sequences and compounds which are capable of inhibiting HTRA1, and their use in treatment of diseases or disorders where HTRA1 is indicated.

SUMMARY

In one aspect, the present disclosure refers to an antisense oligonucleotide of 10 – 30 nucleotides in length, wherein said antisense oligonucleotide targets a HTRA1 nucleic acid, and comprises a contiguous nucleotide region wherein the contiguous nucleotide region is selected from the group selected from: TTCtatctacgcaTTG (SEQ ID NO 67), CTTCttctatctacgcAT (SEQ ID NO 73), and TACTttaatagcTCAA (SEQ ID NO 86); wherein capital letters are LNA nucleotides, and lower case letters are DNA nucleosides, and cytosine residues are optionally 5-methyl cytosine.

The present disclosure relates to oligonucleotides targeting a mammalian HTRA1 nucleic acid, *i.e.* are capable of inhibiting the expression of HTRA1 and to treat or prevent diseases related to the functioning of the HTRA1. The oligonucleotides targeting HTRA1 are antisense oligonucleotides, *i.e.* are complementary to their HTRA1 nucleic acid target.

The oligonucleotide of the disclosure may be in the form of a pharmaceutically acceptable salt, such as a sodium salt or a potassium salt.

Accordingly, the disclosure provides antisense oligonucleotides which comprise a contiguous nucleotide sequence of 10 - 30 nucleotides in length with at least 90% complementarity, such as fully complementary to a mammalian HTRA1 nucleic acid, such as SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3 or SEQ ID NO 4.

In a further example, the disclosure provides pharmaceutical compositions comprising the oligonucleotides of the disclosure and pharmaceutically acceptable diluents, carriers, salts and/or adjuvants.

The disclosure provides LNA antisense oligonucleotides, such as LNA gapmer oligonucleotides, which comprise a contiguous nucleotide sequence of 10 - 30 nucleotides in length with at least 90% complementarity, such as fully complementary to a HTRA1 nucleic acid, such as a sequence selected from the group consisting of SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3 or SEQ ID NO 4.

The disclosure provides for an antisense oligonucleotide comprising a contiguous nucleotide region of at 10 – 30, such as 12 – 22, nucleotides, wherein the contiguous nucleotide region is at least 90% such as 100% complementary to SEQ ID NO 113.

The disclosure provides for an antisense oligonucleotide of 10 – 30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide region of 10 – 30, such as 12 – 22, nucleotides which are at least 90% such as 100% complementarity to SEQ ID NO 113:

5’
GACAGTCAGCATTTGTCTCCTCCTTTAACTGAGTCATCATCTTAGTCCAACTAATGCAGTCG
ATACAATGCGTAGATAGAAGAAGCCCCACGGGAGCCAGGATGGGACTGGTCGTGTTTGTG
CTTTTCTCCAAGTCAGCACCCAAAGGTCAATGCACAGAGACCCCGGGTGGGTGAGCGCTG
GCTTCTCAAACGGCCGAAGTTGCCTCTTTTAGGAATCTCTTTGGAATTGGGAGCACGATGA
CTCTGAGTTTGAGCTATTAAAGTACTTCTTAC 3’.

The reverse complement of SEQ ID NO 113 is SEQ ID NO 119:
GTAAGAAGTACTTTAATAGCTCAAACTCAGAGTCATCGTGCTCCCAATTCCAAAGAGATTCC
TAAAAGAGGCAACTTCGGCCGTTTGAGAAGCCAGCGCTCACCCACCCGGGGTCTCTGTGC
ATTGACCTTTGGGTGCTGACTTGGAGAAAAGCACAAACACGACCAGTCCCATCCTGGCTCC
CGTGGGGCTTCTTCTATCTACGCATTGTATCGACTGCATTAGTTGGACTAAGATGATGACT
CAGTTAAAGGAGGAGACAAATGCTGACTGTC.

The disclosure provides for an antisense oligonucleotide comprising a contiguous nucleotide region of at 10 – 30, such as 12 – 22, nucleotides, wherein the contiguous nucleotide region is at least 90% such as 100% complementary to SEQ ID NO 114.

The disclosure provides for an antisense oligonucleotide of 10 – 30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide region of 10 – 30, such as 12 – 22 nucleotides which are at least 90% such as 100% complementarity to SEQ ID NO 114: 5’
GACAGTCAGCATTTGTCTCCTCCTTTAACTGAGTCATCATCTTAGTCCAACTAATGCAGTCG
ATACAATGCGTAGATAGAAGAAGCCCCACGGGAGCCAGGATGGGACTGGTCGTGTTTGTG
CTTTTCTCCAAGTCAGCACCCAAAGGTCAATGCACAGAGACCCCGGGTGGGTGAGCGCTG
GCTTCTCAAACGGCCGAAGTTGCCTCTTTTAGGAATCTCTTTGGAATTGGGAGCACGATGA
CTCTGAGTTTGAGCTATTAAAGTACTTCTTACACATTGC 3’.

