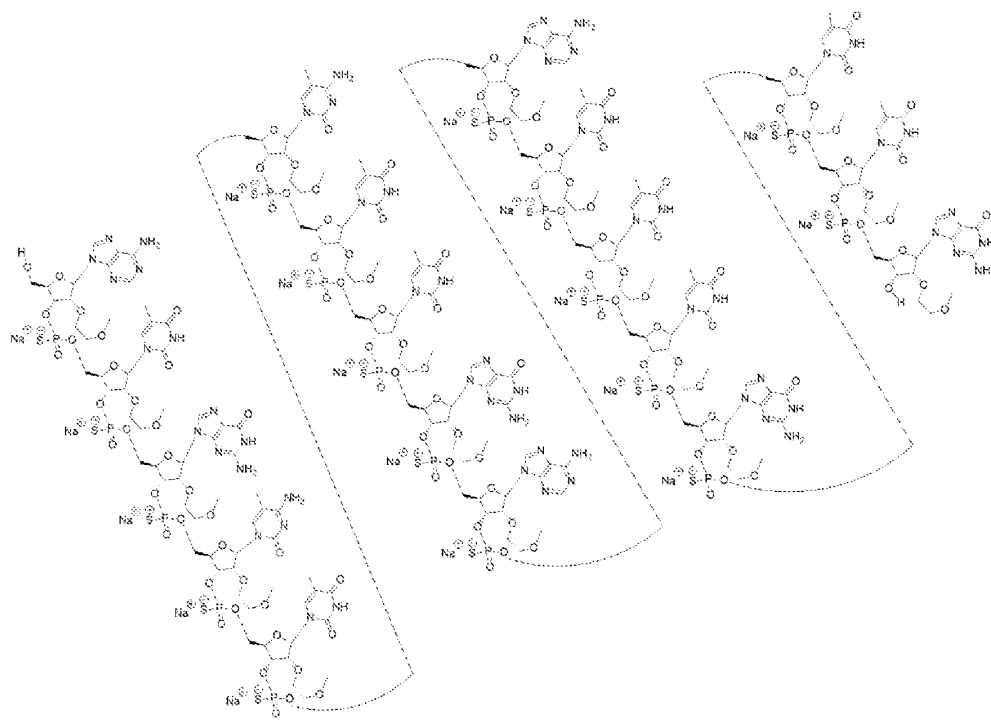




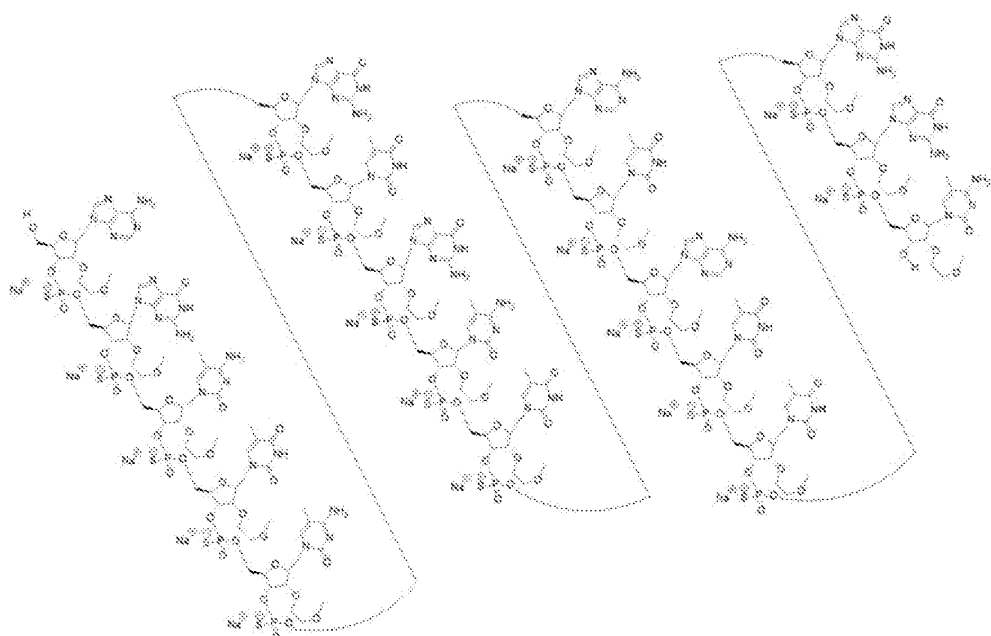
or a pharmaceutically acceptable salt thereof.

The image shows a complex chemical structure of a polyphosphate chain. The backbone consists of a series of phosphate groups (P=O, P-O) linked together. Various nucleoside and nucleotide units are attached to this backbone. These units include sugar rings (ribose or deoxyribose) and nitrogenous bases (adenine, guanine, cytosine, thymine, uracil). Some units are labeled with 'Na' (sodium) and 'H' (hydrogen). The structure is divided into three main sections by dashed lines, suggesting different regions or domains of the molecule. The overall structure is highly branched and complex, with many atoms and bonds represented.

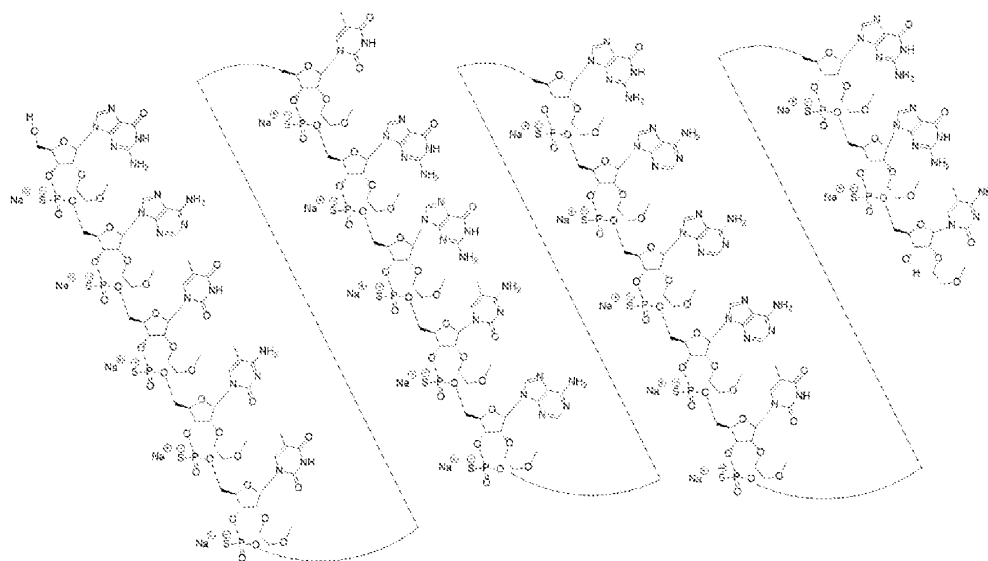




;



; or



104. A pharmaceutical formulation comprising:

(a) an antisense oligomer, wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and

(b) a pharmaceutically acceptable diluent;

wherein the antisense oligomer is dissolved or suspended in a solution at a concentration of about 1 mg/mL to about 200 mg/mL.

