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(54) Title: THERAPEUTIC MOLECULES

Figure 1

gDNA	5'	A	A	T	G	A	A	T	G	C	T	G	G	G	A	G	G	A	T	G	A	3'
mRNA	5'	A	A	U	G	A	A	U	G	C	J	G	G	G	A	G	G	A	U	G	A	3'
ASO1 and 2	3'			A	C	T	T	A	C	G	A	C	C	C	T	C	C	T	A	C	T	5'
ASO 3 and 4	3'	T	T	A	C	T	T	A	C	G	A	C	C	C	T	C	C	T	A			5'

ASO1: 5'<U*C*A*U*C*>C*T*C*C*C*{A}*G*C*<A*U*U*C*A>3'

ASO2: 5'<U*C*A*U*C*>C*T*C*C*{C}*A*{G}*C*<A*U*U*C*A>3'

ASO3: 5'<A*U*C*C*U*>C*C*C*{A}*G*C*A*T*<U*C*A*U*U>3'

ASO4: 5'<A*U*C*C*U*>C*C*{C}*A*{G}*C*A*T*<U*C*A*U*U>3'

CTRL ASO: 5'<C*A*C*C*C*>C*C*A*T*T*C*T*T*<C*A*G*C*C>3'

* = PS
< > = 2'O-MOE
{} = LNA

(57) Abstract: The invention relates to an antisense oligonucleotide comprising a sequence complementary to at least part of a target nucleic acid sequence, wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof. The invention further relates to methods for treating, preventing ameliorating, or slowing progression of Doyne honeycomb macular dystrophy (DHMD) and related medical uses of the antisense oligonucleotides.

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Therapeutic Molecules

Field of invention

The present invention relates to antisense oligonucleotides for use in the treatment of Doyne Honeycomb Macular Dystrophy

Background

Doyne Honeycomb Macular Dystrophy (DHMD) is an autosomal dominant juvenile macular dystrophy for which there is currently no cure or treatments. DHMD is a monogenic disease caused by a dominant variation c.1033C>T, p.(R345W) in the *EFEMP1* gene. This dominant variation results in a toxic gain-of-function by the resultant protein, disrupting the function of the retinal pigment epithelial cells in which it is expressed, and ultimately leading to degeneration of the overlying photoreceptor cells in the retinal macular region and loss of central vision. In this invention, the aim is to develop an RNA-directed therapy that specifically targets the disease-associated allele at the *EFEMP1* locus. This approach is intended to lead to the RNaseH-mediated degradation of the disease allele, thereby rescuing the toxic gain-of-function disease mechanism. There are no existing methods, devices or materials to treat this condition. RNA directed therapies have been developed to treat other diseases. Currently, 15 RNA directed therapies have been approved by the FDA to treat various rare disease, including 1 RNA directed therapy to treat Age-Related Macular Degeneration (Pegaptanib).

Summary of the invention

We provide antisense oligonucleotide (AON) that can specifically target the mRNA of the 1033C>T mutant allele of the *EFEMP1* gene, thereby providing an RNA-directed therapy that specifically targets the disease-associated allele at the *EFEMP1* locus.

According to a first aspect of the present invention, there is provided an antisense oligonucleotide comprising a sequence complementary to at least part of a target nucleic acid sequence, wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof, wherein the antisense oligonucleotide is 100% complementary to position 8 from the 5' end of SEQ ID NO: 1.

In one embodiment, the antisense oligonucleotide may selectively reduce expression of a EFEMP1 gene product comprising a single nucleotide point mutation.

In one embodiment, the antisense oligonucleotide sequence across its entire length may be at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89% complementary upstream and/or downstream of position 8 from the 5' end of SEQ ID NO: 1 to a target nucleic acid sequence, wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

In one embodiment, the antisense oligonucleotide sequence across its entire length may be at least 90% complementary upstream and/or downstream of position 8 from the 5' end of SEQ ID NO: 1 to a target nucleic acid sequence wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

In one embodiment the sequence may be 100% complementary to a target nucleic acid sequence wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 2-16 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 4-16 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 6-12 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 6-10 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 7-9 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide may consist of 10-20 linked nucleotides.

In one embodiment the antisense oligonucleotide may consist of 12-18 linked nucleotides.

In one embodiment the antisense oligonucleotide may consist of 14-16 linked nucleotides.

In one embodiment the antisense oligonucleotide may consist of 16 linked nucleotides.

In one embodiment the antisense oligonucleotide may consist of 15 linked nucleotides.

In one embodiment the antisense oligonucleotide may consist of 14 linked nucleotides.

In one embodiment the antisense oligonucleotide may consist of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 2, 3, 4, or 5.

In one embodiment the antisense oligonucleotide may comprise a contiguous nucleotide sequence of 5'-CTCCCAG-3'.

In one embodiment wherein the antisense oligonucleotide may comprise a contiguous nucleotide sequence of 5'-TCCCA-3'.

In one embodiment the antisense oligonucleotide may comprise a contiguous nucleotide sequence of 5'-GAC-3.

In one embodiment wherein the antisense oligonucleotide may consist of any one of SEQ ID Nos: 2, 3, 4, or 5.

In one embodiment the antisense oligonucleotide may consist of SEQ ID No: 2.

In one embodiment the antisense oligonucleotide may be modified.

In one embodiment the antisense oligonucleotide may be chemically modified.

In one embodiment one or more nucleotide(s) may comprise a modified sugar/s and/or sugar substitutes.

In one embodiment the modified sugar may be 2'-O-methoxyethyl (MOE), 2'-O-(2-N-methylcarbamoylethyl) (MCE), 2'-O-methylation (OMe), or 2'-Fluoro (2F).

In one embodiment the antisense oligonucleotide may comprise a backbone modification, selected from a list comprising phosphorothioate, methyl phosphonate, or methyl phosphorothionate, preferably the antisense oligonucleotide comprises a phosphorothioate backbone.

In one embodiment the antisense oligonucleotide may comprise a wing-gap-wing motif.

In one embodiment the antisense oligonucleotide may comprise:
a gap segment consisting of linked nucleotides;
a 5' wing region consisting of linked nucleotides at a 5' end of the gap segment; and
a 3' wing region consisting of linked nucleotides at a 3' end of the gap segment;
wherein the gap segment is positioned between the 5 ' wing segment and the 3 ' wing segment.

In one embodiment the antisense oligonucleotide may consist of a sequence according to any one of SEQ ID Nos: 2, 3, 4, or 5 and wherein the antisense oligonucleotide comprises:
a gap segment consisting of linked nucleotides;
a 5' wing region consisting of 1-5 linked nucleotides at 5' end of the gap segment; and
a 3' wing region consisting of 1-5 linked nucleotides at 3' end of the gap segment;
wherein the gap segment is positioned between the 5 ' wing segment and the 3 ' wing segment.

In one embodiment the gap segment may comprise a contiguous nucleotide sequence of 5'-CTCCCAG-3'.

In one embodiment the gap segment may comprise a contiguous nucleotide sequence of 5'-TCCCA-3'.

In one embodiment the gap segment may comprise a contiguous nucleotide sequence of 5'-GAC-3'.

In one embodiment the position

6, 7, or 8 of the modified oligonucleotide, as counted from the 5' terminus of the gap, may align with position 8 from the 5' end of the target sequence.

In one embodiment one or more nucleotides of either or both of the wing regions may comprise a modified sugar or sugar substitute.

In one embodiment the modified sugar may be 2'-O-methoxyethyl (MOE), 2'-O-(2-N-methylcarbamoylethyl) (MCE), 2'-O-methylation (OMe), or 2'-Fluoro (2F).

In one embodiment the antisense oligonucleotide may comprise at least one 2'-O-MOE modification.

In one embodiment the nucleotides of the 5' wing region and/or the 3' wing region may comprise at least one 2'-O-MOE modification.

In one embodiment the 5' wing region may consist of 3 nucleotides comprising 2'-O-MOE modifications and the 3' wing region consists of 3 nucleotides comprising 2'-O-MOE modifications.

In one embodiment the antisense oligonucleotide may consist of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 6, 7, 8, or 9.

In one embodiment the antisense oligonucleotide may consist of any one of SEQ ID Nos: 6, 7, 8, or 9.

In one embodiment the antisense oligonucleotide may comprise at least one locked nucleic acid (LNA).

In one embodiment the gap region may comprise at least one LNA.

In one embodiment the LNA may be complementary to position 8 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide consists of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 10, 11, 12, or 13.

In one embodiment the antisense oligonucleotide may consist of any one of SEQ ID Nos: 10, 11, 12, or 13.

In one embodiment the 5' wing region may consist of 3 nucleotides comprising 2'-O-MOE modifications, the 3' wing region may consist of 3 nucleotides comprising 2'-O-MOE modifications, and the gap region may comprise at least one LNA.

In one embodiment the antisense oligonucleotide consists of 10-20 linked nucleotides and may comprise at least 8 contiguous nucleotides of any one of SEQ ID Nos: 6, 7, 8, or 9.

In one embodiment the antisense oligonucleotide may consist of any one of SEQ ID Nos: 6, 7, 8, or 9.

According to a second aspect of the present invention, there is provided a delivery vehicle comprising a copy of the antisense oligonucleotide according to the invention.

According to a third aspect of the present invention, there is provided a host cell comprising the antisense oligonucleotide according to the invention.

According to a fourth aspect of the present invention, there is provided a method of manufacturing an antisense oligonucleotide comprising chemically synthesising an antisense oligonucleotide according to the invention.

In one embodiment the method of manufacturing may comprise an additional step of chemically modifying one or more nucleotides.

According to a fifth aspect of the present invention, there is provided a method of modulating expression of *EFEMP1* gene product in a biological system, the method comprising:
introducing an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention into the biological system.

In one embodiment the expression of *EFEMP1* gene product may be inhibited.

In one embodiment the biological system may be selected from a eukaryotic cell, such as a mammalian cell.

In one embodiment the method may be an *in vitro* or *in vivo* method.

According to a sixth aspect of the present invention, there is provided a pharmaceutical composition comprising an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention.

In one embodiment the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, diluent, enhancer or excipient.

According to a seventh aspect of the present invention, there is provided a method of treating, preventing, ameliorating, or slowing progression of Doyne honeycomb macular dystrophy (DHMD) in a subject comprising administering to the subject an effective amount of the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or a pharmaceutical composition according to the invention.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject may selectively reduce expression of a mutant allele.

In one embodiment the mutant allele may be a c.1033C>T mutation in the *EFEMP1* gene producing a R345W mutant EFEMP1 protein.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD according to the invention wherein administering the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may selectively inhibit expression of R345W mutant EFEMP1 protein expression over wild-type EFEMP1 gene product expression in the subject.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may selectively inhibit expression of R345W mutant EFEMP1 protein by binding to mRNA of the mutant *EFEMP1* allele thereby inhibiting R345W mutant EFEMP1 protein expression.

In one embodiment the expression of *EFEMP1* gene product may be reduced by at least 25-50% in retinal pigment epithelium in a treated subject compared to untreated subjects.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, may comprise contacting a cell with the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention.

In one embodiment the cell may be a retinal pigment epithelium (RPE) cell.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject intravitreally.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject by intravitreal injection.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject by intravitreal injection once every 3 to 12 months.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject by intravitreal injection once every 3 to 6 months.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject at a dosage of the antisense oligonucleotide of 1µg -20mg.

According to an eighth aspect of the present invention, there is provided an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention for use as a medicament.

According to a ninth aspect of the present invention, there is provided an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention for use in treating, preventing, ameliorating, or slowing progression of DHMD in a subject comprising administering to the subject an effective amount of the antisense oligonucleotide.

According to a tenth aspect of the present invention, there is provided an antisense oligonucleotide of the invention or a delivery vehicle according to the invention or pharmaceutical composition of the invention in the manufacture of a medicament for treating, preventing, ameliorating, or slowing progression of DHMD.

According to an eleventh aspect of the present invention, there is provided a kit comprising an antisense oligonucleotide of any one of the invention or a delivery vehicle according to the invention.

The invention is described in the following non-limiting figures and tables.

Figures

Figure 1: Target sequence and ASO design. Upper panel: Top line: The genomic DNA (gDNA) sequence (5'-3') encompassing the mutation of interest (bold underlined, grey fill). Second line: The corresponding messenger RNA (mRNA) target sequence (5'-3') encompassing the mutation of interest (bold underlined, grey fill). Third line: The complementary 18 mer ASO1 (SEQ ID NO. 10) and ASO2 sequence (SEQ ID NO. 11) (5'-TCATCCTCCCAGCATTCA-3') encompassing the mutation of interest (bold underlined, grey fill). Fourth line: The complementary 18 mer ASO3 (SEQ ID NO. 12) and ASO4 sequence

(SEQ ID NO. 13) (5'-ATCCTCCCAGCATTCATT-3') encompassing the mutation of interest (bold underlined, grey fill). The genomic DNA reference sequence for ASO1/2 (SEQ ID NO. 15 (5'-TGAATGCTGGGAGGATGA-3') is located at chr2:55,871,081-55,871,098 and for ASO2/3 (SEQ ID NO. 16 (5'-AATGAATGCTGGGAGGAT-3') at chr:55,871,081-55,871,100 (UCSC Genome Browser on Human (GRCh38/hg38)). The position of the target c.1033C>T, p.(R345W) mutation (bold underlined, grey fill) is chr2:55,871,091. Bottom panel: ASO chemistry. All ASO (5'-3') are 18 mer fully substituted phosphorothioate (PS) gapmers, with a gap of 8 deoxynucleotides flanked at the 5' and 3' end by five nucleotides with 2'O-methoxyethyl (2'O-MOE) ribose sugar modifications (wings). In ASO1 and ASO3, the target nucleotide (c.1033C>T) is a modified linked nucleic acid (LNA). In ASO 2 and 4, the target nucleotide is flanked by a 5' and 3' modified LNA. The sequence and chemistry of the control (CRTL) ASO is shown. * = phosphorothioate (PS) linkages; < > = 2'O-MOE modified ribose sugars; { } = linked nucleic acid (LNA).