The reverse complement of SEQ ID NO 114 is SEQ ID NO 120:
GCAATGTGTAAGAAGTACTTTAATAGCTCAAACTCAGAGTCATCGTGCTCCCAATTCCAAAG
AGATTCCTAAAAGAGGCAACTTCGGCCGTTTGAGAAGCCAGCGCTCACCCACCCGGGGTC
TCTGTGCATTGACCTTTGGGTGCTGACTTGGAGAAAAGCACAAACACGACCAGTCCCATCC
TGGCTCCCGTGGGGCTTCTTCTATCTACGCATTGTATCGACTGCATTAGTTGGACTAAGAT
GATGACTCAGTTAAAGGAGGAGACAAATGCTGACTGTC.
The disclosure provides for an antisense oligonucleotide comprising a contiguous nucleotide region of at 10 – 30, such as 12 – 22, nucleotides, wherein the contiguous nucleotide region is at least 90% such as 100% complementary to SEQ ID NO 115.

The disclosure provides for an antisense oligonucleotide of 10 – 30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide region of 10 – 30,such as 12 – 22 nucleotides which are at least 90% such as 100% complementarity to SEQ ID NO 115: 5’
GACAGTCAGCATTTGTCTCCTCCTTTAACTGAGTCATCATCTTAGTCCAACTAATGCAGTCG

ATACAATGCGTAGATAGAAGAAGCCCCACGGGAGCCAGGATGGGACTGGTCGTGTTTGTG
CTTTTCTCCAAGTCAGCACCCAAAGGTCAATGCACAGAGACCCCGGGTGGGTGAGCGCTG
GCTTCTCAAACGGCCGAAGTTGCCTCTTTTAGGAATCTCTTTGGAATTGGGAGCACGATGA
CTCTGAGTTTGAGCTATTAAAGT 3'.

The reverse complement of SEQ ID NO 115 is SEQ ID NO 121:
ACTTTAATAGCTCAAACTCAGAGTCATCGTGCTCCCAATTCCAAAGAGATTCCTAAAAGAGG
CAACTTCGGCCGTTTGAGAAGCCAGCGCTCACCCACCCGGGGTCTCTGTGCATTGACCTT
TGGGTGCTGACTTGGAGAAAAGCACAAACACGACCAGTCCCATCCTGGCTCCCGTGGGGC
TTCTTCTATCTACGCATTGTATCGACTGCATTAGTTGGACTAAGATGATGACTCAGTTAAAG
GAGGAGACAAATGCTGACTGTC.

The disclosure provides for an antisense oligonucleotide comprising a contiguous nucleotide region of at 10 – 30, such as 12 – 22, nucleotides, wherein the contiguous nucleotide region is at least 90% such as 100% complementary to SEQ ID NO 116.

The disclosure provides for an antisense oligonucleotide of 10 – 30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide region of 10 – 30, such as 12 – 22 nucleotides which are at least 90% such as 100% complementarity to SEQ ID NO 116: 5'
CAACTAATGCAGTCGATACAATGCGTAGATAGAAGAAGCCCCACGGGAGCCAGGATGGGA
CTGGTCGTGTTTGTGCTTTTCTCCAAGTCAGCACCCAAAGGTCAATGCACAGAGACCCCGG
GTGGGTGAGCGCTGGCTTCTCAAACGGCCGAAGTTGCCTCTTTTAGGAATCTCTTTGGAAT
TGGGAGCACGATGACTCTGAGTTTGAGCTATTAAAGTACTTCTTACACATTGC 3'.

The reverse complement of SEQ ID NO 116 is SEQ ID NO 122:
GCAATGTGTAAGAAGTACTTTAATAGCTCAAACTCAGAGTCATCGTGCTCCCAATTCCAAAG
AGATTCCTAAAAGAGGCAACTTCGGCCGTTTGAGAAGCCAGCGCTCACCCACCCGGGGTC
TCTGTGCATTGACCTTTGGGTGCTGACTTGGAGAAAAGCACAAACACGACCAGTCCCATCC
TGGCTCCCGTGGGGCTTCTTCTATCTACGCATTGTATCGACTGCATTAGTTG.