105. A pharmaceutical formulation comprising:

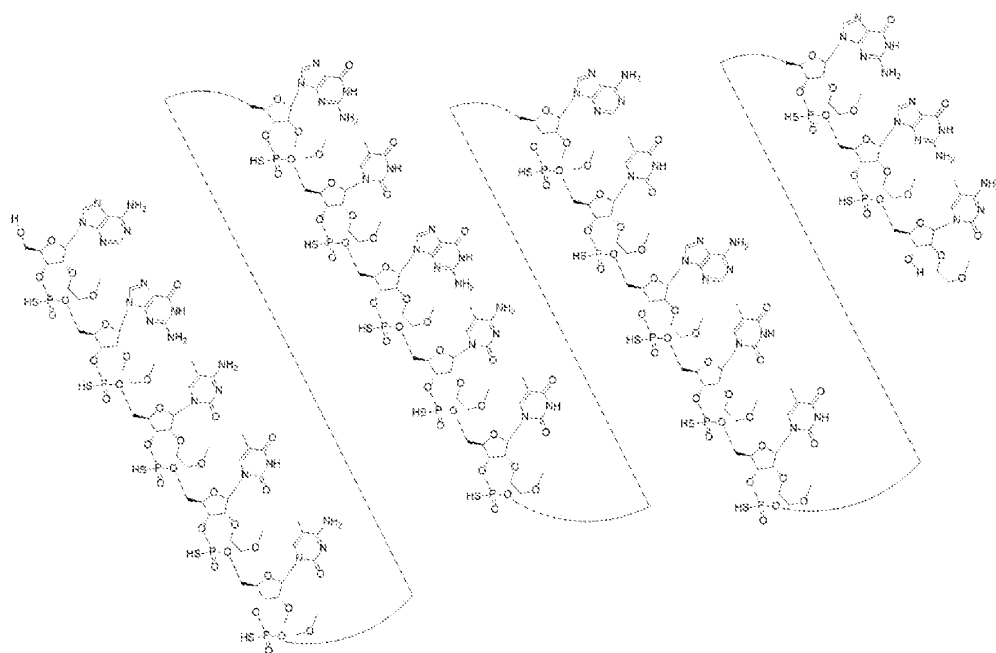
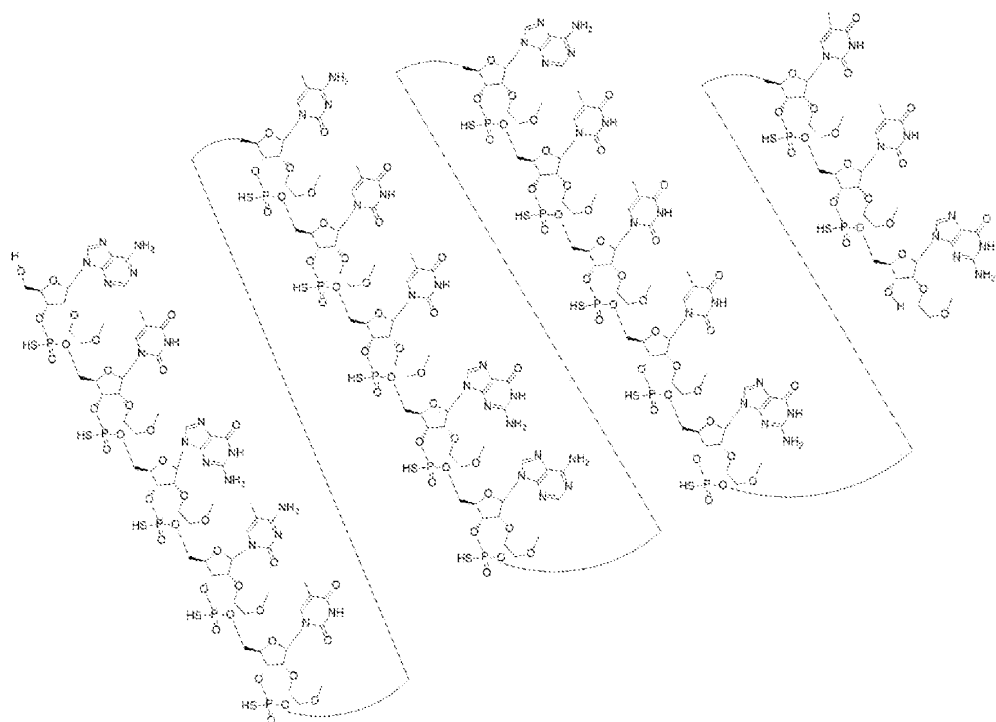
(a) an antisense oligomer; and

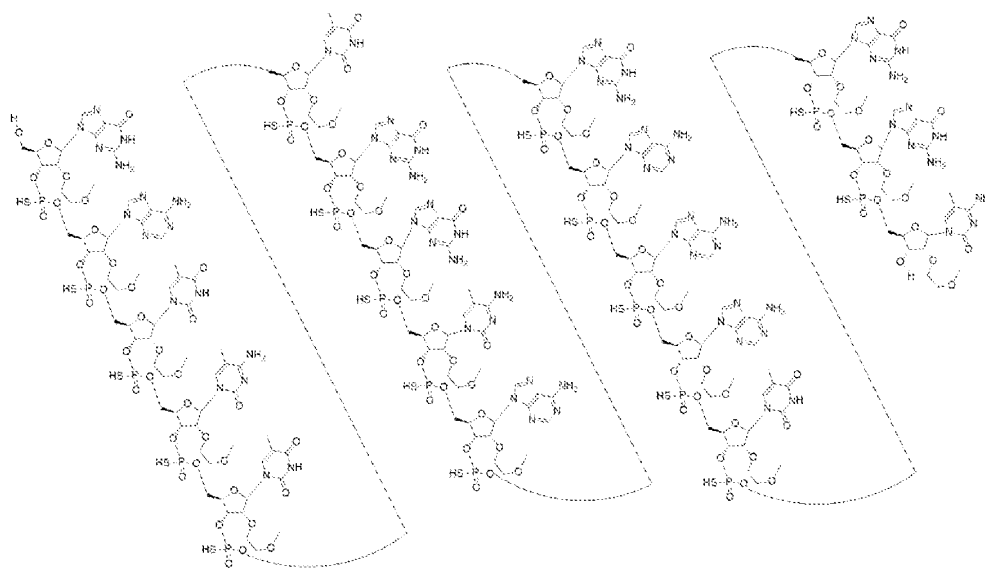
(b) a pharmaceutically acceptable diluent;

wherein the antisense oligomer is dissolved or suspended in a solution, and

wherein the antisense oligomer is a compound according to any one of the following chemical structures:







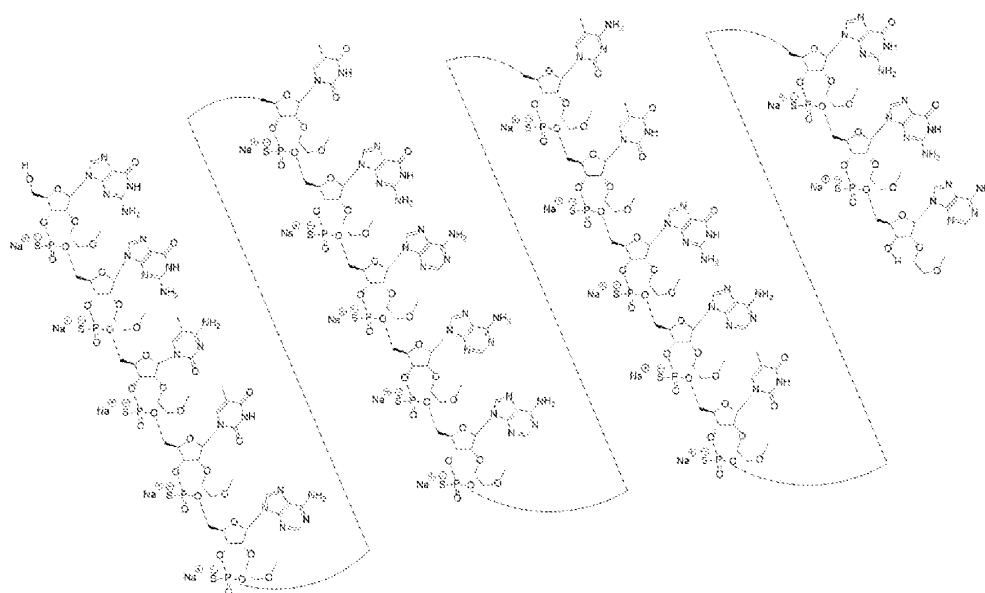
or a pharmaceutically acceptable salt thereof.

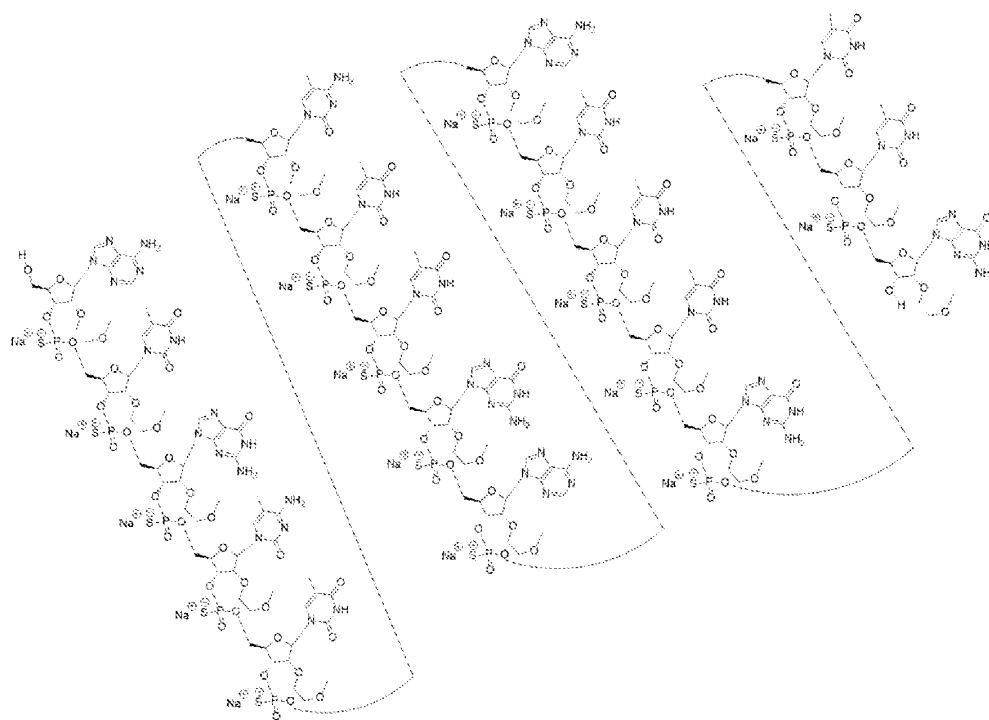
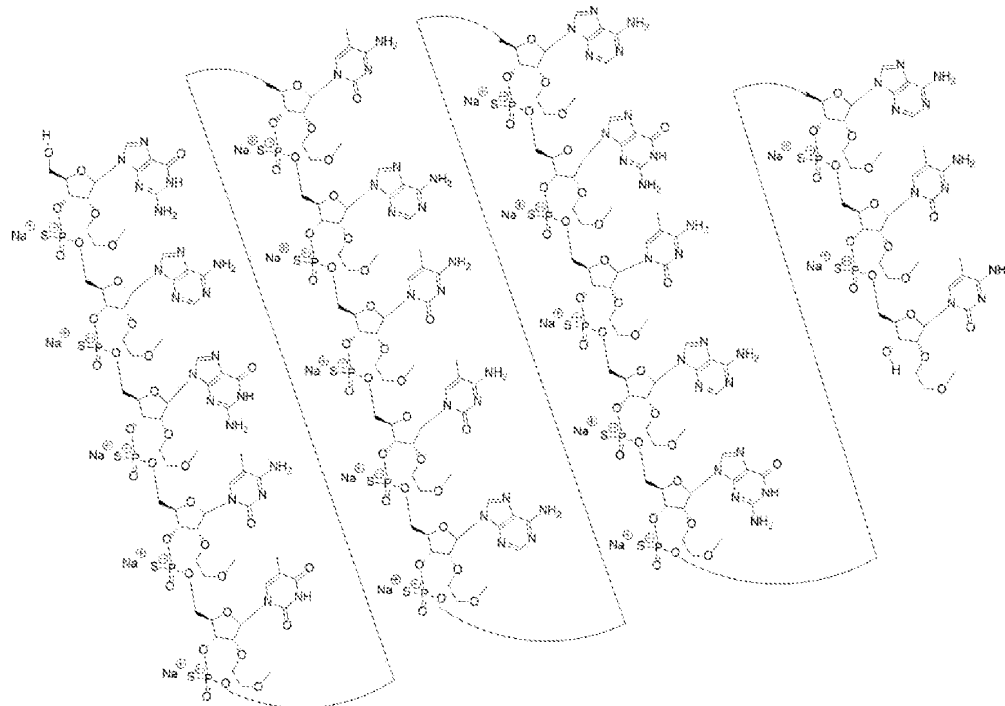
106. A pharmaceutical formulation comprising:

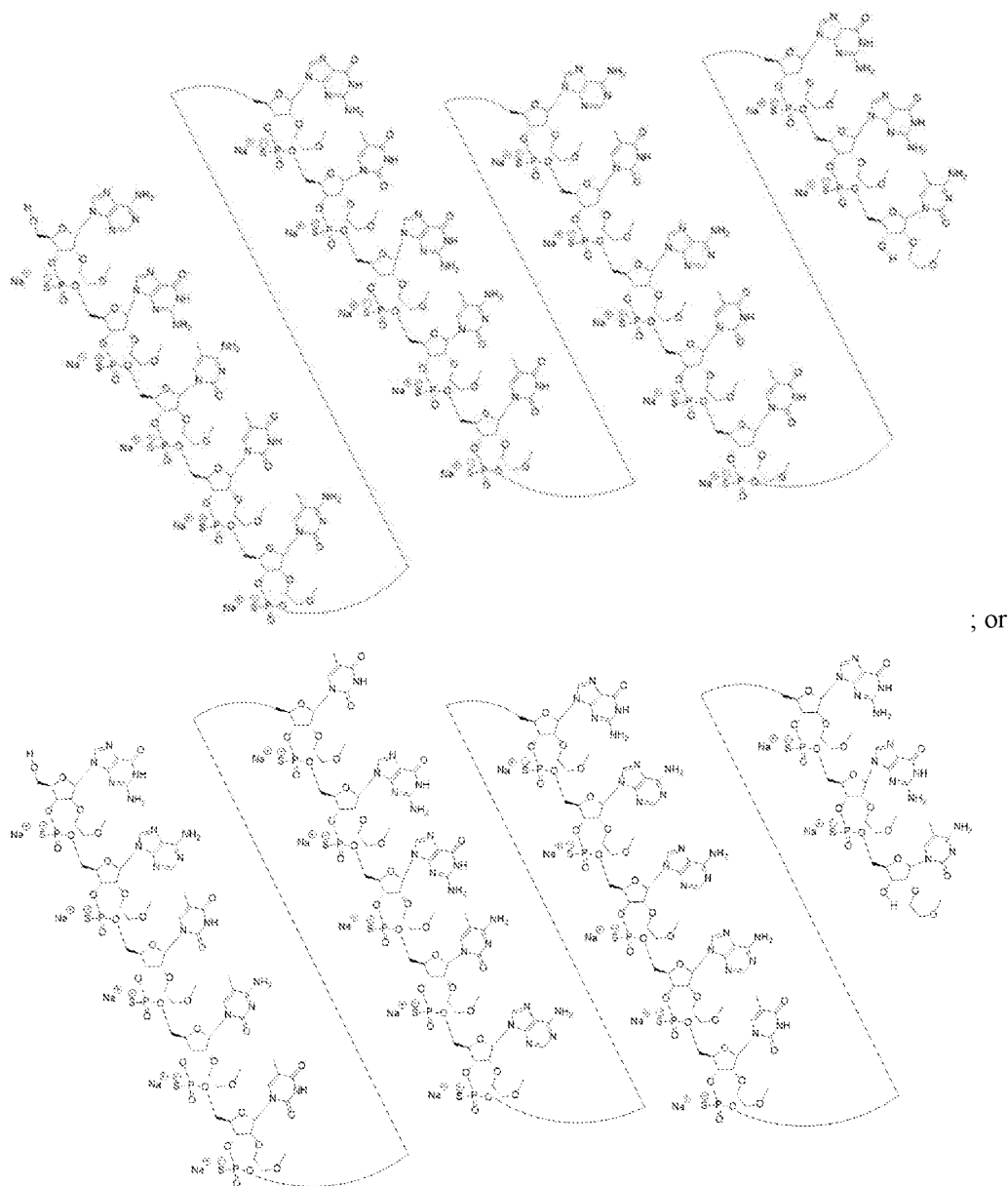
- (a) an antisense oligomer; and
- (b) a pharmaceutically acceptable diluent;

wherein the antisense oligomer is dissolved or suspended in a solution, and

wherein the antisense oligomer has any one of the following chemical structures:







107. The pharmaceutical formulation of claim 105 or 106, wherein the antisense oligomer is present in the solution at a concentration of about 1 mg/mL to about 200 mg/mL.

108. The pharmaceutical formulation of claim 104 or 107, wherein the antisense oligomer is present in the solution at a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to

about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL.

109. The pharmaceutical formulation of claim 104, wherein the antisense oligomer is present in the solution at a concentration of about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

110. The pharmaceutical formulation of any one of claims 104-109, wherein the pharmaceutical composition is prepared by diluting a concentrate comprising the antisense oligomer.

111. The pharmaceutical formulation of claim 110, wherein the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40

mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

112. The pharmaceutical composition of claim 110, wherein the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

113. The pharmaceutical formulation of any one of claims 104-112, wherein the concentrate is phosphate buffered.

114. The pharmaceutical formulation of any one of claims 104-113, wherein the antisense oligomer is solubilized or diluted in a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

115. The pharmaceutical formulation of any one of claims 104-113, wherein the antisense oligomer is solubilized or diluted in a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

116. The pharmaceutical formulation of any one of claims 104-113, wherein the antisense oligomer is solubilized or diluted in an isotonic solution.

117. The pharmaceutical formulation of any one of claims 104-116, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered solution with at least pH 5.8.

118. The pharmaceutical formulation of any one of claims 104-116, wherein the antisense oligomer is solubilized or diluted in a phosphate-buffered (pH 6.6 – 7.6) solution.

119. The pharmaceutical formulation of any one of claims 104-118, wherein the pharmaceutical formulation does not comprise a preservative.

120. The pharmaceutical formulation of any one of claims 104-119, wherein the pharmaceutical formulation is suitable for an intravitreal injection.

121. The pharmaceutical formulation of any one of claims 104-120, wherein the pharmaceutical formulation is packaged in a single-use vial.

122. A kit comprising:

- (i) a concentrate comprising an antisense oligomer (ASO), wherein the ASO comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299; and
- (ii) a diluent, wherein the concentrate is miscible with the diluent; and
- (iii) instructions for diluting the concentrate with the diluent.

123. The kit of claim 122, wherein the antisense oligomer is present in the concentrate at a concentration of about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, or about 40 mg/mL to about 60 mg/mL.

124. The kit of claim 122, wherein the antisense oligomer is present in the concentrate at a concentration of about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL.

125. The kit of any one of claims 122-124, wherein the concentrate is phosphate buffered.

126. The kit of any one of claims 122-125, wherein the diluent is a solution comprising one or more of sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, or water for injection.

127. The kit of any one of claims 122-125, wherein the diluent is a solution comprising sodium chloride, sodium phosphate dibasic, potassium phosphate monobasic, and water for injection.

128. The kit of any one of claims 122-127, wherein the diluent comprises an isotonic solution.

129. The kit of any one of claims 122-128, wherein the diluent comprises a phosphate-buffered solution with at least pH 5.8.

130. The kit of any one of claims 122-128, wherein the diluent comprises a phosphate-buffered (pH 6.6 - 7.6) solution.

131. The kit of any one of claims 122-130, wherein the concentrate or the diluent does not comprise a preservative.

132. The kit of any one of claims 122-131, wherein the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 2 mg/mL to 200 mg/mL in the diluent.

133. The kit of any one of claims 122-132, wherein the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the ASO to a concentration of about 5 mg/mL to about 200 mg/mL, about 10 mg/mL to about 200 mg/mL, about 15 mg/mL to about 200 mg/mL, about 20 mg/mL to about 200 mg/mL, about 25 mg/mL to about 200 mg/mL, about 30 mg/mL to about 200 mg/mL, about 35 mg/mL to about 200 mg/mL, about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, about 60 mg/mL to about 200 mg/mL, about 80 mg/mL to about 200 mg/mL, about 100 mg/mL to about 200 mg/mL, about 150 mg/mL to about 200 mg/mL, about 180 mg/mL to about 200 mg/mL, 2 mg/mL to about 150 mg/mL, about 5 mg/mL to about 150 mg/mL, about 10 mg/mL to about 150 mg/mL, about 15 mg/mL to about 150 mg/mL, about 20 mg/mL to about 150 mg/mL, about 25 mg/mL to about 150 mg/mL, about 30 mg/mL to about 150 mg/mL, about 35 mg/mL to about 150 mg/mL, about 40 mg/mL to about 150 mg/mL, about 50 mg/mL to about 150 mg/mL, about 60 mg/mL to about 150 mg/mL, about 80 mg/mL to about 150 mg/mL, about 100 mg/mL to about 150 mg/mL, 2 mg/mL to about 100 mg/mL, about 5 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 15 mg/mL to about 100 mg/mL, about 20 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 30 mg/mL to about 100 mg/mL, about 35 mg/mL to about 100 mg/mL, about 40 mg/mL to about 100 mg/mL, about 50 mg/mL to about 100 mg/mL, about 60 mg/mL to about 100 mg/mL, about 80 mg/mL to about 100 mg/mL, 2 mg/mL to about 80 mg/mL, about 5 mg/mL to about 80 mg/mL, about 10 mg/mL to about 80 mg/mL, about 15 mg/mL to about 80 mg/mL, about 20 mg/mL to about 80 mg/mL, about 25 mg/mL to about 80 mg/mL, about 30 mg/mL to about 80 mg/mL, about 35 mg/mL to about 80 mg/mL, about 40 mg/mL to about 80 mg/mL, about 60 mg/mL to about 80 mg/mL, 2 mg/mL to about 60 mg/mL, about 5 mg/mL to about 60 mg/mL, about 10 mg/mL to about 60 mg/mL, about 15 mg/mL to about 60 mg/mL, about 20 mg/mL to about 60 mg/mL, about 25 mg/mL to about 60 mg/mL, about 30 mg/mL to about 60 mg/mL, about 35 mg/mL to about 60 mg/mL, about 40 mg/mL to about 60 mg/mL, 2 mg/mL to about 40 mg/mL, about 5 mg/mL to about 40 mg/mL, about 10 mg/mL to about 40 mg/mL, about 15 mg/mL to about 40 mg/mL, about 20 mg/mL to about 40 mg/mL, or about 25 mg/mL to about 40 mg/mL in the diluent.