Figure 2: *In vitro* screen of ASO1-4 in a HEK293T heterologous expression system: HEK293T cells were transfected with pEFEMP1(WT)-FLAG3X alone, pEFEMP1(R345W)-mScarlet alone, pEFEMP1(WT)-FLAG3X + pEFEMP1(R345W)-mScarlet, and with control (CTRL) ASO or increasing amounts (25 nM, 50 nM, 100 nM, 200 nM) of ASO1, ASO2, ASO3 and ASO4. Reverse transcriptase quantitative PCR (qPCR) using primer pairs specific for WT *EFEMP1* (*EFEMP1* (FLAG)), R345W *EFEMP1* (*EFEMP1* (mScarlet)) or total *EFEMP1* show an ASO1-mediated reduction in all *EFEMP1* levels compared to the CTRL ASO and untreated cells (plasmids only) (pink bars). Reduced levels of all *EFEMP1* with ASO2 (blue bars) are evident only at the highest and lowest concentrations. No reduction of *EFEMP1* levels is evident with ASO3 (yellow to red bars) or with ASO 4 (green bars). Results were normalised to plasmid only = 1. No expression of *EFEMP1* was detected in cells that were not transfected (NT) with the EFEMP1 heterologous expression plasmids.

Figure 3: ASO refinement: Upper box: Summary of ASO1 (18 mer) parameters from which all further ASO were derived, including gDNA reference sequence (5'-3'), mRNA target sequence (5'-3'), complementary 18 mer ASO1 sequence (5'-3') and chemistry. Target mutation: bold underlined (grey fill). Chemistry: * = phosphorothioate (PS) linkages; < > =

2'O-MOE modified ribose sugars; { } = linked nucleic acid (LNA). Middle box: 16 mer ASO1A (SEQ ID NO. 6), 15 mer ASO1B (SEQ ID NO. 7), 15 mer ASO1C (SEQ ID NO. 8) and 14 mer ASO1D (SEQ ID NO. 9). The mRNA target sequence (5'-3') for ASO1 is shown at the top. The complementary ASO1A, 1B, 1C and 1D sequences aligned to the target sequence are shown underneath. Target mutation: bold underlined, grey fill. Lower box: Summary of ASO1A-1D target sequence (5'-3') (left) and ASO1A-1D sequence (5'-3') (right). The ASO1A-1D sequences (5'-3') are bold underlined. The ASO chemistry is shown underneath. All ASO (1A-1D) consist of a fully substituted phosphorothioate (PS) backbone with a gap of deoxynucleotides of variable length (10 nucleotides in ASO1A, 9 deoxynucleotides in ASO1B and 1C, 8 deoxynucleotides in ASO1D) and flanked by three 2'O-MOE ribose sugar modified nucleotides at the 5' and 3' end (where 5=5', i=internal, r=ribose, 3=3'). The target nucleotide (c.1033C>T) in all ASO is a modified LNA (indicated by a +). The chemistry and sequence of the control (CTRL) ASO is shown. * = phosphorothioate (PS) linkages; < > = 2'O-MOE modified ribose sugars.

Figure 4: *In vitro* screen of ASO1A-1D in a HEK293T heterologous expression system. Levels of *EFEMP1* (total), R345W *EFEMP1* (MUT) and WT *EFEMP1* (WT) measured by qPCR following RNA purification and reverse transcription. HEK293T cells were transfected with pEFEMP1(WT)-FLAG3X + pEFEMP1(R345W)-mScarlet (EFEMP1, black and hatched bars), pEFEMP1(R345W)-mScarlet alone (MUT, light grey bars) or pEFEMP1(WT)-FLAG3X alone (WT, dark grey bars), and with control (CTRL) ASO or increasing amounts (25 nM, 50 nM, 100 nM, 200 nM) of ASO1A (blue), ASO1B (green), ASO1C (red) and ASO1D (yellow). All results show the comparison with plasmid transfected cells treated with CTRL ASO or that were not treated with ASO (NT). All ASO, with the exception of ASO1B, show a concentration-dependent decline in all *EFEMP1* levels and the highest level of *EFEMP1* reduction is achieved with 200 nM ASO1A. Whilst ASO1B can effectively reduce *EFEMP1* levels at all concentrations tested, the ASO1B-mediated reduction in *EFEMP1* levels is not concentration-dependent. All ASO (1A-1B) mediated a greater reduction in R345W *EFEMP1* compared to WT *EFEMP1*.

Figure 5: ASO screening in a clinically relevant iPSC-RPE disease model: Immunofluorescent (left) and brightfield (right) images of isogenic control iPSC-RPE (HDR) and patient iPSC-RPE (R345W). CRISPR-Cas9 homology directed repair (HDR) was used to repair the autosomal dominant heterozygous c.1033C>T mutation in patient-derived iPSC

reprogrammed from renal epithelial (RE) cells to create an isogenic control iPSC repaired line. The isogenic control and patient iPSC were differentiated to RPE (protocol based on Michelet *et al*., 2020). Brightfield microscopy at 72 days of iPSC-RPE differentiation show a typical homogeneous honeycomb cobblestone morphology of isogenic control iPSC-RPE (HDR) cells. In contrast, patient iPSC-RPE (R345W) show a heterogeneous population of mixed cells with evidence of epithelial-mesenchymal transition (EMT). Fluorescence microscopy shows efficient delivery of 5' 6-FAM conjugated ASO to the iPSC-RPE. Graph: Patient iPSC-RPE were treated with ASO1A, 1B, 1C, 1D or control (CT) ASO (200 nM) at 72 days differentiation and analysed 48 hours later for *EFEMP1* expression by qPCR following RNA purification and reverse transcription. Results were compared to non-treated (white bar) and control ASO treated (grey bar) iPSC-RPE. N=3 independent experiments, n=2 independent transfections (per experiment).

Figure 6: ASO screening in a clinically relevant iPSC-RPE disease model: Sanger sequencing chromatograms of *EFEMP1* amplicons from untreated patient iPSC-RPE or patient iPSC-RPE treated with control (CTRL) ASO, ASO1A, ASO1B, ASO1C or ASO1D (200 nM) at day 72 of differentiation and analysed 48 hours after treatment. N=3 independent experiments, n=2 independent transfections (per experiment). The chromatograms from 1 experiment (traces from 2 independent transfections) are shown for each condition. The heterozygous C/T peak in the untreated and CTRL treated patient iPSC-RPE is resolved to a homozygous WT C peak in all ASO treated samples (ASO1A-1D).

Figure 7: Quantitation of ASO-mediated allele-specific targeting by next generation sequencing (NGS): Illumina MiSeq next generation sequencing (NGS) (paired end reads, read depth ~150,000) of *EFEMP1* amplicons from untreated patient iPSC-RPE (NT) or patient iPSC-RPE treated with control (CTRL) ASO, ASO1A, ASO1B, ASO1C or ASO1D (200 nM) at day 72 of differentiation and analysed 48 hours after treatment. All results shown for N=3 independent experiments, n=2 independent transfections (per experiment). Illumina MiSeq NGS results for gDNA and cDNA from corresponding patient renal epithelial (RE) cells is also shown. In patient RE cells, there is a near equilibrium 50/50 frequency of the C/T alleles. In untreated (NT) and control (Ctrl) ASO-treated patient iPSC-RPE, slight allele skewing in favour of the WT C allele is evident. In patient iPSC-RPE cells treated with ASO1A, 1B, 1C or 1D, there is preferential ASO-mediated *EFEMP1* c.1033C>T allele-specific targeting, with the mutant allele (T) accounting for ~12,5% and the wild type allele (C) accounting for ~87.5% of total *EFEMP1* levels.

Figure 8: ASO-mediated rescue of EMT: EMT markers *VIM*, *TGFBI*, *ZEB1* and *SNAI2* in patient iPSC-RPE following treatment with control (CTRL) ASO, ASO1A or ASO1B (200 nM) at 72 days differentiation and qPCR analysis 48 hours after treatment. Results were compared to untreated (NT) (black bars) and control (CTRL) ASO treated (grey bars) patient iPSC-RPE. ASO1A treated cells = blue bars. ASO1B treated cells = orange bars. N=3 independent experiments, n=2 independent transfections (per experiment). Rescue of *ZEB1* levels, a master regulator of EMT that was significantly increased in patient iPSC-RPE compared to isogenic control iPSC-RPE, was evident with both ASO1A and ASO1B (results not yet analysed for ASO1C and 1D).

Detailed description

The present disclosure will now be further described. In the following passages, different aspects of the disclosure are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, pathology, oncology, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Green and Sambrook et al., Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012);

Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard

techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code.

As used in the description of the invention and the appended claims, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Also as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

The term “about,” as used herein when referring to a measurable value such as an amount of polypeptide, dose, time, temperature, enzymatic activity or other biological activity and the like, is meant to encompass variations of ± 20%, ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

The term “enhance” or “increase” refers to an increase in the specified parameter of at least about 1.25-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, twelve-fold, or even fifteen-fold.

The term “inhibit” or “reduce” or grammatical variations thereof as used herein refers to a decrease or diminishment in the specified level or activity of at least about 15%, 25%, 35%, 40%, 50%, 60%, 75%, 80%, 90%, 95% or more. In particular embodiments, the inhibition or reduction results in little or essentially no detectible activity (at most, an insignificant amount, e.g., less than about 10% or even 5%).

As used herein, “nucleic acid,” “nucleotide sequence,” and “polynucleotide" are used interchangeably and encompass both RNA and DNA, including cDNA, genomic DNA,

mRNA, synthetic (e.g., chemically synthesized) DNA or RNA and chimeras of RNA and DNA. The term polynucleotide, nucleotide sequence, or nucleic acid refers to a chain of nucleotides without regard to length of the chain. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be a sense strand or an antisense strand. The nucleic acid can be synthesized using oligonucleotide analogs or derivatives (e.g., inosine or phosphorothioate nucleotides). Such oligonucleotides can be used, for example, to prepare nucleic acids that have altered base-pairing abilities or increased resistance to nucleases. The present invention further provides a nucleic acid that is the complement (which can be either a full complement or a partial complement) of a nucleic acid, nucleotide sequence, or polynucleotide of this invention. When dsRNA is produced synthetically, less common bases, such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others can also be used for antisense, dsRNA, and ribozyme pairing. For example, polynucleotides that contain C-5 propyne analogues of uridine and cytidine have been shown to bind RNA with high affinity and to be potent antisense inhibitors of gene expression. Other modifications, such as modification to the phosphodiester backbone, or the 2'-hydroxy in the ribose sugar group of the RNA can also be made.

An “isolated polynucleotide” is a nucleotide sequence (e.g., DNA or RNA) that is not immediately contiguous with nucleotide sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. Thus, in one embodiment, an isolated nucleic acid includes some or all of the 5' non-coding (e.g., promoter) sequences that are immediately contiguous to a coding sequence. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment), independent of other sequences. It also includes a recombinant DNA that is part of a hybrid nucleic acid encoding an additional polypeptide or peptide sequence. An isolated polynucleotide that includes a gene is not a fragment of a chromosome that includes such gene, but rather includes the coding region and regulatory regions associated with the gene, but no additional genes naturally found on the chromosome.

The term “isolated” can refer to a nucleic acid, nucleotide sequence or polypeptide that is substantially free of cellular material, viral material, and/or culture medium (when produced

by recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Moreover, an “isolated fragment” is a fragment of a nucleic acid, nucleotide sequence or polypeptide that is not naturally occurring as a fragment and would not be found in the natural state. “Isolated” does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the polypeptide or nucleic acid in a form in which it can be used for the intended purpose.

An “isolated cell” refers to a cell that is separated from other components with which it is normally associated in its natural state. For example, an isolated cell can be a cell in culture medium and/or a cell in a pharmaceutically acceptable carrier of this invention. Thus, an isolated cell can be delivered to and/or introduced into a subject.

As used herein, the term “gene” refers to a nucleic acid molecule capable of being used to produce mRNA, antisense RNA, miRNA, and the like. Genes may or may not be capable of being used to produce a functional protein. Genes can include both coding and non-coding regions (e.g., introns, regulatory elements, promoters, enhancers, termination sequences and 5’ and 3’ untranslated regions). A gene may be “isolated” by which is meant a nucleic acid that is substantially or essentially free from components normally found in association with the nucleic acid in its natural state. Such components include other cellular material, culture medium from recombinant production, and/or various chemicals used in chemically synthesizing the nucleic acid.

As used herein, the term "allele" is one member of a pair of genes or one member of a series of different forms of a DNA sequences that can exist at a single locus or marker on a specific chromosome. For a diploid organism or cell or for autosomal chromosomes, each allelic pair will normally occupy corresponding positions (loci) on a pair of homologous chromosomes, one inherited from the mother and one inherited from the father. If these alleles are identical, the organism or cell is said to be ’homozygous’ for that allele; if they differ, the organism or cell is said to be ’heterozygous’ for that allele. "Major allele" refers to an allele containing the nucleotide present in a statistically significant proportion of individuals in the human population. "Minor allele" refers to an allele containing the nucleotide present in a relatively small proportion of individuals in the human population. "Wild type allele" refers to the genotype typically not associated with disease or dysfunction of the gene product.

As used herein, the term "mutant allele" refers to one of the pair of genes or DNA sequence existing at a single locus comprising a single point mutation and is the genotype associated with disease or dysfunction of the gene product. For example, a 1033C>T mutation in the *EFEMP1* gene which causes DHMD.

According to a first aspect of the present invention, there is provided an antisense oligonucleotide comprising a sequence complementary to at least part of a target nucleic acid sequence, wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof, wherein the antisense oligonucleotide is 100% complementary to position 8 from the 5' end of SEQ ID NO: 1.

"Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%», and 100% complementary).

The antisense oligonucleotide can hybridize to a target nucleic acid sequence. The antisense oligonucleotide can hybridize to an EFEMP1 gene product (mRNA) comprising a single nucleotide point mutation. A gene product is the biochemical material, either RNA or protein, resulting from expression of a gene. For instance, the antisense oligonucleotide can hybridize to EFEMP1 RNA comprising a single nucleotide point mutation. This single point mutation may result in a R345W mutant EFEMP1 protein. For instance, the antisense oligonucleotide can hybridize to mutant EFEMP1 mRNA which would express a R345W mutant EFEMP1 protein. The hybridization may result in a reduction in R345W mutant EFEMP1 protein. The hybridization thus effectively targets the mutant *EFEMP1* allele comprising a c.1033C>T mutation and modulates its expression.

In one embodiment, the antisense oligonucleotide may selectively reduce expression of a *EFEMP1* gene product (mRNA) comprising a single nucleotide point mutation. Degradation of this specific mRNA selectively reduces expression of protein comprising the mutation.

In one embodiment, the antisense oligonucleotide sequence across its entire length may be at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89% complementary

upstream and/or downstream of position 8 from the 5' end of SEQ ID NO: 1 to a target nucleic acid sequence, wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

In one embodiment, the antisense oligonucleotide sequence across its entire length may be at least 90% complementary upstream and/or downstream of position 8 from the 5' end of SEQ ID NO: 1 to a target nucleic acid sequence wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

In one embodiment the sequence may be 100% complementary to a target nucleic acid sequence wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof. In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 2-16 of SEQ ID NO: 1. In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 4-16 of SEQ ID NO: 1. In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 6-12 of SEQ ID NO: 1. In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 6-10 of SEQ ID NO: 1. In one embodiment the antisense oligonucleotide may comprise a sequence complementary to at least nucleotides at positions 7-9 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide may consist of 10-20 linked nucleotides. As used herein, "linked" or "linkage" means two entities are bound to one another by any physicochemical means. Any linkage known to those of ordinary skill in the art, covalent or non-covalent, is embraced. Natural linkages, which are those ordinarily found in nature connecting the individual units of a nucleic acid, are most common. The individual units of a nucleic acid may be linked, however, by synthetic or modified linkages.

In one embodiment the antisense oligonucleotide may consist of 12-18 linked nucleotides. In one embodiment the antisense oligonucleotide may consist of 14-16 linked nucleotides. In one embodiment the antisense oligonucleotide may consist of 16 linked nucleotides. In one embodiment the antisense oligonucleotide may consist of 15 linked nucleotides. In one embodiment the antisense oligonucleotide may consist of 14 linked nucleotides. In one embodiment the antisense oligonucleotide may consist of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 2, 3, 4, or 5.

Preferably the antisense oligonucleotide is single stranded. The antisense oligonucleotide may be double stranded.

In one embodiment the antisense oligonucleotide may comprise a contiguous nucleotide sequence of 5'-CTCCCAG-3'. In one embodiment wherein the antisense oligonucleotide may comprise a contiguous nucleotide sequence of 5'-TCCCA-3'. In one embodiment the antisense oligonucleotide may comprise a contiguous nucleotide sequence of 5'-GAC-3'.

In one embodiment wherein the antisense oligonucleotide may consist of any one of SEQ ID Nos: 2, 3, 4, or 5. In one embodiment the antisense oligonucleotide may consist of SEQ ID No: 2.

In one embodiment the antisense oligonucleotide may be modified.

In one embodiment the antisense oligonucleotide may be chemically modified. In order to improve the pharmacodynamic, pharmacokinetic, and biodistribution properties of antisense oligonucleotides (ASOs), the antisense oligonucleotides can be designed and engineered to comprise one or more chemical modifications (e.g. a modified inter-nucleoside linker, a modified nucleoside, or a combination thereof). In some embodiments, the antisense oligonucleotide comprises one or more modifications. In certain embodiments, the modification comprises a modified inter-nucleoside linker, a modified nucleoside, or a combination thereof.

The antisense oligonucleotides, as described, can comprise one or more nucleotides comprising a modified sugar moiety, wherein the modified sugar moiety is a modification of the sugar moiety when compared to the ribose sugar moiety found in deoxyribose nucleic acid (DNA) and RNA. Numerous nucleotides with modification of the ribose sugar moiety can be utilized, primarily with the aim of improving certain properties of oligonucleotides, such as affinity and/or nuclease resistance. Such modifications include those where the ribose ring structure is modified. These modifications include replacement with a hexose ring (HNA), a bicyclic ring having a biradicle bridge between the C2 and C4 carbons on the ribose ring (e.g. locked nucleic acids (LNA)), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (e.g. UNA). Other sugar modified nucleosides include, for example, bicyclohexose nucleic acids or tricyclic nucleic acids. Modified nucleosides also include

nucleosides where the sugar moiety is replaced with a non-sugar moiety, for example in the case of peptide nucleic acids (PNA), or morpholino nucleic acids.

Sugar modifications also include modifications made by altering the substituent groups on the ribose ring to groups other than hydrogen, or the 2'-OH group naturally found in DNA and RNA nucleotides. Substituents may, for example be introduced at the 2', 3', 4' or 5' positions. Nucleosides with modified sugar moieties also include 2' modified nucleosides, such as 2' substituted nucleosides. Indeed, much focus has been spent on developing 2' substituted nucleosides, and numerous 2' substituted nucleosides have been found to have beneficial properties when incorporated into oligonucleotides, such as enhanced nucleoside resistance and enhanced affinity. A 2' sugar modified nucleoside is a nucleoside that has a substituent other than H or — OH at the 2' position (2' substituted nucleoside) or comprises a 2' linked biradicle, and includes 2' substituted nucleosides and LNA (2'-4' biradicle bridged) nucleosides. Examples of 2' substituted modified nucleosides are 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2 '-alkoxy -RNA, 2'-O- methoxyethyl-oligos (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, and 2'-F-ANA nucleoside. In some embodiments, the antisense oligonucleotide comprises one or more modified sugars. In some embodiments, the antisense oligonucleotide comprises only modified sugars. In certain embodiments, the antisense oligo comprises greater than 10%, 25%, 50%, 75%, or 90% modified sugars. In some embodiments, the modified sugar is a bicyclic sugar. In some embodiments, the modified sugar comprises a 2'-O-methoxyethyl (MOE) group.
Modifications to the ribose sugar or nucleobase can also be utilized to increase pharmacodynamic, pharmacokinetic, and biodistribution properties. Nucleoside modifications prevent or reduce degradation by cellular nucleases, thus increasing the pharmacokinetics and bioavailability of the antisense oligonucleotide. Generally, a modified nucleoside includes the introduction of one or more modifications of the sugar moiety or the nucleobase moiety.

In one embodiment one or more nucleotide(s) may comprise a modified sugar/s and/or sugar substitutes.

In one embodiment the modified sugar may be 2’-O-methoxyethyl (MOE), 2′-O-(2-N-methylcarbamoylethyl) (MCE), 2'-O-methylation (OMe), or 2’-Fluoro (2F).

In one embodiment, the antisense oligonucleotide comprises a modified backbone. Examples of such backbones are provided by morpholino backbones, carbamate backbones,

siloxane backbones, sulfide, sulfoxide and sulfone backbones, formacetyl and thioformacetyl backbones, methyleneformacetyl backbones, riboacetyl backbones, alkene containing backbones, sulfamate, sulfonate and sulfonamide backbones, methyleneimino and methylenehydrazino backbones, and amide backbones. In one embodiment, the antisense oligonucleotide may comprise a backbone modification, selected from a list comprising phosphorothioate, methyl phosphonate, or methyl phosphorothionate, preferably the antisense oligonucleotide comprises a phosphorothioate backbone.

In one embodiment the antisense oligonucleotide may comprise a phosphorothioate backbone. The antisense oligonucleotide may comprise a single-stranded phosphorothioate (PS) backbone. Modification of the inter-nucleoside linker (i.e. backbone) can be utilized to increase pharmacodynamic, pharmacokinetic, and biodistribution properties. For example, inter-nucleoside linker modifications prevent or reduce degradation by cellular nucleases, thus increasing the pharmacokinetics and bioavailability of the antisense oligonucleotide. Generally, a modified inter-nucleoside linker includes any linker other than phosphodiester (PO) liners, that covalently couples two nucleosides together. In some embodiments, the modified inter-nucleoside linker increases the nuclease resistance of the antisense oligonucleotide compared to a phosphodiester linker. For naturally occurring antisense oligonucleotides, the inter-nucleoside linker includes phosphate groups creating a phosphodiester bond between adjacent nucleosides. Modified inter-nucleoside linkers are particularly useful in stabilizing antisense oligonucleotides for in vivo use and may serve to protect against nuclease cleavage. The phosphorothioate backbone is important for RNAse activity.

In some embodiments, the antisense oligonucleotide comprises one or more inter-nucleoside linkers modified from the natural phosphodiester to a linker that is for example more resistant to nuclease attack. In some embodiments all of the inter-nucleoside linkers of the antisense oligonucleotide, or contiguous nucleotide sequence thereof, are modified. In some embodiments all of the inter-nucleoside linkers of the antisense oligonucleotide, or contiguous nucleotide sequence thereof, are nuclease resistant inter-nucleoside linkers. In some embodiments the inter-nucleoside linkage comprises sulphur (S), such as a phosphorothioate inter-nucleoside linkage.

Phosphorothioate inter-nucleoside linkers are particularly useful due to nuclease resistance and improved pharmacokinetics. In some embodiments, one or more of the inter-nucleoside

linkers of the antisense oligonucleotide, or contiguous nucleotide sequence thereof, comprise a phosphorothioate inter-nucleoside linker. In some embodiments, all of the inter-nucleoside linkers of the antisense oligonucleotide, or contiguous nucleotide sequence thereof, comprise a phosphorothioate inter-nucleoside linker.

In some embodiments, the antisense oligonucleotide comprises both inter-nucleoside linker modifications and nucleoside modifications.

In one embodiment the antisense oligonucleotide may comprise a wing-gap-wing motif. This motif is also known as a “Gapmer”. Gapmers are antisense oligonucleotide structures with RNA-like segments on both sides of a DNA sequence. Gapmers are designed to be complementary to a target RNA and silence the gene by hybridizing to the target sequence and inducing RNase H cleavage. The internal region may be referred to as the "gap" and the external regions may be referred to as the "wings." In one embodiment, the gap may comprise linked DNA nucleotides and the wings may comprise linked RNA nucleotides.

In one embodiment the antisense oligonucleotide may comprise a gap segment consisting of linked nucleotides, a 5' wing region consisting of linked nucleotides at a 5’ end of the gap segment, and a 3’ wing region consisting of linked nucleotides at a 3’ end of the gap segment, wherein the gap segment is positioned between the 5 ' wing segment and the 3 ' wing segment.

In one embodiment the antisense oligonucleotide may consist of a sequence according to any one of SEQ ID Nos: 2, 3, 4, or 5 and wherein the antisense oligonucleotide comprises a gap segment consisting of linked nucleotides, a 5' wing region consisting of 1-5 linked nucleotides at 5’ end of the gap segment, and a 3’ wing region consisting of 1-5 linked nucleotides at 3’ end of the gap segment, wherein the gap segment is positioned between the 5 ' wing segment and the 3 ' wing segment.

In one embodiment the gap segment may comprise a contiguous nucleotide sequence of 5’-CTCCCAG-3’. In one embodiment the gap segment may comprise a contiguous nucleotide sequence of 5’-TCCCA-3’. In one embodiment the gap segment may comprise a contiguous nucleotide sequence of 5’-GAC-3’. In one embodiment the position 6, 7, or 8 of the modified oligonucleotide, as counted from the 5’ terminus of the gap, may align with position 8 from the 5’ end of the target sequence.

In one embodiment one or more nucleotides of either or both of the wing regions may comprise a modified sugar or sugar substitute. In one embodiment the modified sugar may be 2'-O-methoxyethyl (MOE), 2'-O-(2-N-methylcarbamoylethyl) (MCE), 2'-O-methylation (OMe), or 2'-Fluoro (2F). In one embodiment the antisense oligonucleotide may comprise at least one 2'-O-MOE modification. In one embodiment the nucleotides of the 5' wing region and/or the 3' wing region may comprise at least one 2'-O-MOE modification. In one embodiment the 5' wing region may consist of 3 nucleotides comprising 2'-O-MOE modifications and the 3' wing region consists of 3 nucleotides comprising 2'-O-MOE modifications. In one embodiment the antisense oligonucleotide may consist of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 6, 7, 8, or 9. In one embodiment the antisense oligonucleotide may consist of any one of SEQ ID Nos: 6, 7, 8, or 9.

In one embodiment the antisense oligonucleotide may comprise at least one locked nucleic acid (LNA). As used herein, "locked nucleic acid " or "LNA" means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH 2 -0-2'bridge. Locked nucleic acids are described eg. In J. Wengel, Acc. Chem. Res., 120, 5458-5463 (1999) or J. Wengel et al., nucleosides & nucleotides, 18(6&7), S. 1365-1370. In one embodiment the gap region may comprise at least one LNA. In one embodiment the LNA may be complementary to position 8 of SEQ ID NO: 1.

In one embodiment the antisense oligonucleotide consists of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 10, 11, 12, or 13. In one embodiment the antisense oligonucleotide may consist of any one of SEQ ID Nos: 10, 11, 12, or 13.

In one embodiment the 5' wing region may consist of 3 nucleotides comprising 2'-O-MOE modifications, the 3' wing region may consist of 3 nucleotides comprising 2'-O-MOE modifications, and the gap region may comprise at least one LNA. In one embodiment the antisense oligonucleotide consists of 10-20 linked nucleotides and may comprise at least 8 contiguous nucleotides of any one of SEQ ID Nos: 6, 7, 8, or 9. In one embodiment the antisense oligonucleotide may consist of any one of SEQ ID Nos: 6, 7, 8, or 9.