The disclosure provides for an antisense oligonucleotide comprising a contiguous nucleotide region of at 10 – 30, such as 12 – 22, nucleotides, wherein the contiguous nucleotide region is at least 90% such as 100% complementary to SEQ ID NO 117.

The disclosure provides for an antisense oligonucleotide of 10 – 30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide region of 10 – 30, such as 12 – 22 nucleotides which are at least 90% such as 100% complementarity to SEQ ID NO 117: 5'
CAACTAATGCAGTCGATACAATGCGTAGATAGAAGAAGCCCCACGGGAGCCAGGATGGGA
CTGGTCGTGTTTGTGCTTTTCTCCAAGTCAGCACCCAAAGGTCAATGCACAGAGACCCCGG

GTGGGTGAGCGCTGGCTTCTCAAACGGCCGAAGTTGCCTCTTTTAGGAATCTCTTTGGAAT TGGGAGCACGATGACTCTGAGTTTGAGCTATTAAAGTTACTTCTTAC 3'.

The reverse complement of SEQ ID NO 117 is SEQ ID NO 123:
GTAAGAAGTAACTTTAATAGCTCAAACTCAGAGTCATCGTGCTCCCAATTCCAAAGAGATTC CTAAAAGAGGCAACTTCGGCCGTTTGAGAAGCCAGCGCTCACCCACCCGGGGTCTCTGTG CATTGACCTTTGGGTGCTGACTTGGAGAAAAGCACAAACACGACCAGTCCCATCCTGGCTC CCGTGGGGCTTCTTCTATCTACGCATTGTATCGACTGCATTAGTTG.

In some embodiments the antisense oligonucleotide of the disclosure is not of sequence 5' gcaatgtgtaagaagt 3' (SEQ ID NO 112). In some embodiments the antisense oligonucleotide of the disclosure does not comprise or consist of sequence 5' gcaatgtgtaagaagt 3'. In some embodiments the antisense oligonucleotide of the disclosure does not comprise or consist of 10 or more contiguous nucleotides present in sequence 5' gcaatgtgtaagaagt 3'. In some embodiments the oligonucleotide of the disclosure is other than 5' GCAatgtgtaagaAGT 3', wherein Capital letters represent LNA nucleosides (beta-D-oxy LNA nucleosides were used), all LNA cytosines are 5-methyl cytosine, lower case letters represent DNA nucleosides, DNA cytosines preceded with a superscript m represents a 5-methyl C-DNA nucleoside. All internucleoside linkages are phosphorothioate internucleoside linkages.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10 contiguous nucleotides present in any one of SEQ ID NOs 5 – 111. The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 12 contiguous nucleotides present in any one of SEQ ID NOs 5 – 111. The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 14 contiguous nucleotides present in any one of SEQ ID NOs 5 – 111. The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 15 or 16 contiguous nucleotides present in any one of SEQ ID NOs 5 – 111. The disclosure provides an antisense oligonucleotide, wherein the contiguous nucleotide sequence of the oligonucleotide comprises or consists of a nucleobase sequence selected from the group consisting of any one of SEQ ID NOs 5 – 111.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, at least 13, or at least 14 or at least 15 or at least 16 contiguous nucleotides present SEQ ID NO 118: 5' CTTCTTCTATCTACGCATTG 3'. The reverse complement of SEQ ID NO 118 is SEQ ID NO 231: CAATGCGTAGATAGAAGAAG.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, at least 13, or at least 14 or at least 15 or at least 16 contiguous nucleotides complementary to SEQ ID NO 231.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, or at least 13, or at least 14 or at least 15 or 16 contiguous nucleotides present SEQ ID NO 67.

The invention provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, or at least 13, or at least 14 or at least 15 or 16 contiguous nucleotides present SEQ ID NO 86.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, or at least 13, or at least 14 or at least 15 or at least 16 or at least 17 or 18 contiguous nucleotides present SEQ ID NO 73.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, or at least 13, or at least 14 or at least 15 or 16 contiguous nucleotides complementary to SEQ ID NO 186.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, or at least 13, or at least 14 or at least 15 or 16 contiguous nucleotides complementary to SEQ ID NO 205.

The disclosure provides an antisense oligonucleotide which comprises a contiguous nucleotide region of at least 10, or at least 12, or at least 13, or at least 14 or at least 15 or at least 16 or at least 17 or 18 contiguous nucleotides complementary to SEQ ID NO 192.