134. The kit of any one of claims 122-132, wherein the instructions for diluting the concentrate with the diluent comprise instructions for diluting or solubilizing the antisense oligomer to a concentration of about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 12 mg/mL, about 14

mg/mL, about 15 mg/mL, about 16 mg/mL, about 18 mg/mL, about 20 mg/mL, about 22 mg/mL, about 24 mg/mL, about 26 mg/mL, about 28 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 80 mg/mL, about 100 mg/mL, about 120 mg/mL, about 140 mg/mL, about 160 mg/mL, about 180 mg/mL, or about 200 mg/mL in the diluent.

135. The pharmaceutical composition or kit of any one of claims 104 or 108-134, wherein the antisense oligomer comprises a nucleotide sequence having at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 6-275 or 280-299.

136. The pharmaceutical composition or kit of any one of claims 104 or 108-134, wherein the antisense oligomer comprises a nucleotide sequence having at least 80%, at least 90%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36, 236, 242, 250, 280-283, 288, and 290-292.

137. The pharmaceutical composition or kit of any one of claims 104 or 108-136, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

138. The pharmaceutical composition or kit of any one of claims 104 or 108-137, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, a 2'-O-N-methyl-acetamide (2'-NMA), or a 2'-O-methoxyethyl moiety.

139. The pharmaceutical composition or kit of any one of claims 104 or 108-138, wherein the antisense oligomer comprises at least one modified sugar moiety.

140. The pharmaceutical composition or kit of claim 139, wherein each sugar moiety is a modified sugar moiety.

141. The pharmaceutical composition or kit of any one of claims 104 or 108-140, wherein the antisense oligomer comprises a 5'-methylcytosine (5'-MeC).

142. The pharmaceutical composition or kit of any one of claims 104 or 108-141, wherein each cytosine of the antisense oligomer is a 5'-methylcytosine (5'-MeC).

143. The pharmaceutical composition or kit of any one of claims 104 or 108-142, wherein the antisense oligomer comprises a 5'-methyluracil (5'-MeU).

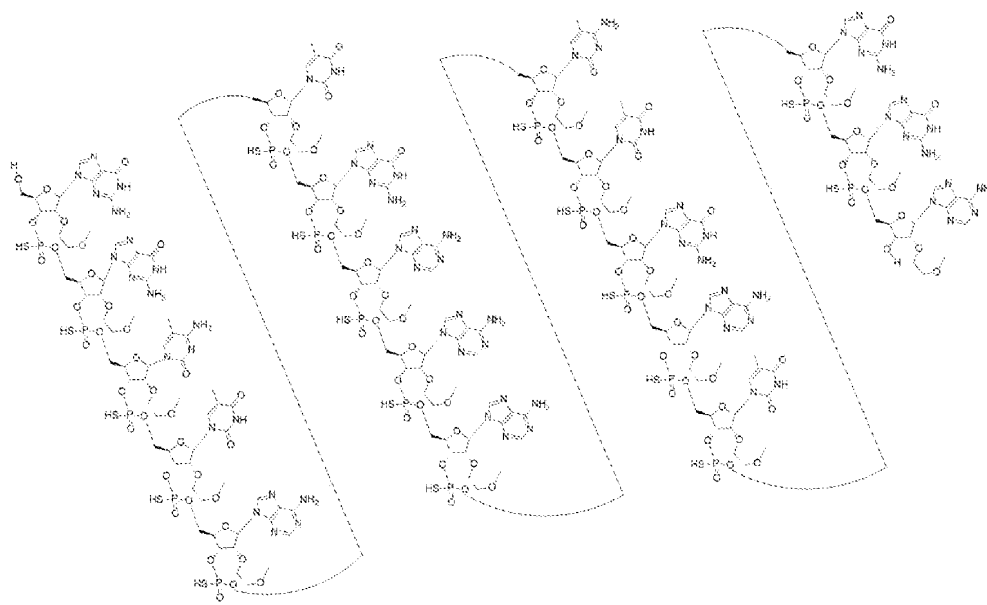
144. The pharmaceutical composition or kit of any one of claims 104 or 108-143, wherein the antisense oligomer comprises a phosphorothioate linkage.