Vector Delivery Vehicle

According to a second aspect of the present invention, there is provided a delivery vehicle comprising a copy of the antisense oligonucleotide according to the invention. The selection of the delivery vehicle may be readily selected by one of skill in the art. The selection of the delivery vehicle is not considered to be a limitation of this invention. For example, suitable delivery vehicles may be a vector, a liposome, a nanoparticle, or a micelle.

Host cell

According to a third aspect of the present invention, there is provided a host cell comprising the antisense oligonucleotide according to the invention. Also provided is a host cell comprising the delivery vehicle as described above. The host cell may be mammalian, viral, bacterial, a plant or yeast cell.

Method of manufacture

According to a fourth aspect of the present invention, there is provided a method of manufacturing an antisense oligonucleotide according to the invention.

In one embodiment the method of manufacturing may comprise chemically synthesising an antisense oligonucleotide according to the invention.

In one embodiment the method of manufacturing may comprise an additional step of chemically modifying one or more nucleotides.

Method of modulating R345W expression

According to a fifth aspect of the present invention, there is provided a method of modulating expression of *EFEMP1* gene product in a biological system, the method comprising:

introducing an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention into the biological system.

The antisense oligonucleotide provided herein is useful for targeting nucleic acid expressed from the mutant *EFEMP1* allele. In order to achieve effective targeting of nucleic acid produced from the mutant *EFEMP1* allele (e.g. messenger RNA), the antisense oligonucleotides disclosed herein comprise a sequence complementary to a target nucleic acid sequence wherein the target sequence comprises SEQ ID NO:1 or a portion thereof.

Wherein the complementary sequence of the antisense oligonucleotide binds and/or hybridizes to a sequence of the mRNA expressed from the mutant *EFEMP1* allele comprising SEQ ID NO:1 or a portion thereof. For example, mRNA transcripts.

In one embodiment the expression of *EFEMP1* gene product may be inhibited. The term inhibited is used to indicate a decrease or downregulation of expression or activity. As will be appreciated by the skilled person, the phrase "inhibit expression" does not necessarily require that the expression of the gene be entirely silenced. In embodiments, the method may result in substantially complete inhibition of expression of the gene or RNA (i.e. 100% inhibition or near 100% of gene expression). However, in alternative embodiments, the method of the present invention may result in partial, e.g. a slight or moderate reduction in the expression of the target gene or RNA. For example, the method can result in expression of the gene or RNA being inhibited /downregulated by at least 10%, 20%, 30%, 40% or 50% compared to normal or wildtype expression.

In one embodiment the method may be an *in vitro* or *in vivo* method. In one embodiment the method may be performed *in vitro*. For example, in a cell, tissue, blood sample or other sample from a human, plant or animal subject. In such embodiments, inhibition of gene expression may be required for research purposes.

In one embodiment the method may be performed *in vivo*. For example, wherein the subject may be a human or animal subject. In such embodiments, inhibition of gene expression may result in an altered phenotype in said human or animal subject. For example, inhibition of gene expression, where the gene is disease linked, may result in treatment of that disease.

In one embodiment the biological system may be selected from a eukaryotic cell, such as a mammalian cell. As will be appreciated by the skilled person, the biological system may be a cell or plurality of cells, for example a eukaryotic cell/cells, a sample from a subject. The biological system may comprise a retina of a subject. The biological system may comprise retinal pigment epithelium. In certain embodiments, the subject may be a human or animal subject, for example a subject in which inhibition of gene expression is required. In embodiments, the subject may comprise a synthetic biological system, created from component parts *in vitro* or created *in silico*, for example.

Pharmaceutical composition

According to a sixth aspect of the present invention, there is provided a pharmaceutical composition comprising an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention.

In one embodiment the pharmaceutical composition may further comprise a pharmaceutically acceptable carrier, diluent, enhancer or excipient.

The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions are, for example, in tablet or powder form. The term "carrier" refers to a diluent, adjuvant or excipient, with which a drug antisense oligonucleotide according to the invention is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, when administered to an animal, the antisense oligonucleotide of the present invention or compositions and pharmaceutically acceptable carriers are sterile. Water is a preferred carrier when the drug antisense oligonucleotide according to the invention is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The pharmaceutical composition of the invention can be in the form of a liquid, e.g., a solution, emulsion or suspension. The liquid can be useful for delivery by injection (e.g. intravitreal), infusion (e.g., IV infusion) or subcutaneously.

When intended for oral administration, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, corn starch and the like; lubricants such as magnesium stearate; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the composition is in the form of a capsule (e. g. a gelatin capsule), it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

The composition can be in the form of a liquid, e. g. an elixir, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection. When intended for oral administration, a composition can comprise one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.
Compositions can take the form of one or more dosage units.

The pharmaceutical composition of the present invention may be administered orally, topically, by inhalation, insufflation or parenterally. Preferably the pharmaceutical composition comprising the antisense oligonucleotide may be administered intravitreally. The pharmaceutical composition comprising the antisense oligonucleotide may be administered by intravitreal injection. The compositions and formulations of said antisense oligonucleotides may be administered topically to the eye. In an embodiment they may be formulated for topical administration to the corneal surface of the eye. Application to the corneal surface may, for example be in the form of eyedrops, a gel, lotion, cream or ocular inserts. In one embodiment, the pharmaceutical composition may be administered topically. In a further embodiment, the pharmaceutical composition may be administered by a contact lens impregnated with the pharmaceutical composition. In one embodiment, the contact lens may be a slow release contact lens. Other administration forms to the eye may include injection into the eye.

The amount of the therapeutic that is effective/active in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition and the animal to be treated and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account.

Toxicity and therapeutic efficacy of the compounds, therapies, combinations and compositions of the invention, administered alone or in combination, can be determined by any number of systems or means. For example, the toxicity and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index (LD50/ ED50). The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds or therapies, alone or in combination, lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration.

The pharmaceutical composition may be specifically formulated for delivery of DNA or RNA molecules using non-viral vectors such as exosomes, nanoparticles or liposomes or viral vectors for example retroviral vectors, adenoviral vectors or herpes simplex viral vectors or lipid conjugated. Other potential delivery methods are listed elsewhere herein.

Methods of delivery of the antisense oligonucleotide, pharmaceutical composition, or vehicle include injection of naked antisense oligonucleotide, physical delivery such as electroporation, gene gun, sonoporation, magnetofection, hydrodynamic delivery, and chemical methods to enhance delivery such as inorganic nanoparticles and cell-penetrating peptides. Preferably the antisense oligonucleotide may be administered as a bare molecule in a carrier. The carrier may be a liquid. The antisense oligonucleotide may be conjugated to

another moiety to aid its delivery. The moiety may be selected from a small molecule, for example a chemical, nanoparticle, small molecule, liposome or extracellular vesicle.

The antisense oligonucleotide of the invention may be complexed with membrane disruptive agents and/or a cationic lipid or helper lipid molecule.

The pharmaceutical composition may further comprise a further DHMD therapy.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) a disease state. The pharmaceutically effective dose generally depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize.

Generally, an amount of 1µg -30mg may be administered. An amount of 1µg -30mg may be administered by intravitreal injection. The pharmaceutical composition may be administered once every 3 to 12 months. The pharmaceutical composition may be administered once every 3 to 6 months.

Methods of treatment

According to a seventh aspect of the present invention, there is provided a method of treating, preventing, ameliorating, or slowing progression of Doyne honeycomb macular dystrophy (DHMD) in a subject comprising administering to the subject an effective amount of the antisense oligonucleotide according to the invention or a delivery vector according to the invention or a pharmaceutical composition according to the invention.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject may selectively reduce expression of a mutant allele. In one embodiment the mutant allele may be a c.1033C>T mutation in the *EFEMP1* gene producing a R345W mutant EFEMP1 protein.

Advantageously, administering the antisense oligonucleotide may improve, preserve, or prevent worsening of visual function, visual field, retinal function, retinal pigment epithelium,

function, electroretinogram (ERG) response, or visual acuity compared to an untreated subject. The inventors have demonstrated that antisense oligonucleotide reduced c.1033C>T, p.R345W mutant allele expression in a heterologous HEK in vitro model and iPSC-retinal pigment epithelial cells derived from a molecularly confirmed c.1033C>T, p.R345W Doyne Honeycomb Macular Dystrophy patient. Administering the antisense oligonucleotide may improve retinal structure and/or function and this can be measured by techniques known in the art such as ERG, OCT, microperimetry, FST, adaptive optics, pupillometry.

Administering the antisense oligonucleotide may inhibit, prevent, or delay progression of retinal pigment epithelial cell loss or deterioration of the retina outer nuclear layer in a subject compared to an untreated subject.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD according to the invention wherein administering the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may selectively inhibit expression of R345W mutant EFEMP1 protein expression over wild-type EFEMP1 gene product expression in the subject.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may selectively inhibit expression of R345W mutant EFEMP1 protein by binding to mRNA of the *EFEMP1* gene thereby inhibiting R345W mutant EFEMP1 protein expression.

In one embodiment, the expression of *EFEMP1* gene product may be reduced by at least 25-50% in retinal pigment epithelium in a treated subject compared to untreated subjects.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, may comprise contacting a cell with the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention.

In one embodiment the cell may be a retinal pigment epithelium (RPE) cell.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject intravitreally.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject by intravitreal injection.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject by intravitreal injection once every 3 to 12 months. The antisense oligonucleotide or pharmaceutical composition comprising the antisense oligonucleotide may be administered by intravitreal injection at regular intervals.

In one embodiment the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject by intravitreal injection once every 3 to 6 months. The antisense oligonucleotide or pharmaceutical composition comprising the antisense oligonucleotide may be administered by intravitreal injection at regular intervals.

The antisense oligonucleotide or pharmaceutical composition comprising the antisense oligonucleotide may be administered at a dosage determined by the skilled person. Determination of the dosage may be based on techniques well known in the art and may vary dependent on the subject, desired effect, frequency of administration and safety. In one embodiment the method of treating, preventing, ameliorating, or slowing progression of

DHMD in a subject according to the invention, wherein the antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention may be administered to the subject at a dosage of the antisense oligonucleotide of 1µg - 30mg. The antisense oligonucleotide or pharmaceutical composition comprising the antisense oligonucleotide may be administered initially in a loading dose and subsequently as a maintenance dose. The loading dose may comprise a greater concentration of the antisense oligonucleotide than a subsequent maintenance dose of the antisense oligonucleotide.

Medical Uses and methods

According to an eighth aspect of the present invention, there is provided an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention for use as a medicament.

According to a ninth aspect of the present invention, there is provided an antisense oligonucleotide according to the invention or a delivery vehicle according to the invention or the pharmaceutical composition according to the invention for use in treating, preventing, ameliorating, or slowing progression of DHMD in a subject comprising administering to the subject an effective amount of the antisense oligonucleotide.

In other embodiments the antisense oligonucleotide may be administered together with a second therapy. The present invention therefore also provides a combination therapy comprising the administration of the antisense oligonucleotide and another active ingredient such as, for example, another DHMD therapy. Other therapies may include cell stress modifiers (inhibitors and activators) or neuroprotective strategies. The therapies may be administered at the same time (for example in the same medicament) or at a different time (for example in a different medicament). The therapies may be provided as different medicaments administered sequentially. The antisense oligonucleotide may be administered prior to or after the other DHMD therapeutic.

According to a tenth aspect of the present invention, there is provided an antisense oligonucleotide of the invention or a delivery vehicle according to the invention or pharmaceutical composition of the invention in the manufacture of a medicament for treating, preventing, ameliorating, or slowing progression of DHMD.

Kit

According to an eleventh aspect of the present invention, there is provided a kit comprising an antisense oligonucleotide of any one of the invention or a delivery vehicle according to the invention. In one embodiment the kit comprises instructions for use.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. While the foregoing disclosure provides a general description of the subject matter encompassed within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are provided to further enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

Examples

Target sequence and ASO design (Figure 1)

Two antisense oligonucleotides (ASO) were designed targeting the EGF containing fibulin extracellular matrix protein 1 (EFEMP1, OMIM 601548) gene located at 2p16.1 (genomic coordinates (GRCh38): 2:55,865,967-55,923,782 (NCBI)). ASO target sequences were selected based on *in silico* prediction of target RNA secondary structure and accessibility,

and optimal *in silico* properties of the complementary ASO (%GC, TM, G, maxG, secondary structure formation and stability). The reference gDNA sequence for ASO1 and ASO2 (SEQ ID NO. 15) (5'TGAATGCTGGGAGGATGA3') and for ASO3 and ASO4 (SEQ ID NO. 16) (5'AATGAATGCTGGGAGGAT3') are located at chr2:55,871,081-55,871,098 and chr2:55,871,083-55,871,100 respectively (UCSC Genome Browser on Human (GRCh38/hg38)) and incorporate the c.1033C>T, p.(R345W) EFEMP1 variation at chr2:55,871,091. The ASO sequences are as follows: ASO1 (SEQ ID NO 10) and 2 (SEQ ID NO. 11): (5'TCATCCTCCCAGCATTCA3') ASO 3 (SEQ ID NO. 12) and 4 (SEQ ID NO. 13) (5'ATCCTCCCAGCATTCATT3'): The ASO chemistry for all four 18 mer ASO is a 'gapmer' configuration with a fully substitutes phosphorothioate (PS) backbone, and a gap of 8 deoxynucleotides (dDNA bases) flanked on either side by 5 nucleotides with 2'O-methoxyethyl (2'O-MOE) ribose sugar modifications. In ASO1 and ASO2, the target nucleotide (c.1033C>T) is located at position 6 of the 'gap' whereas in ASO3 and ASO4, it is located at position 4 of the 'gap'. In ASO1 and ASO3, the target nucleotide itself is a linked nucleic acid (LNA). In ASO2 and ASO4, the target nucleotide is flanked on the 5' and 3' side by an LNA.

