

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. 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). In some embodiments, 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.

**[0032]** In some embodiments, the subject is a human. In some embodiments, the subject is a non-human animal. In some embodiments, the subject is a fetus, an embryo, or a child. In some embodiments, the cells that the methods and compositions described herein are applicable to are ex vivo. In some embodiments, 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. In some embodiments, the therapeutic agent is administered by intravitreal injection. In some embodiments, the method disclosed herein treats the disease or condition.

**[0032a]** Described herein, in some aspects, is a pharmaceutical composition comprising a therapeutic agent or a vector encoding the therapeutic agent, wherein the therapeutic agent comprises an antisense oligomer that comprises a sequence selected from the group consisting of SEQ ID NOs: 6-275 and 280-299, and a pharmaceutically acceptable excipient.

**[0032b]** Described herein, in some aspects, is a composition comprising: (a) an antisense oligomer that comprises 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; or (b) a vector encoding the antisense oligomer.

**[0032c]** Described herein, in some aspects, is the pharmaceutical composition as described herein or the composition as described herein for use in treating or preventing a disease or condition.

**[0032d]** Described herein, in some aspects, is a use of the pharmaceutical composition as described herein or the composition as described herein in the manufacture of a medicament for the treatment of a disease or condition, wherein the disease or condition (i) is associated with a loss-of-function mutation in an OPA1 gene; or (ii) 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.

**[0032e]** Described herein, in some aspects, is a use of the pharmaceutical composition as described herein or the composition as described herein in the manufacture of a medicament for modulating expression of an OPA1 protein in a cell having a pre-mRNA that is transcribed from an OPA1 gene and that comprises a non-sense mediated RNA decay-inducing exon (NMD exon), wherein the use comprises contacting the composition or the pharmaceutical composition to the cell, whereby the therapeutic 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.

**[0032f]** Described herein, in some aspects, is a use of a therapeutic agent or a vector encoding the therapeutic agent in the manufacture of a medicament for 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, wherein the use comprises contacting the therapeutic agent or the vector encoding the therapeutic agent to the cell, whereby the therapeutic 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.

## **INCORPORATION BY REFERENCE**

**[0033]** 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**

[0034] 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:

**[0035] FIGs. 1A-1C** depict a schematic representation of a target mRNA that contains a non-sense 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. **FIG. 1A** shows a cell divided into nuclear and cytoplasmic compartments. In the nucleus, a pre-mRNA transcript of a target gene undergoes splicing to generate mRNA, and this mRNA is exported to the cytoplasm and translated into target protein. For this target gene, some fraction of the mRNA contains a nonsense-mediated mRNA decay-inducing exon (NMD exon mRNA) that is degraded in the cytoplasm, thus leading to no target protein production. **FIG. 1B** shows an example of the same cell divided into nuclear and cytoplasmic compartments. Treatment with a therapeutic agent, such as an antisense oligomer (ASO), promotes the exclusion of the nonsense-mediated mRNA decay-inducing exon and results in an increase in mRNA, which is in turn translated into higher levels of target protein. **FIG. 1C** shows an example schematic of a Novel NMD exon inclusion event (Exon X) identified in the *OPAI* gene which leads to the introduction of a premature termination codon (PTC) resulting in a non-productive mRNA transcript degraded by non-sense mediated decay (NMD).

**[0036] FIG. 2** depicts identification of an exemplary nonsense-mediated mRNA decay (NMD)-inducing exon in the *OPAI* gene. The identification of the NMD-inducing exon in the *OPAI* gene using RNA sequencing is shown, visualized in the UCSC genome browser. The upper panel shows a graphic representation of the *OPAI* gene to scale. Peaks corresponding to RNA sequencing reads were identified in intron GRCh38/hg38: chr3 193626204 to 193631611, shown in the middle panel. Bioinformatic analysis identified an exon-like sequence (bottom panel, sequence highlighted in uppercase; GRCh38/hg38: chr3 193628509 to 193628616) flanked by 3' and 5' splice sites. Inclusion of this exon leads to the introduction of a premature termination codon rendering the transcript a target of NMD. FIG. 2 discloses SEQ ID NO: 300.

**[0037] FIG. 3** depicts identification of an exemplary nonsense-mediated mRNA decay (NMD)-inducing exon in the *OPAI* gene. The identification of the NMD-inducing exon in the *OPAI* gene using RNA sequencing is shown, visualized in the UCSC genome browser. The upper panel shows a graphic representation of the *OPAI* gene to scale. Peaks corresponding to RNA sequencing reads were identified in intron GRCh38/hg38: chr3 193593374 to 193614710, shown in the middle panel. Bioinformatic analysis identified an exon-like sequence (bottom panel, sequence highlighted in uppercase; GRCh38/hg38: chr3 193603500 to 193603557) flanked by 3'

and 5' splice sites. Inclusion of this exon leads to the introduction of a premature termination codon rendering the transcript a target of NMD. FIG. 3 discloses SEQ ID NO: 301.

**[0038] FIG. 4** depicts confirmation of NMD-inducing exon via puromycin or cycloheximide treatment in various cell lines, as well as the confirmation of NMD-inducing exon in brain and retina samples. RT-PCR analysis using total RNA from water-treated, DMSO-treated, puromycin-treated, or cycloheximide-treated cells confirmed the presence of a band corresponding to the NMD-inducing exon 7x (GRCh38/hg38: chr3 193628509 to 193628616) of *OPA1* gene

**[0039] FIG. 5** depicts an exemplary ASO walk around *OPA1* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region. A graphic representation of an ASO walk performed for around *OPA1* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region targeting sequences upstream of the 3' splice site, across the 3' splice site, exon 7x, across the 5' splice site, and downstream of the 5' splice site is shown. ASOs were designed to cover these regions by shifting 5 nucleotides at a time or 3 nucleotides across the splice site regions. FIG. 5 discloses SEQ ID NOS 302-304, respectively, in order of appearance.