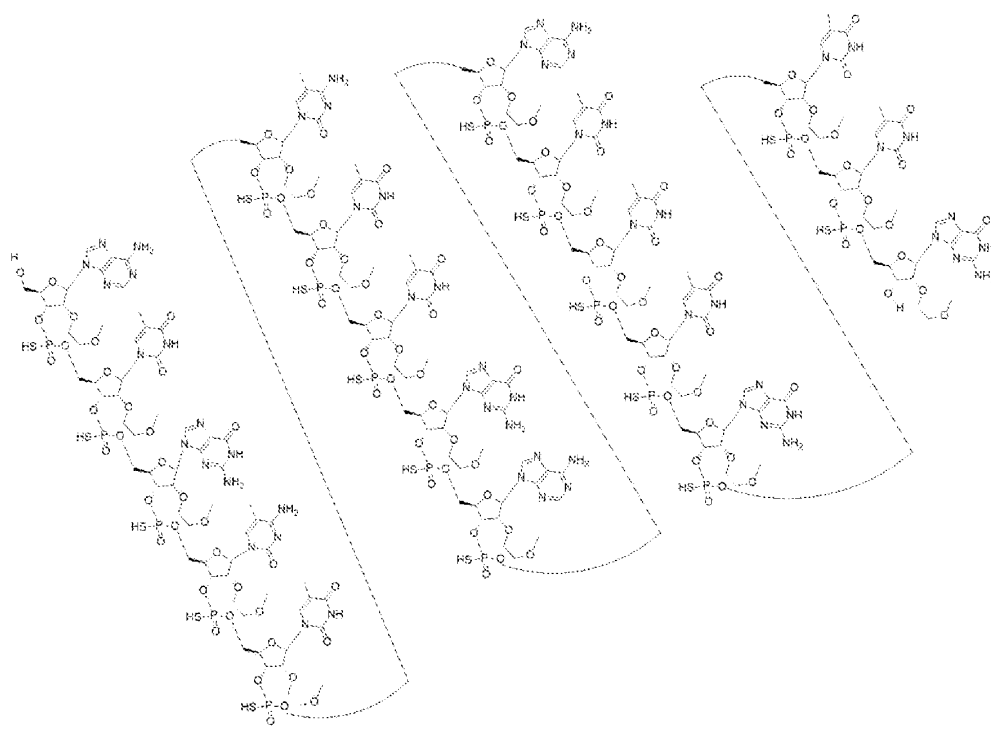
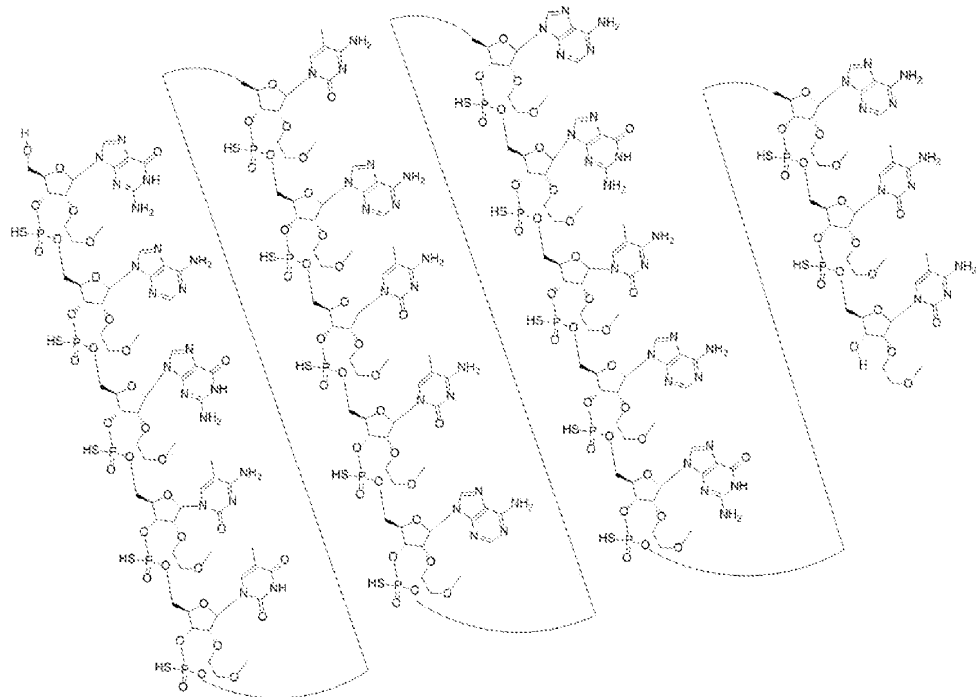
145. The pharmaceutical composition or kit of any one of claims 104 or 108-144, wherein each internucleoside linkage of the ASO is a phosphorothioate linkage.

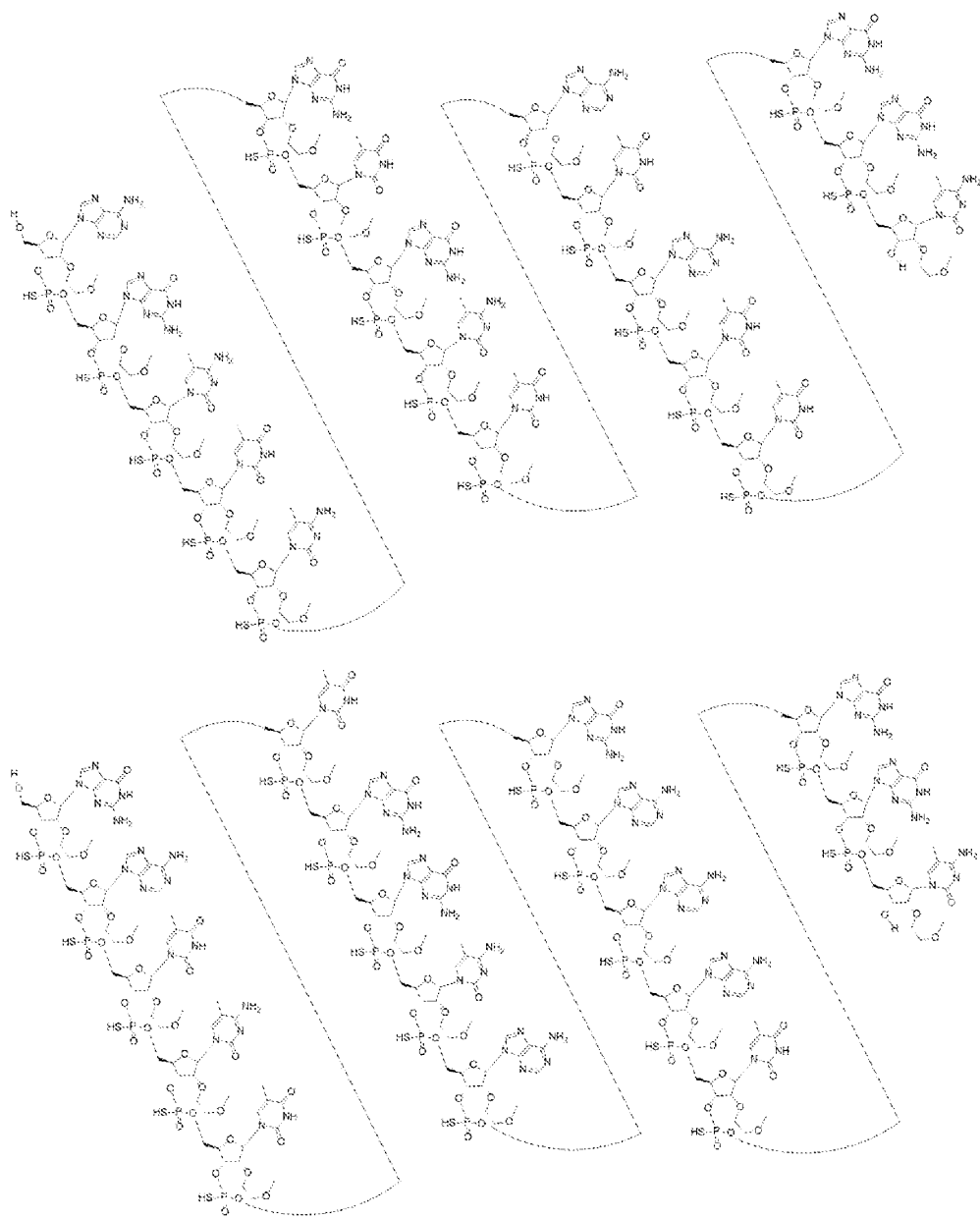
146. The pharmaceutical composition or kit of any one of claims 104 or 108-145, wherein the antisense oligomer comprises a locked nucleic acid (LNA).

147. The pharmaceutical composition or kit of any one of claims 104 or 108-146, wherein the antisense oligomer is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases in length.