<u>In vitro screen of ASO1-4 in a HEK293T heterologous expression system</u>

HEK293T cells were transfected with pEFEMP1(WT)-FLAG3X alone, pEFEMP1(R345W)-mScarlet alone, pEFEMP1(WT)-FLAG3X + pEFEMP1(R345W)-mScarlet, and with control (CTRL) ASO or increasing amounts (25 nM, 50 nM, 100 nM, 200 nM) of ASO1, ASO2, ASO3 and ASO4. Reverse transcriptase quantitative PCR (qPCR) of purified RNA was conducted using primer pairs specific for EFEMP1(WT) (common EFEMP1 primer and FLAG-specific primer), EFEMP1(R345W) (common EFEMP1 primer and mScarlet-specific primer) or both (EFEMP1) (EFEMP1-specific primer pair) and the results compared to non-transfected (NT) cells and cells transfected with plasmids only (non-treated) (N=2 independent transfections). Reduced levels of EFEMP1 were detected with all concentrations of ASO1 relative to the CTRL ASO or plasmid only control (no ASO), reduced levels of EFEMP1 were detected only at the lowest and highest concentrations with ASO2, whereas no reduction in EFEMP1 levels was achieved with ASO3 or ASO4 (Figure 2).

<u>ASO refinement (Figure 3)</u>

ASO1 (18 mer) was selected as the lead ASO for further refinement of the ASO design to improve specificity, stability and efficiency. ASO1A is a 16 mer ASO, ASO1B and C are 15 mer ASO and ASO1D is a 14 mer ASO. All ASO (1A-1D) are fully substituted phosphorothioate (PS) gapmers in which the target nucleotide (c.1033C>T) is a modified LNA. In all ASO (1A-1D), the wings flanking the gap are comprised of three 2'O-MOE ribose modified nucleotides (shortened from the previous 5 nucleotide wings in ASO1). The gap in ASO1A is 10 deoxynucleotides in length, 9 deoxynucleotides in ASO1B and 1C, and 8 deoxynucleotides in the shortest 14 mer ASO1D. The position of the target nucleotide LNA within the gap shifts according to the alignment of the gapmer configuration to the target sequence, and is located at position 7, 8, 6 and 6 of the gap respectively for ASO1A, 1B, 1C and 1D.

In vitro screen of ASO1A-1D in a HEK293T heterologous expression system

HEK293T cells were transfected with pEFEMP1(WT)-FLAG3X alone, pEFEMP1(R345W)-mScarlet alone or pEFEMP1(WT)-FLAG3X + pEFEMP1(R345W)-mScarlet, and with control (CTRL) ASO or increasing amounts (25 nM, 50 nM, 100 nM, 200 nM) of ASO1A, ASO1B, ASO1C or ASO1D. Reverse transcriptase quantitative PCR (qPCR) of purified RNA was conducted using primer pairs specific for EFEMP1(WT), EFEMP1(R345W) or both (EFEMP1) and the results compared to cells transfected with plasmid only (NT; non-treated) or treated with control ASO (CTRL ASO). Whilst all ASO were effective at reducing EFEMP1 levels in comparison to untreated cells (NT) and cells treated with control (CTRL) ASO, a concentration-dependent reduction was seen for ASO1A, 1C and 1D, and preferential reduction of EFEMP1(R345W) was evident for all ASO (1A-1D) (Figure 4).

ASO screening in a clinically relevant iPSC-RPE disease model

Renal epithelial (RE) cells were retrieved from a molecularly confirmed c.1033C>T, p.R345W Doyne Honeycomb Macular Dystrophy patient. The RE cells were reprogrammed to induced pluripotent stem cells (iPSC) via ectopic expression of the 'Yamanaka factors' and the pluripotency and trilineage differentiation potential of the iPSC characterised by immunocytochemistry (ICC) of pluripotency markers (TRA-1-60, OCT4, NANOG, SSEA-4) and RT-qPCR of markers for trilineage potential following directed differentiation, respectively. CRISPR-Cas9 homology directed repair (HDR) through nucleofection of patient iPSC with EFEMP1-specific crisprRNA/tracrRNA/ribonucleotide protein (RNP) and ssODN

repair template was employed to correct the c.1033C>T variation in the patient iPSC line, thereby generating an isogenic corrected control. The patient and corrected iPSC were differentiated to retinal pigment epithelial cells (RPE), using a modified protocol based on Michelet et al., 2020. Significant morphological disruption of the iPSC-RPE cells from the patient compared to the isogenic control were evident (data not shown). The isogenic control cells formed a uniform honeycomb monolayer of cuboidal shaped epithelial cells characterised by ZO-1 tight junctions and specific markers of RPE differentiation (e.g. MERTK) (Figure 5). The patient-derived iPSC-RPE formed a heterogeneous population of mixed morphology epithelial and non-epithelial cells indicative of upregulation of epithelial-mesenchymal transition (EMT), a clinically relevant phenotype. Reverse transcriptase qPCR of purified RNA confirmed the significant downregulation of RPE markers (BEST1, MERTK, RPE65 and PMEL) in the patient iPSC-RPE compared to the isogenic control, and the significant upregulation of EMT markers (ZEB1, TGFB1, SNAI2). The transfection of iPSC-RPE with 5' 6-FAM conjugated ASO confirmed successful uptake of ASO into the iPSC-RPE (Figure 5). At day 72 of differentiation, patient iPSC-RPE cells were transfected with control ASO (CT) or ASO1A, 1B, 1C and 1D (200 nM) and the EFEMP1 levels analysed 48 hours later by reverse transcription of purified RNA followed by qPCR and the results compared to non-treated cells and cells treated with control (CTRL) ASO (N=3 independent experiments, n=2 independent treatments for each experiment) (Figure 5 lower figure). Significant reduction in *EFEMP1* levels was achieved with ASO1A, 1B, 1C and 1D assessed by RT-qPCR. Sanger sequencing of *EFEMP1* spanning the c.1033C>T variation confirmed the preferential reduction of the mutant allele compared to the wild type (WT) allele (Figure 6). Notably, a heterozygous C/T peak was detected in the non-treated and CTRL ASO treated samples (expected as the patient harbours an autosomal dominant heterozygous c.1033C>T variation). Treatment of patient iPSC-RPE with ASO1A-1D completely resolved the 'T' peak to a homozygous 'C' peak indicating significant preferential targeting and knockdown of the expression of the mutant allele by Sanger sequencing.

<u>EFEMP1 c.1033C>T, p.R345W allele-specificity confirmed by quantitative next generation sequencing (NGS)</u>

The patient iPSC-RPE were treated with ASO1A, 1B, 1C, 1D or control (CTRL) ASO (200 nM) at day 72 of differentiation and analysed 48 h later. RNA was purified from non-treated (NT) and treated cells and reverse transcribed to cDNA. The target (spanning *EFEMP1* c.1033C>T) was amplified and sequenced (paired-end reads) by Illumina MiSeq next

generation sequencing (NGS) (read depth ~150,000). The target locus was also amplified and quantified from patient renal epithelial cells (gDNA and cDNA). The data show a small allele bias (favouring the wild type C allele) in patient iPSC-RPE (non-treated and CTRL ASO treated) (Figure 7). Treatment of patient iPSC-RPE with ASO1A-1D significantly decreased the levels of the mutant allele to ~12.5% of the total levels of EFEMP1 (with the wild type EFEMP1 allele accounting for ~87.5% of total EFEMP1 levels), confirming mutant allele-specific targeting by these ASO. Moreover, ASO1A and 1B (data not collected for ASO1C or 1D) mediated the rescue of ZEB1 (a master regulator of EMT) levels, assessed by RT qPCR 48 hours after treatment (Figure 8).

Sequences

SEQ ID NO.	Description	Sequence
1	Target sequence	UGAAUGCUGGGAGGAUGA
2	ASO 1A	CATCCTCCCAGCATTC
3	ASO 1B	TCATCCTCCCAGCAT
4	ASO 1C	ATCCTCCCAGCATTC
5	ASO 1D	ATCCTCCCAGCATT
6	ASO 1A i2MOE and LNA modifications only	/52MOErC//i2MOErA//i2MOErT/ CCTCCC+AGCA/i2MOErT //i2MOErT//32MOErC/
7	ASO 1B i2MOE and LNA modifications only	/52MOErT//i2MOErC//i2MOErA/ TCCTCCC+AG /i2MOErC//i2MOErA//32MOErT/
8	ASO 1C i2MOE and LNA modifications only	/52MOErA//i2MOErT//i2MOErC/ CTCCC+AGCA /i2MOErT//i2MOErT//32MOErC/
9	ASO 1D i2MOE and LNA modifications only	/52MOErA//i2MOErT//i2MOErC/ CTCCC+AGC /i2MOErA//i2MOErT//32MOErT/
10	ASO 1	/52MOErT//i2MOErC//i2MOErA//i2MOErT//i2MOErC/ CTCCC+AGC /i2MOErA//i2MOErT//i2MOErT//i2MOErC//32MOErA/
11	ASO 2	/52MOErT//i2MOErC//i2MOErA//i2MOErT//i2MOErC/ CTCC+CA+GC /i2MOErA//i2MOErT//i2MOErT//i2MOErC//32MOErA/
12	ASO 3	/52MOErA//i2MOErT//i2MOErC//i2MOErC//i2MOErT/ CCC+AGCAT /i2MOErT//i2MOErC//i2MOErA//i2MOErT//32MOErT/
13	ASO 4	/52MOErA//i2MOErT//i2MOErC//i2MOErC//i2MOErT/ CC+CA+GCAT /i2MOErT//i2MOErC//i2MOErA//i2MOErT//32MOErT/
14	CTRL ASO	/52MOErC//i2MOErA//i2MOErC//i2MOErC//i2MOErC/ CCATTCTT /i2MOErC//i2MOErA//i2MOErG//i2MOErC//i2MOErC/
15	Reference genomic DNA sequence of	TGAATGCTGGGAGGATGA

	ASO 1 and 2	
16	Reference genomic DNA sequence of ASO 3 and 4	AATGAATGCTGGGAGGAT

+ = LNA
2MOEr = 2'O-MOE modification
i=internal
5=5'
3=3'
r=ribose

Claims

1. An antisense oligonucleotide comprising a sequence complementary to at least part of a target nucleic acid sequence, wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof, wherein the antisense oligonucleotide is 100% complementary to position 8 from the 5' end of SEQ ID NO: 1.

2. The antisense oligonucleotide according to claim 1, wherein the antisense oligonucleotide selectively reduces expression of a EFEMP1 gene product comprising a single nucleotide point mutation c.1033C>T p.R345W.

3. The antisense oligonucleotide according to claim 1 or claim 2 wherein the antisense oligonucleotide sequence across its entire length is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89% complementary upstream and/or downstream of position 8 from the 5' end of SEQ ID NO: 1 to a target nucleic acid sequence, wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

4. The antisense oligonucleotide according to claim 1 or claim 2 wherein the antisense oligonucleotide sequence across its entire length is at least 90% complementary upstream and/or downstream of position 8 from the 5' end of SEQ ID NO: 1 to a target nucleic acid sequence wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

5. The antisense oligonucleotide according to claim 1 or claim 2 wherein the sequence is 100% complementary to a target nucleic acid sequence wherein the target sequence comprises SEQ ID NO: 1 or a portion thereof.

6 The antisense oligonucleotide according to any one of claims 1 to 5 wherein the antisense oligonucleotide comprises a sequence complementary to at least nucleotides at positions 2-18 of SEQ ID NO: 1.

7. The antisense oligonucleotide according to any one of claims 1 to 5, wherein the antisense oligonucleotide comprises a sequence complementary to at least nucleotides at positions 4-16 of SEQ ID NO: 1.

8. The antisense oligonucleotide according to any one of claims 1 to 5, wherein the antisense oligonucleotide comprises a sequence complementary to at least nucleotides at positions 6-12 of SEQ ID NO: 1.

9. The antisense oligonucleotide according to claims 1 to 4, wherein the antisense oligonucleotide comprises a sequence complementary to at least nucleotides at positions 6-10 of SEQ ID NO: 1.

10. The antisense oligonucleotide according to claims 1 to 4, wherein the antisense oligonucleotide comprises a sequence complementary to at least nucleotides at positions 7-9 of SEQ ID NO: 1.

11. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide consists of 10-20 linked nucleotides.

12. The antisense oligonucleotide according to any one of claims 1-11, wherein the antisense oligonucleotide consists of 12-18 linked nucleotides.

13. The antisense oligonucleotide according to any one of claims 1-11, wherein the antisense oligonucleotide consists of 14-16 linked nucleotides.

14. The antisense oligonucleotide according to any one of claims 1-11, wherein the antisense oligonucleotide consists of 16 linked nucleotides.

15. The antisense oligonucleotide according to any one of claims 1-11, wherein the antisense oligonucleotide consists of 15 linked nucleotides.

16. The antisense oligonucleotide according to any one of claims 1-11, wherein the antisense oligonucleotide consists of 14 linked nucleotides.

17. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide consists of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 2, 3, 4, or 5.

18. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence of 5'-CTCCCAG-3'.

19. The antisense oligonucleotide according to any one of claims 1 to 18, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence of 5'-TCCCA-3'.

20. The antisense oligonucleotide according to any one of claims 1 to 18, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence of 5'-GAC-3'.

21. The antisense oligonucleotide according to any one of the preceding claims, wherein the antisense oligonucleotide consists of any one of SEQ ID Nos: 2, 3, 4, or 5.

22. The antisense oligonucleotide according to claim 4, wherein the antisense oligonucleotide consists of SEQ ID No: 2.

23. The antisense oligonucleotide according to any previous claim wherein the antisense oligonucleotide is modified.

24. The antisense oligonucleotide according to claim 23, wherein the antisense oligonucleotide is chemically modified.

25. The antisense oligonucleotide according to claim 23 or claim 24, wherein one or more nucleotide(s) comprise a modified sugar/s and/or sugar substitutes.