**[0040] FIG. 6** depicts an *OPA1* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region ASO walk evaluated by Taqman RT-qPCR. Graphs of fold-change of the *OPA1* productive mRNA product relative to Sham are plotted.

**[0041] FIG. 7** depicts an *OPA1* exon 7x (GRCh38/hg38: chr3 193628509 193628616) region ASO walk evaluated by Taqman RT-qPCR. Graphs of fold-change of the *OPA1* productive mRNA product relative to Sham are plotted.

**[0042] FIG. 8** illustrates expression of *OPA1* transcripts containing the NMD exon in HEK293 cells treated with increasing amounts of cycloheximide.

**[0043] FIG. 9A** illustrates RT-PCR data from the posterior segment of the eye of *Chlorocebus sabaeus* (green monkey) at postnatal data P93 (3 months) and postnatal day P942 (2.6 years). Fig. 9A confirms expression of *OPA1* transcripts containing the NMD exon in these cells.

**[0044] FIG. 9B** illustrates quantification of the NMD exon abundance from **FIG. 9A**.

**[0045] FIG. 10A** illustrates RT-PCR of the productive and non-productive *OPA1* mRNA after treatment of HEK293 cells with various ASOs and cycloheximide.

**[0046] FIG. 10B** illustrates quantification of the data in **FIG. 10A**.

**[0047] FIG. 11** illustrates expression of productive *OPA1* mRNA by quantitative PCR in HEK293 cells treated with various ASOs and not treated with cycloheximide.

**[0048] FIG. 12A** illustrates RT-PCR for non-productive *OPA1* mRNAs in HEK293 cells after treatment with ASO-14 and cycloheximide.

**[0049] FIG. 12B** illustrates quantification of productive *OPA1* mRNAs in HEK293 cells after treatment with ASO-14 in the absence of cycloheximide.

[0050] **FIG. 12C** illustrates protein expression of OPA1 in HEK293 cells after treatment with ASO-14 in the absence of cycloheximide.

[0051] **FIG. 13A** illustrates mRNA and protein levels of *OPA1* gene in *OPA1* haploinsufficient (*OPA1*<sup>+/-</sup>) HEK293 cells.

[0052] **FIG. 13B** illustrates OPA1 protein expression in the *OPA1* haploinsufficient (*OPA1*<sup>+/-</sup>) HEK293 cells after treatment with ASO-14.

[0053] **FIG. 13C** illustrates quantification of OPA1 protein expression in the *OPA1* haploinsufficient (*OPA1*<sup>+/-</sup>) HEK293 cells after treatment with ASO-14.

[0054] **FIG. 14A** illustrates study design for the *in vivo* rabbit experiment of Example 14.

[0055] **FIG. 14B** illustrates levels of productive and non-productive *OPA1* mRNA and protein.

[0056] **FIG. 14C** illustrates quantification of the data from **FIG. 14B**.

[0057] **FIG. 15** illustrates exemplary OPA1 ASOs of this disclosure. The right two columns in the chart illustrate the chemical modifications of the exemplary ASOs. Each nucleotide of all the ASOs has 2'-O-methoxyethyl (2'MOE) modification ("MOE") unless otherwise noted, for instance, letters of larger font size (*e.g.*, **G**) are locked nucleic acids ("LNA"), underlined letters (*e.g.*, C) are 5' methyl-cytosines that have 2'-MOE moiety ("5MeC-MOE"), and some ASOs are noted as phosphorodiamidate morpholino oligomers ("PMO"). FIG. 15 discloses SEQ ID NOS 6-148, 148, 148, 149, 149, 149, 150, 150, 150-151, 151, 151, 123, 152, 152, 152-153, 153, 153-154, 154, 154, 144-146, 93, 81-82, 36, 155, 155-156, 156-157, 157-161, 125, 162, 126, 163-166, 92, 167-179, 156, 180, 157, 159, 181, 160, 182, 161, 183-275, and 305-607 respectively, in order of column.

[0058] **FIG. 16A** illustrates RT-PCR results for *OPA1* mRNAs using probes spanning exon 7 and exon 8 in HEK293 cells after treatment with ASO-14 and cycloheximide.

[0059] **FIG. 16B** illustrates quantification of *OPA1* mRNAs in HEK293 cells after treatment with ASO-14 in the absence of cycloheximide based on qPCR using probes spanning exons 6 and 8, probes spanning exons 7 and 8, or probes spanning exons 23 and 24.

[0060] **FIG. 16C** illustrates sequencing data on the relative amount of various *OPA1* mRNA transcripts in HEK293 cells transfected with ASO-14.

[0061] **FIG. 17A** illustrates RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 in HEK293 cells after treatment with various exemplary OPA1 ASOs.

[0062] **FIG. 17B** illustrates relative ratio of *OPA1* mRNA transcripts having exons 6, 7, and 8 in tandem ("6-7-8") over the total amount of "6-7-8" transcripts and transcripts having

exons 6 and 8 in tandem (“6-8”), in HEK293 cells after treatment with various exemplary OPA1 ASOs.

**[0063] FIGs. 17C and 17D** illustrate quantification of *OPA1* mRNAs using probes spanning exons 6 and 8, and probes spanning exons 7 and 8, respectively, in HEK293 cells after treatment with various exemplary OPA1 ASOs.

**[0064] FIG. 18A** illustrates RT-PCR results for *OPA1* mRNAs using probes spanning exon 6 and exon 8 (“Exon 6-8 PCR”), or probes spanning exon 7x and exon 8 (“Exon 7x-8 PCR”), in