148. The pharmaceutical composition or kit of any one of claims 104 or 108-134, wherein the antisense oligomer is a compound according to any one of the following chemical structures:

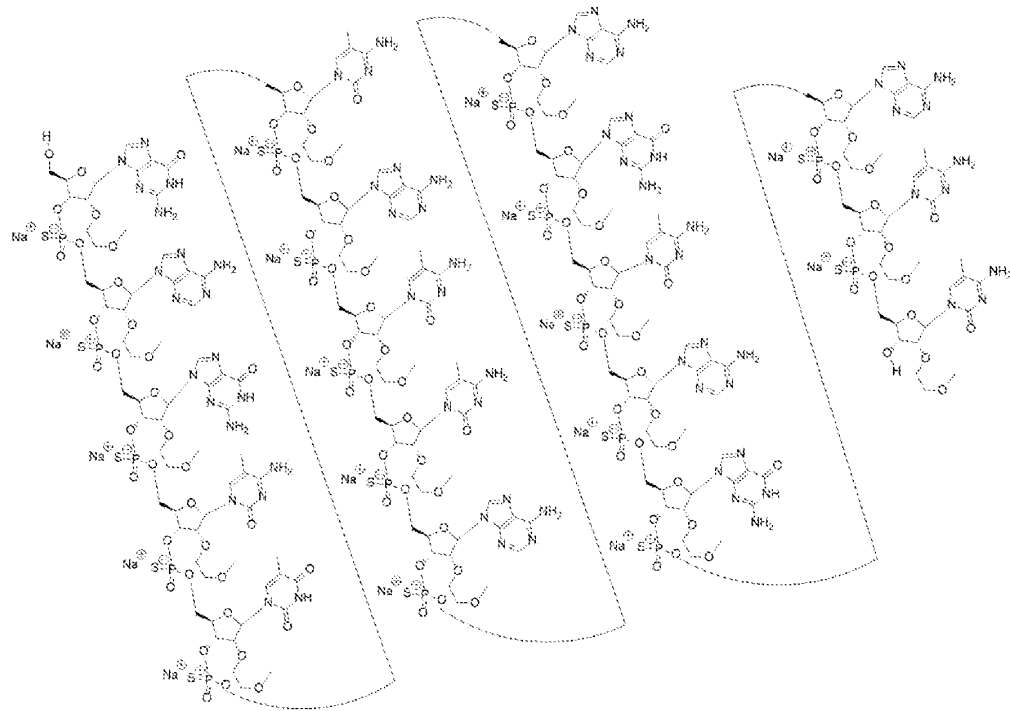
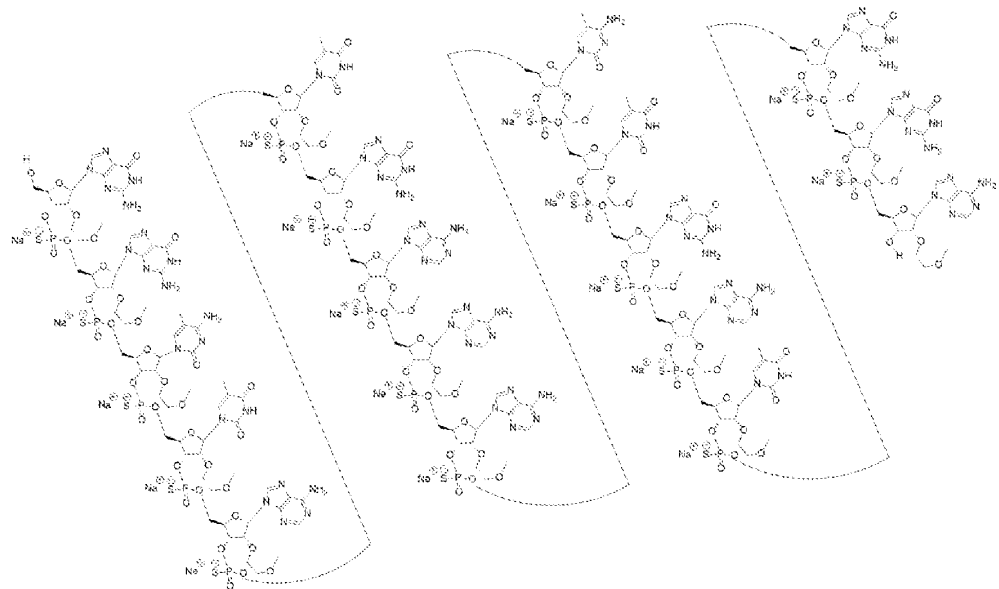


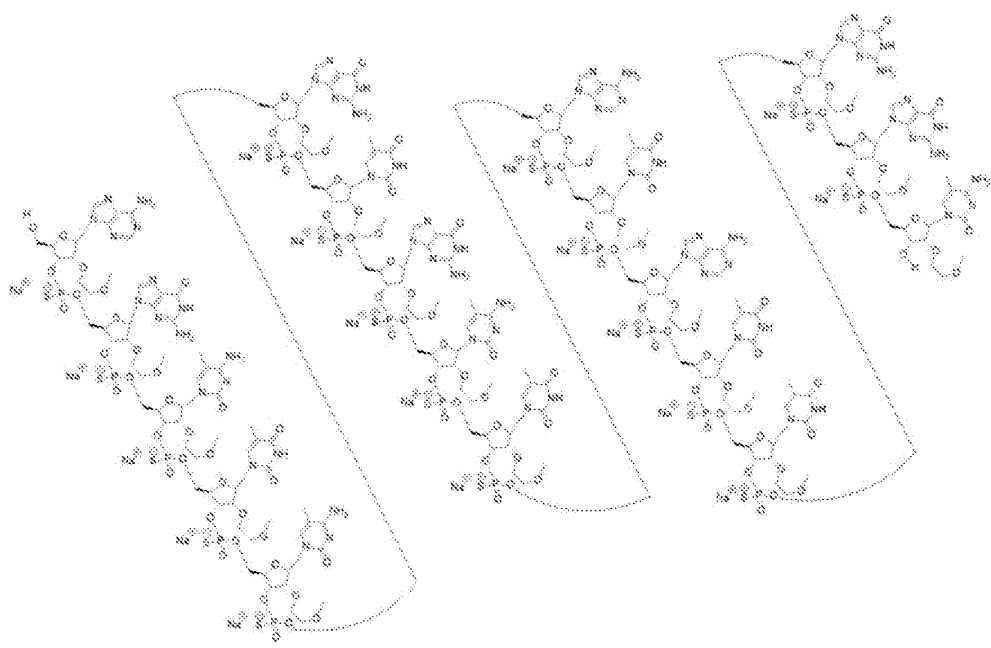
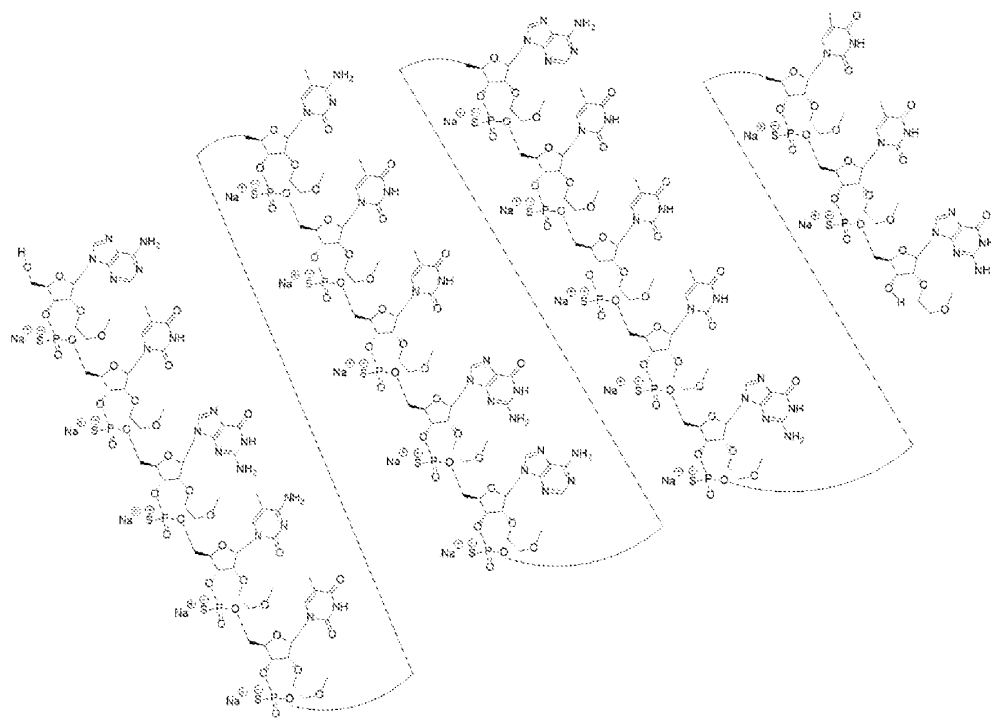