26. The antisense oligonucleotide according to claim 25, wherein the modified sugar is 2'-O-methoxyethyl (MOE), 2'-O-(2-N-methylcarbamoylethyl) (MCE), 2'-O-methylation (OMe), or 2'-Fluoro (2F).

27. The antisense oligonucleotide according to any previous claim, wherein the antisense oligonucleotide comprises a backbone modification, selected from a list comprising phosphorothioate, methyl phosphonate, or methyl phosphorothionate, preferably the antisense oligonucleotide comprises a phosphorothioate backbone.

28. The antisense oligonucleotide according to any previous claim, wherein the antisense oligonucleotide comprises a wing-gap-wing motif.

29. The antisense oligonucleotide according to claim 28, wherein the antisense oligonucleotide comprises:
a gap segment consisting of linked nucleotides;
a 5' wing region consisting of linked nucleotides at a 5' end of the gap segment; and
a 3' wing region consisting of linked nucleotides at a 3' end of the gap segment;
wherein the gap segment is positioned between the 5 ' wing segment and the 3 ' wing segment.

30. The antisense oligonucleotide according to claim 29, wherein the antisense oligonucleotide consists of a sequence according to any one of SEQ ID Nos: 2, 3, 4, or 5 and wherein the antisense oligonucleotide comprises:
a gap segment consisting of linked nucleotides;
a 5' wing region consisting of 1-5 linked nucleotides at 5' end of the gap segment; and
a 3' wing region consisting of 1-5 linked nucleotides at 3' end of the gap segment;
wherein the gap segment is positioned between the 5 ' wing segment and the 3 ' wing segment.

31. The antisense oligonucleotide according to any one of claims 28 to 30, wherein the gap segment comprises a contiguous nucleotide sequence of 5'-CTCCCAG-3'.

32. The antisense oligonucleotide according to any one of claims 28 to 30, wherein the gap segment comprises a contiguous nucleotide sequence of 5'-TCCCA-3'.

33. The antisense oligonucleotide according to any one of claims 28 to 30, wherein the gap segment comprises a contiguous nucleotide sequence of 5'-GAC-3'.

34. The antisense oligonucleotide according to any one of claims 28 to 30, wherein position
6, 7, or 8 of the modified oligonucleotide, as counted from the 5' terminus of the gap, aligns with position 8 from the 5' end of the target sequence.

35. The antisense oligonucleotide according to any one of claims 30 to 34, wherein one or more nucleotides of either or both of the wing regions comprise a modified sugar or sugar substitute.

36. The antisense oligonucleotide according to claim 35, wherein the modified sugar is 2'-O-methoxyethyl (MOE), 2'-O-(2-N-methylcarbamoylethyl) (MCE), 2'-O-methylation (OMe), or 2'-Fluoro (2F).

37. The antisense oligonucleotide according to claim 36, wherein the antisense oligonucleotide comprises at least one 2'-O-MOE modification.

38. The antisense oligonucleotide according to claim 37, wherein the nucleotides of the 5' wing region and/or the 3' wing region comprise at least one 2'-O-MOE modification.

39. The antisense oligonucleotide according to claim 38, wherein the 5' wing region consists of 3 nucleotides comprising 2'-O-MOE modifications and the 3' wing region consists of 3 nucleotides comprising 2'-O-MOE modifications.

40. The antisense oligonucleotide according to claim 39, wherein the antisense oligonucleotide consists of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 2, 3, 4, or 5, wherein the antisense oligonucleotide comprises at least one 2'-O-MOE modification.

41. The antisense oligonucleotide according to claim 40, wherein the antisense oligonucleotide consists of any one of SEQ ID Nos: 2, 3, 4, or 5, wherein the antisense oligonucleotide comprises at least one 2'-O-MOE modification.

42. The antisense oligonucleotide according to any one of claims 23 to 41, wherein the antisense oligonucleotide comprises at least one locked nucleic acid (LNA).

43. The antisense oligonucleotide according to any one of claims 28 to 42, wherein the gap region comprises at least one LNA.

44. The antisense oligonucleotide according to claim 43, wherein the LNA is complementary to position 8 of SEQ ID NO: 1.

45. The antisense oligonucleotide according to claims 43 or claim 44, wherein the antisense oligonucleotide consists of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 2, 3, 4, or 5, wherein the antisense oligonucleotide comprises at least one 2'-O-MOE modification, and wherein the LNA is complementary to position 8 of SEQ ID NO: 1.

46. The antisense oligonucleotide according to claim 45, wherein the antisense oligonucleotide consists of any one of SEQ ID Nos: 2, 3, 4, or 5, wherein the antisense oligonucleotide comprises at least one 2'-O-MOE modification, and wherein the LNA is complementary to position 8 of SEQ ID NO: 1.

47. The antisense oligonucleotide according to any one of claims 23 to 46, wherein the 5' wing region consists of 3 nucleotides comprising 2'-O-MOE modifications, the 3' wing region consists of 3 nucleotides comprising 2'-O-MOE modifications, and the gap region comprises at least one LNA.

48. The antisense oligonucleotide according to claim 47, wherein the antisense oligonucleotide consists of 10-20 linked nucleotides and comprises at least 8 contiguous nucleotides of any one of SEQ ID Nos: 6, 7, 8, or 9.

49. The antisense oligonucleotide according to claim 48, wherein the antisense oligonucleotide consists of any one of SEQ ID Nos: 6, 7, 8, or 9.

50. A delivery vehicle comprising a copy of the antisense oligonucleotide according to any of claims 1 to 49.

51. A host cell comprising the antisense oligonucleotide according to claims 1 to 49.

52. A method of manufacturing an antisense oligonucleotide comprising chemically synthesising an antisense oligonucleotide according to claims 1 to 49

53. The method of manufacturing according to claim 52, wherein the method of manufacturing comprises an additional step of chemically modifying one or more nucleotides.

54. A method of modulating expression of *EFEMP1* gene product in a biological system, the method comprising:
introducing an antisense oligonucleotide according claims 1 to 49 or a delivery vehicle according to claim 50 into the biological system.

55. The method of modulating expression of *EFEMP1* gene product according to claims 54, wherein expression of *EFEMP1* gene product is inhibited.

56. The method of modulating expression of *EFEMP1* gene product according to claim 54 or claim 55, wherein the biological system is selected from a eukaryotic cell, such as a mammalian cell.

57. The method of modulating expression of *EFEMP1* gene product according to claims 54 to 56, wherein the method is an *in vitro* or *in vivo* method.

58. A pharmaceutical composition comprising an antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50.

59. A pharmaceutical composition according to claim 58, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent, enhancer or excipient.

60. A method of treating, preventing, ameliorating, or slowing progression of Doyne honeycomb macular dystrophy (DHMD) in a subject comprising administering to the subject an effective amount of the antisense oligonucleotide according to 1 to 49 or a delivery vehicle according to claim 50 or a pharmaceutical composition according to claims 58 to 59.

61. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claim 60, wherein the method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject selectively reduces expression of a mutant allele.

62. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claim 60 or claim 61, wherein the mutant allele is a c.1033C>T mutation in the *EFEMP1* gene producing a R345W mutant EFEMP1 protein.

63. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claims 60 to 62, wherein administering the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 selectively inhibits expression of R345W mutant EFEMP1 protein expression over wild-type EFEMP1 protein expression in the subject.

64. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claims 60 to 63, wherein the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 selectively inhibits expression of R345W mutant EFEMP1 protein by binding to mRNA of the mutant *EFEMP1* allele thereby inhibiting R345W mutant EFEMP1 protein expression.

65. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claims 60 to 64, wherein expression of R345W mutant *EFEMP1* protein is reduced by at least 25-50% in retinal pigment epithelium in a treated subject compared to untreated subjects.

66. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claims 60 to 65, comprises contacting a cell with the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59.

67. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claim 66, wherein the cell is a retinal pigment epithelium (RPE) cell.

68. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claims 60 to 67, wherein the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 is administered to the subject intravitreally.

69. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claim 68, wherein the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 is administered to the subject by intravitreal injection.

70. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claim 69, wherein the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 is administered to the subject by intravitreal injection once every 3 to 12 months.

71. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claim 69, wherein the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 is administered to the subject by intravitreal injection once every 3 to 6 months.

72. The method of treating, preventing, ameliorating, or slowing progression of DHMD in a subject according to claims 60 to 71, wherein the antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 is administered to the subject at a dosage of the antisense oligonucleotide of 1µg-20mg.

73. An antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 for use as a medicament.

74. An antisense oligonucleotide according to claims 1 to 49 or a delivery vehicle according to claim 50 or the pharmaceutical composition according to claims 58 to 59 for use in treating, preventing, ameliorating, or slowing progression of DHMD in a subject comprising administering to the subject an effective amount of the antisense oligonucleotide.

75. The use of an antisense oligonucleotide of any one of claims 1 to 49 or a delivery vehicle according to claim 50 or pharmaceutical composition of claims 58 to 59 in the manufacture of a medicament for treating, preventing, ameliorating, or slowing progression of DHMD.

76. A kit comprising an antisense oligonucleotide of any one of claims 1 to 49 or a delivery vehicle according to claim 50 or pharmaceutical composition of claims 58 to 59.

Figure 1

gDNA	5'	A	A	T	G	A	A	T	G	C	T	G	G	G	A	G	G	A	T	G	A	3'
mRNA	5'	A	A	U	G	A	A	U	G	C	U	G	G	G	A	G	G	A	U	G	A	3'
ASO1 and 2	3'			**A**	**C**	**T**	**T**	**A**	**C**	**G**	**A**	**C**	**C**	**C**	**T**	**C**	**C**	**T**	**A**	**C**	**T**	5'
ASO 3 and 4	3'	**T**	**T**	**A**	**C**	**T**	**T**	**A**	**C**	**G**	**A**	**C**	**C**	**C**	**T**	**C**	**C**	**T**	**A**			5'

ASO1: 5'**<U*C*A*U*C*>**C*T*C*C*C*{A}*G*C***<A*U*U*C*A>**3'

ASO2: 5'**<U*C*A*U*C*>**C*T*C*C*{C}*A*{G}*C***<A*U*U*C*A>**3'

ASO3: 5'**<A*U*C*C*U*>**C*C*C*{A}*G*C*A*T***<U*C*A*U*U>**3'

ASO4: 5'**<A*U*C*C*U*>**C*C*{C}*A*{G}*C*A*T***<U*C*A*U*U>**3'

CTRL ASO: 5'**<C*A*C*C*C*>**C*C*A*T*T*C*T*T***<C*A*G*C*C>**3'

* = PS
< > = 2'O-MOE
{ } = LNA

Figure 2

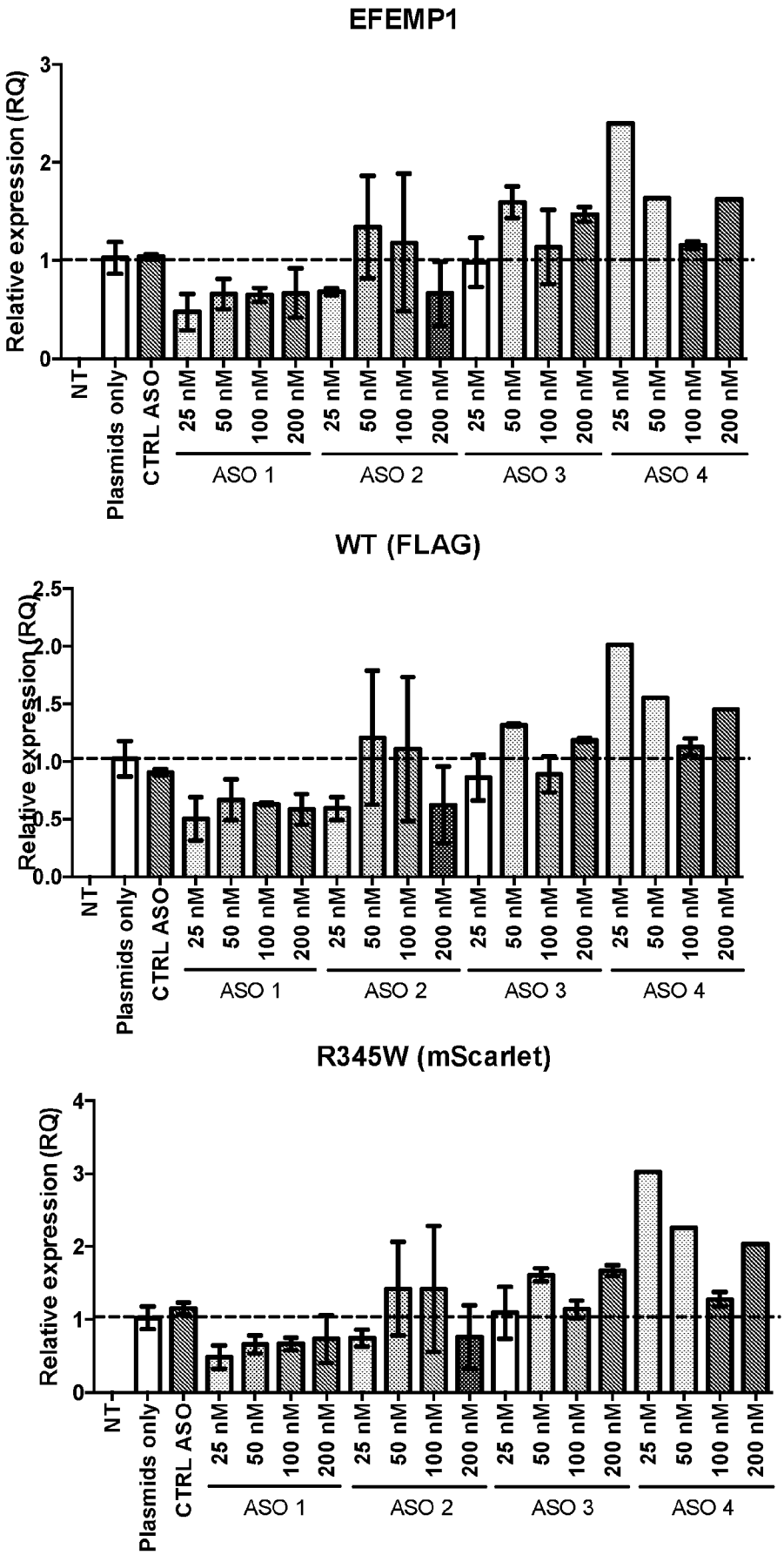

EFEMP1
Relative expression (RQ)
0
1
2
3
NT
Plasmids only
CTRL ASO
25 nM
50 nM
100 nM
200 nM
ASO 1
ASO 2
ASO 3
ASO 4
WT (FLAG)
Relative expression (RQ)
0.0
0.5
1.0
1.5
2.0
2.5
R345W (mScarlet)
Relative expression (RQ)
0
1
2
3
4