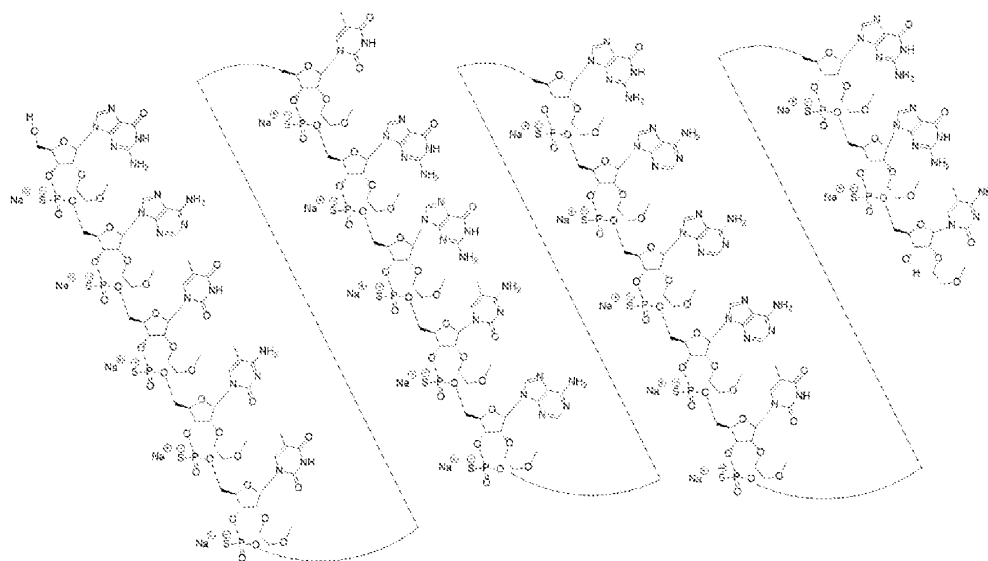
or a pharmaceutically acceptable salt thereof.

149. The pharmaceutical composition or kit of any one of claims 104 or 108-134, wherein the antisense oligomer has any one of the following chemical structures:





; or



150. Use of an antisense oligomer for the manufacture of a medicament for treating a disease or condition characterized by a reduced expression or function of OPA1 protein in a human subject in need thereof, or reducing likelihood of the subject developing the disease or condition, wherein the medicament is administered to one eye of the subject at a dose of about 0.005 mg to about 20 mg, and wherein the antisense oligomer comprises a sequence with at least 80% sequence identity to any one of SEQ ID NOs: 6-275 or 280-299.

151. The use of claim 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.005 mg to about 15 mg, about 0.005 mg to about 10 mg, about 0.005 mg to about 5 mg, about 0.005 mg to about 1 mg, about 0.01 mg to about 15 mg, about 0.01 mg to about 10 mg, about 0.01 mg to about 5 mg, about 0.01 mg to about 2.5 mg, about 0.01 mg to about 1.0 mg, about 0.01 mg to about 0.5 mg, about 0.01 mg to about 0.25 mg, about 0.01 mg to about 0.1 mg, about 0.01 mg to about 0.05 mg, about 0.05 mg to about 10 mg, about 0.05 mg to about 5 mg, about 0.05 mg to about 2.5 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.25 mg, about 0.05 mg to about 0.1 mg, about 0.1 mg to about 5 mg, about 0.1 mg to about 2.5 mg, about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.5 mg, or about 0.1 mg to about 0.25 mg of the antisense oligomer.

152. The use of claim 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.005 mg, about 0.01 mg, about 0.05 mg, about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.75 mg, about 2.0 mg, about 2.25 mg, about 2.5 mg, about 2.75 mg, about 3 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, about 5.0 mg, about 5.5 mg, about 6.0 mg, about 7.0 mg, about 8.0 mg, about 9.0 mg, about 10 mg, about 12.5 mg, about 15 mg, about 17.5 mg, or about 20 mg of the antisense oligomer.

153. The use of claim 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.1 mg to about 1.5 mg, about 0.1 mg to about 1.4 mg, about 0.1 mg to about 1.2 mg, about 0.1 mg to about 1.0 mg, about 0.1 mg to about 0.8 mg, about 0.1 mg to about 0.7 mg, about 0.1 mg to about 0.5 mg, about 0.1 mg to about 0.3 mg, about 0.2 mg to about 1.5 mg, about 0.2 mg to about 1.4 mg, about 0.2 mg to about 1.2 mg, about 0.2 mg to about 1.0 mg, about 0.2 mg to about 0.8 mg, about 0.2 mg to about 0.7 mg, about 0.2 mg to about 0.5 mg, about 0.3 mg to about 1.5 mg, about 0.3 mg to about 1.4 mg, about 0.3 mg to about 1.2 mg, about 0.3 mg to about 1.0 mg, about 0.3 mg to about 0.8 mg, about 0.3 mg to about 0.7 mg, about 0.3 mg to about 0.5 mg, about 0.5 mg to about 1.5 mg, about 0.5 mg to about 1.4 mg, about 0.5 mg to about 1.2 mg, about 0.5 mg to about 1.0 mg, about 0.5 mg to about 0.8 mg, about 0.5 mg to about 0.7 mg, about 0.7 mg to about 1.5 mg, about 0.7 mg to about 1.4 mg, about 0.7 mg to about 1.2 mg, about 0.7 mg to about 1.0 mg, about 0.8 mg to about 1.5 mg, about 0.8 mg to about 1.4 mg, about 0.8 mg to about 1.2 mg, about 0.8 mg to about 1.0 mg, about 1.0 mg to about 1.5 mg, about 1.0 mg to about 1.4 mg, about 1.0 mg to about 1.2 mg, about 1.2 mg to about 1.5 mg, or about 1.2 mg to about 1.4 mg of the antisense oligomer.

154. The use of claim 150, wherein the medicament is administered to the one eye of the subject at a dose of about 0.1 mg, about 0.2 mg, about 0.3 mg, 0.4 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1.0 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, or about 1.5 mg of the antisense oligomer.