Figure 3

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
gDNA	5'	T	G	A	A	T	G	C	T	G	G	G	A	G	G	A	T	G	A	3'
mRNA	5'	U	G	A	A	U	G	C	U	G	G	G	A	G	G	A	U	G	A	3'
ASO1	5'	T	C	A	T	C	C	T	C	C	C	A	G	C	A	T	T	C	A	3'
ASO1 chemistry	5'	<U*	C*	A*	U*	C*>	C	T*	C*	C*	C*	(A*)	G*	G*	<A*	T*	T*	C*	A>	3'

mRNA			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Target sequence		5'	U	G	A	A	U	G	C	U	G	G	G	A	G	G	A	U	G	A	3'
ASO 1A	16m	3'		C	T	T	A	C	G	A	C	C	C	T	C	C	T	A	C		5'
ASO 1B	15m	3'				T	A	C	G	A	C	C	C	T	C	C	T	A	C	T	5'
ASO 1C	15m	3'		C	T	T	A	C	G	A	C	C	C	T	C	C	T	A			5'
ASO 1D	14m	3'			T	T	A	C	G	A	C	C	C	T	C	C	T	A			5'

ASO 1A: Target: 5'-GAAUGCUGGGAGGAUG-3' ASO 1A (16m): 5'-**CATCCTCCCAGCATTC**-3'
ASO 1B: Target: 5'-AUGCUGGGAGGAUGA-3' ASO 1B (15m): 5'-**TCATCCTCCCAGCAT**-3'
ASO 1C: Target: 5'-GAAUGCUGGGAGGAU-3' ASO 1C (15m): 5'-**ATCCTCCCAGCATTC**-3'
ASO 1D: Target: 5'-AAUGCUGGGAGGAU-3' ASO 1D (15m): 5'-**ATCCTCCCAGCATT**-3'
ASO 1A (16m): /52MOErC//i2MOErA//i2MOErT/CCTCCC+AGCA/i2MOErT//i2MOErT//32MOErC/
ASO 1B (15m): /52MOErT//i2MOErC//i2MOErA/TCCTCCC+AG/i2MOErC//i2MOErA//32MOErT/
ASO 1C (15m): /52MOErA//i2MOErT//i2MOErC/CTCCC+AGCA/i2MOErT//i2MOErT//32MOErC/
ASO 1D (14m): /52MOErA//i2MOErT//i2MOErC/CTCCC+AGC/i2MOErA//i2MOErT//32MOErT/

CTRL ASO: <C*A*C*C*C*>C*C*A*T*T*C*T*T<*C*A*G*C*C>

Figure 4

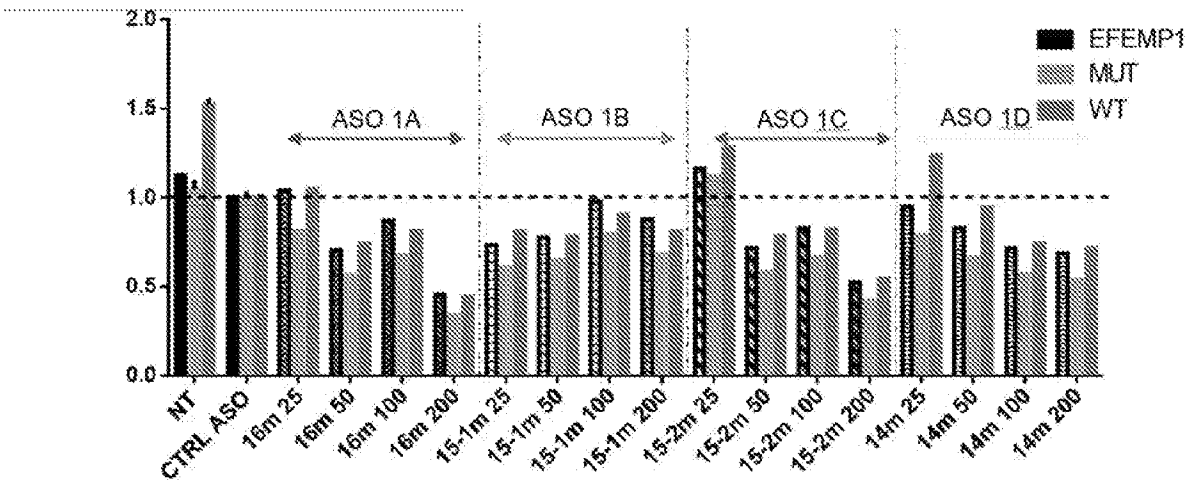
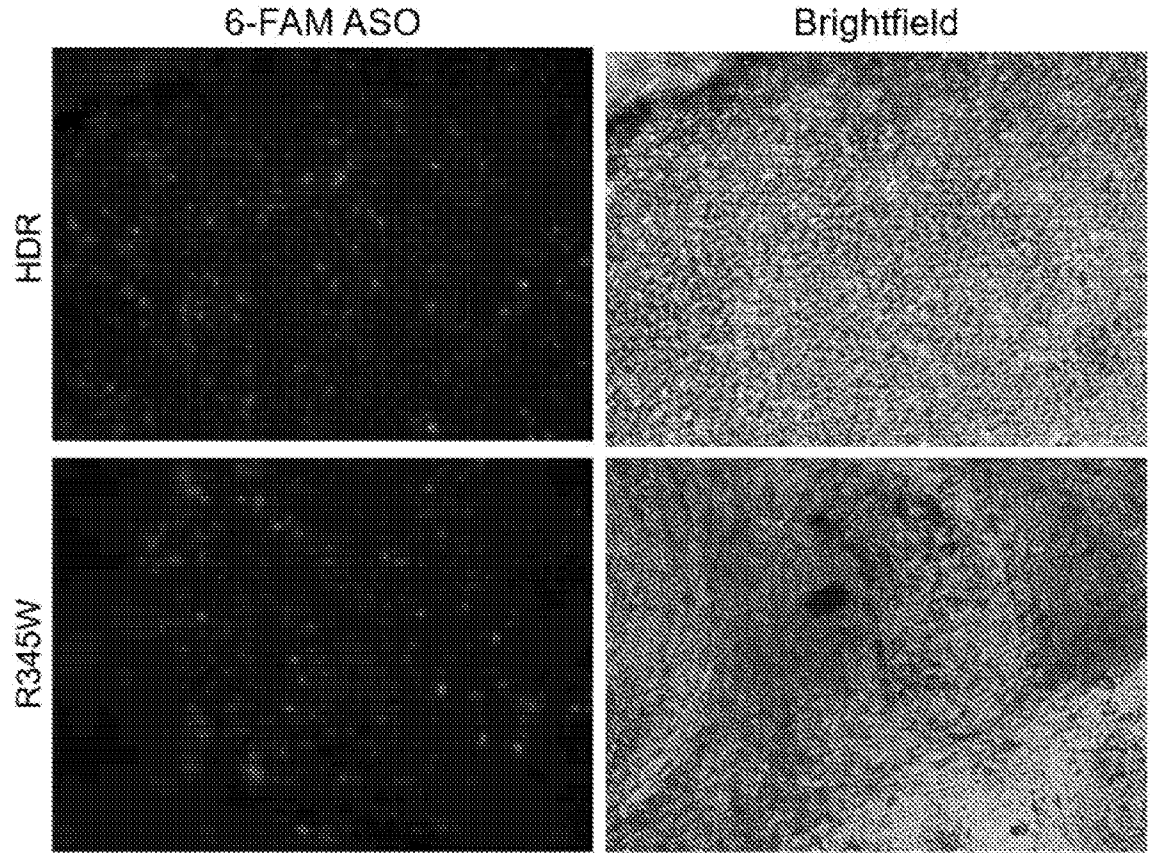


Figure 5


6-FAM ASO
Brightfield
HDR
R345W

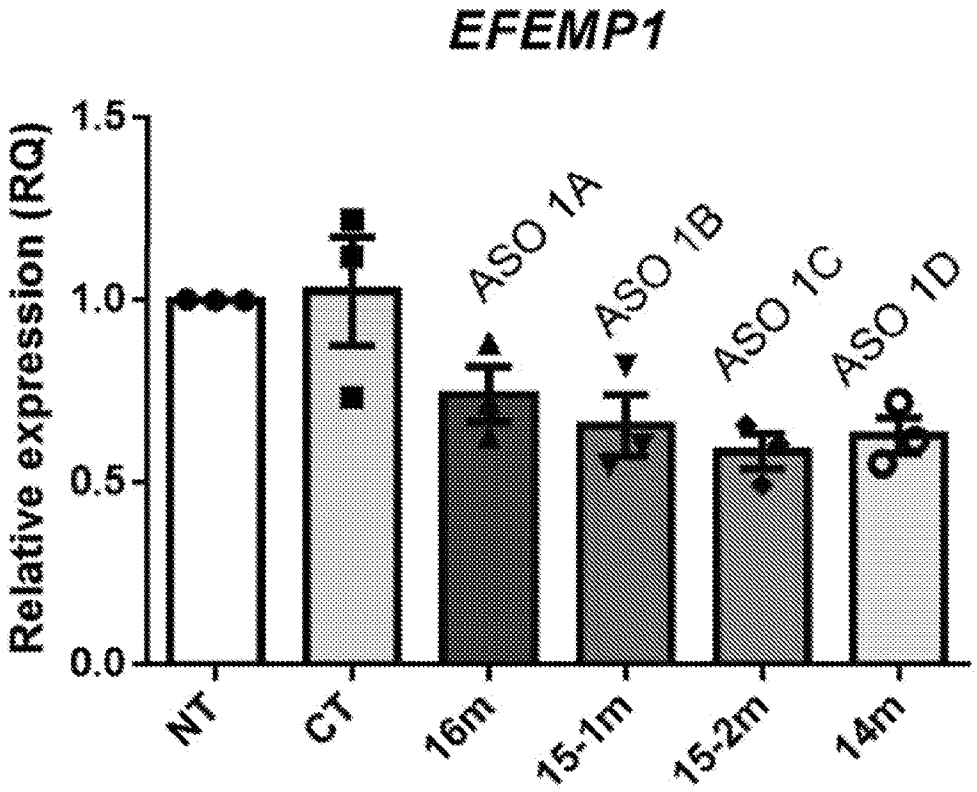
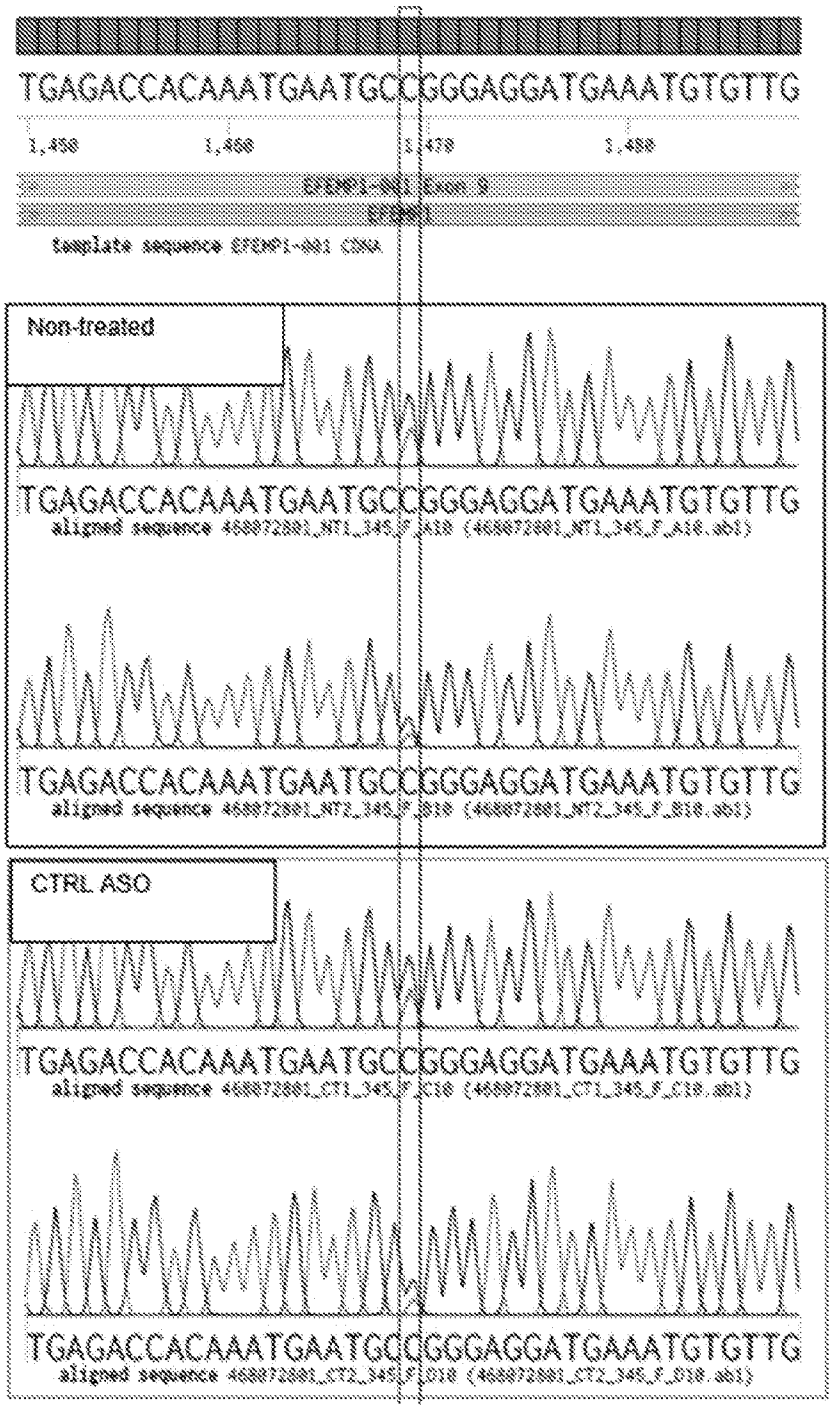
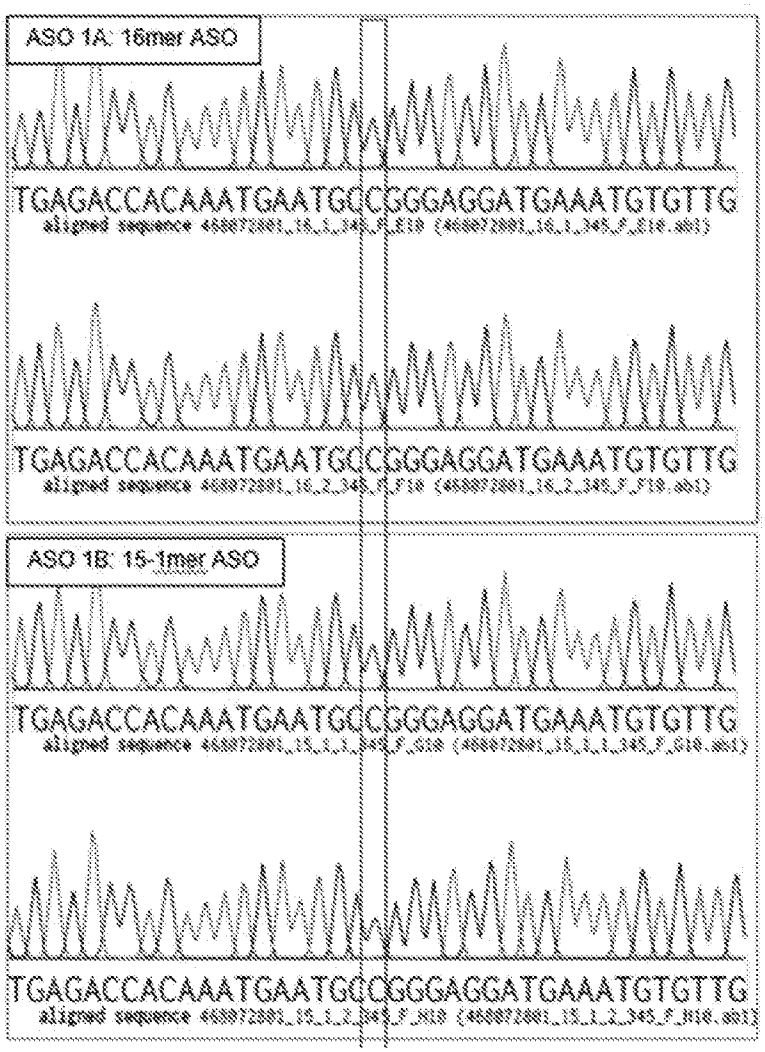

EFEMP1
Relative expression (RQ)
1.5
1.0
0.5
0.0
ASO 1A
ASO 1B
ASO 1C
ASO 1D
NT
CT
16m
15-1m
15-2m
14m

Figure 6


TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
1,458
1,468
1,478
1,488
Non-treated
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
CTRL ASO
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG


ASO 1A: 16mer ASO
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
ASO 1B: 15-1mer ASO
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG

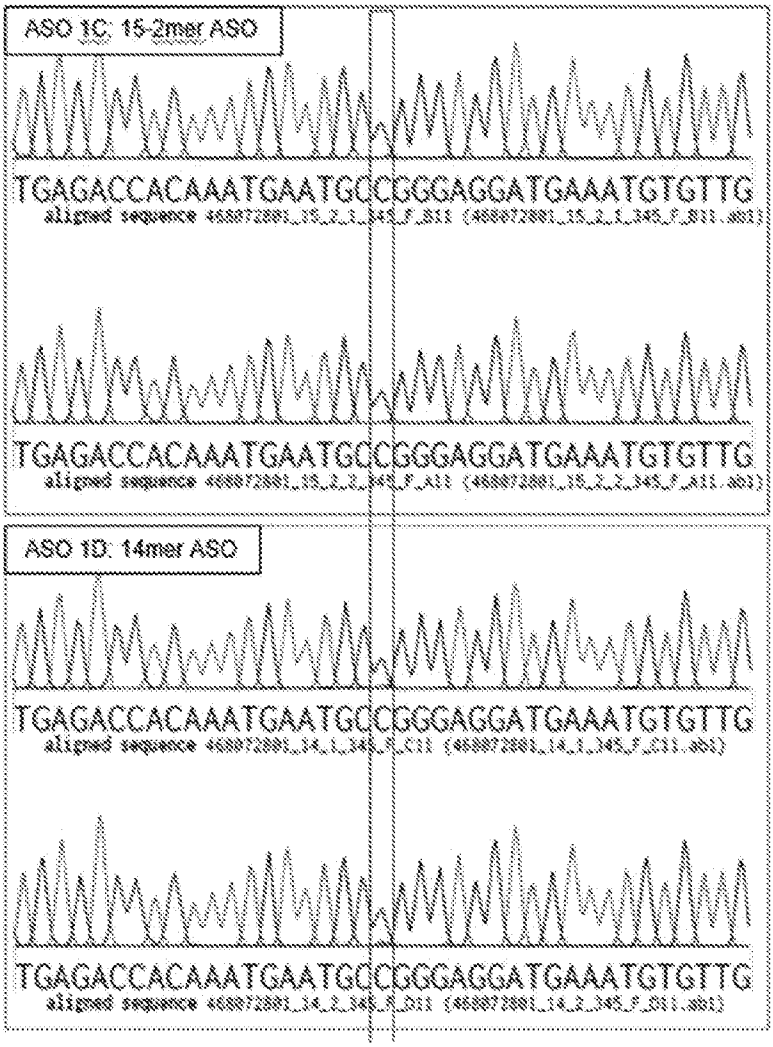

ASO 1C: 15-2mer ASO
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
ASO 1D: 14mer ASO
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG
TGAGACCACAAATGAATGCCGGGAGGATGAAATGTGTTG

Figure 7

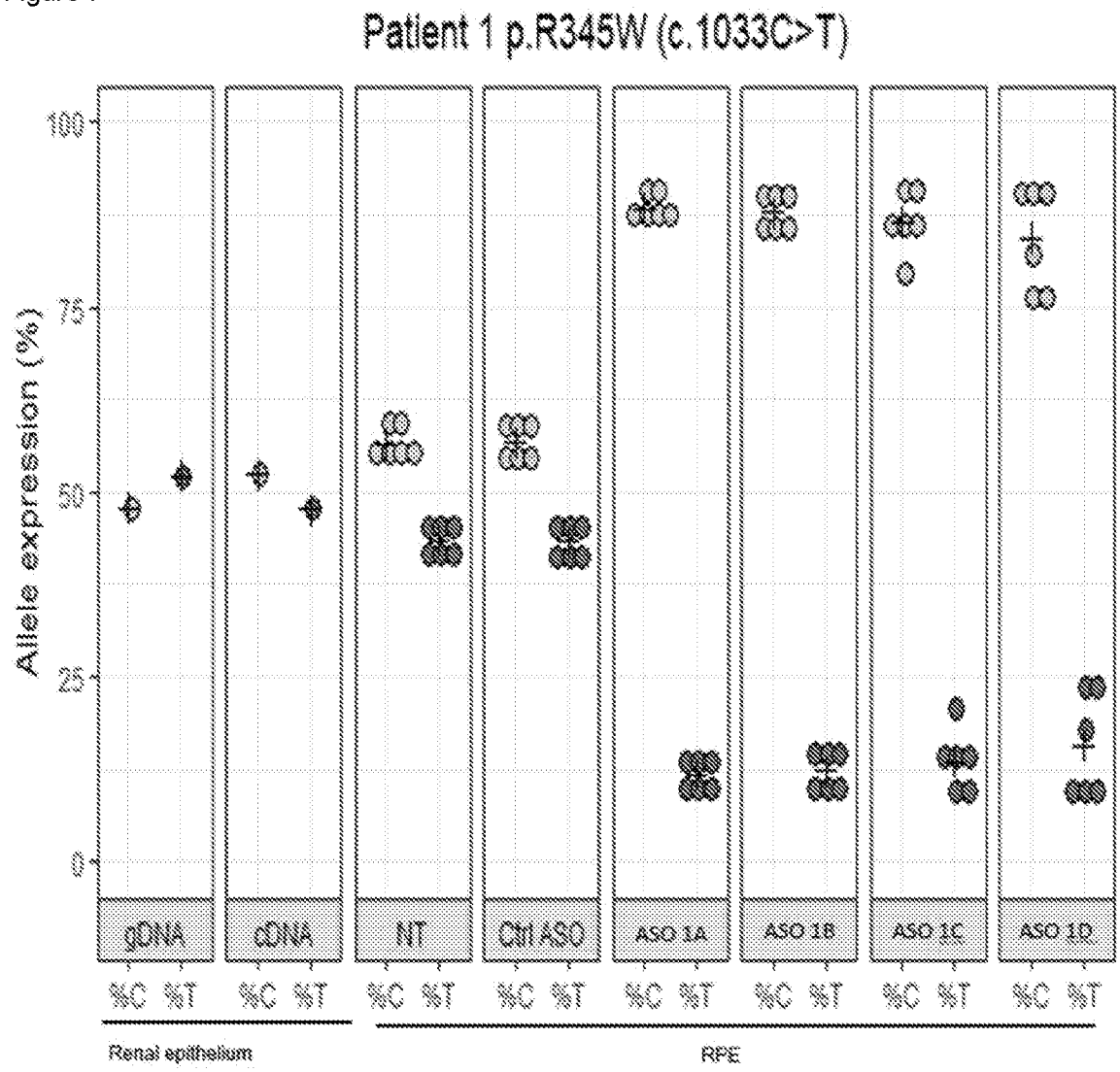
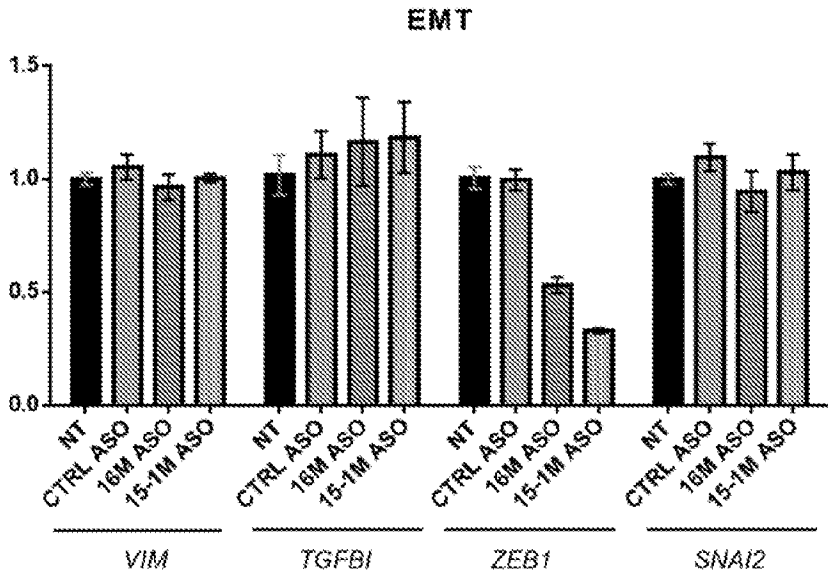


Figure 8



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/052082

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/712 A61K31/7125
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/173637 A1 (RANA THERAPEUTICS INC [US]; GEN HOSPITAL CORP D B A [US]) 21 November 2013 (2013-11-21) sequences 1305699,1305697	1-4, 6-13,15, 17-19,75
X	WO 2019/183630 A2 (UNIV COLUMBIA [US]) 26 September 2019 (2019-09-26)	1-20, 23-40, 42-45, 47,48, 50-76
A	example 7	21,22, 41,46,49
X,P	WO 2023/154964 A1 (ALLOY THERAPEUTICS INC [US]) 17 August 2023 (2023-08-17)	1-20, 23-40, 42-45, 47,48, 50-76
	the whole document	

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 2024	25/10/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Romano, Alper

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2024/052082

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2024/052082

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013173637 A1	21-11-2013	EP 2850184 A1	25-03-2015
		JP 2016522674 A	04-08-2016
		US 2015141320 A1	21-05-2015
		WO 2013173637 A1	21-11-2013
WO 2019183630 A2	26-09-2019	CN 112153990 A	29-12-2020
		EP 3768327 A2	27-01-2021
		EP 4186921 A1	31-05-2023
		IL 277526 A	30-11-2020
		JP 2021519067 A	10-08-2021
		JP 2024038518 A	19-03-2024
		US 2021017509 A1	21-01-2021
		WO 2019183630 A2	26-09-2019
WO 2023154964 A1	17-08-2023	NONE	