The disclosure provides for an oligonucleotide comprising or consisting of an oligonucleotide selected from the group consisting of :

$T_sT_s{}^mC_st_sa_st_sc_st_sa_s{}^mc_sg_sc_sa_sT_sT_sG$ (SEQ ID NO 67,1),

${}^mC_sT_sT_s{}^mC_st_st_sc_st_sa_st_sc_st_sa_s{}^mc_sg_sc_sA_sT$ (SEQ ID NO 73,1), and

$T_sA_s{}^mC_sT_st_st_sa_sa_st_sa_sg_sc_sT_s{}^mC_sA_sA$ (SEQ ID NO 86,1);

wherein capital letters represent beta-D-oxy LNA nucleosides, lower case letters are DNA nucleosides, subscript s represents a phosphorothioate internucleoside linkage, and ${}^mC$ represent 5 methyl cytosine beta-D-oxy LNA nucleosides, and ${}^mc$ represents 5 methyl cytosine DNA nucleosides.

The disclosure provides for an oligonucleotide of formula:

$T_sT_s{}^mC_st_sa_st_sc_st_sa_s{}^mc_sg_sc_sa_sT_sT_sG$ (SEQ ID NO 67,1),

wherein capital letters represent beta-D-oxy LNA nucleosides, lower case letters are DNA nucleosides, subscript s represents a phosphorothioate internucleoside linkage,

and ^{m}C represent 5 methyl cytosine beta-D-oxy LNA nucleosides, and ^{m}c represents 5 methyl cytosine DNA nucleosides.

The disclosure provides for an oligonucleotide of formula:

$^{m}C_sT_sT_s{}^{m}C_st_st_sc_st_sa_st_sc_st_sa_s{}^{m}c_sg_sc_sA_sT$ (SEQ ID NO 73,1)

wherein capital letters represent beta-D-oxy LNA nucleosides, lower case letters are DNA nucleosides, subscript s represents a phosphorothioate internucleoside linkage, and ^{m}C represent 5 methyl cytosine beta-D-oxy LNA nucleosides, and ^{m}c represents 5 methyl cytosine DNA nucleosides.

The disclosure provides for an oligonucleotide of formula:

$T_sA_s{}^{m}C_sT_st_st_sa_sa_st_sa_sg_sc_sT_s{}^{m}C_sA_sA$ (SEQ ID NO 86,1)

wherein capital letters represent beta-D-oxy LNA nucleosides, lower case letters are DNA nucleosides, subscript s represents a phosphorothioate internucleoside linkage, and ^{m}C represent 5 methyl cytosine beta-D-oxy LNA nucleosides, and ^{m}c represents 5 methyl cytosine DNA nucleosides.

The disclosure provides for the oligonucleotides provided in the examples.

The disclosure provides for a conjugate comprising the oligonucleotide according to the disclosure, and at least one conjugate moiety covalently attached to said oligonucleotide. In a further aspect, the present disclosure refers to a conjugate comprising the oligonucleotide as defined herein, and at least one conjugate moiety covalently attached to said oligonucleotide. In a further aspect, the present disclosure refers to a pharmaceutical composition comprising the oligonucleotide as defined herein or the conjugate as defined herein and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

The disclosure provides for a pharmaceutically acceptable salt of the oligonucleotide or conjugate of the disclosure. In another aspect, the present disclosure relates to a pharmaceutically acceptable salt of any one of the oligonucleotide of the disclosure.

In a further example, the disclosure provides methods for *in vivo* or *in vitro* method for modulation of HTRA1 expression in a cell which is expressing HTRA1, by administering an oligonucleotide, conjugate or composition of the disclosure in an effective amount to said cell. In a further aspect, the present disclosure relates to an in vitro method for modulating HTRA1 expression in a target cell which is expressing HTRA1, said method comprising administering

an oligonucleotide as defined herein or the conjugate as defined herein or the pharmaceutical composition as defined herein in an effective amount to said cell.

In a further example the disclosure provides methods for treating or preventing a disease, disorder or dysfunction associated with *in vivo* activity of HTRA1 comprising administering a therapeutically or prophylactically effective amount of the oligonucleotide of the disclosure, or conjugate thereof, to a subject suffering from or susceptible to the disease, disorder or dysfunction.

In a further example the oligonucleotide or composition of the disclosure is used for the treatment or prevention of macular degeneration, and other disorders where HTRA1 is implicated.

In another aspect, the present disclosure relates to the oligonucleotide of the disclosure or the conjugate of the disclosure or the pharmaceutical composition of the disclosure for use in medicine.

The disclosure provides for the oligonucleotide or conjugate of the disclosure, for use in the treatment of a disease or disorder selected from the list comprising of Duchenne muscular

dystrophy, arthritis, such as osteoarthritis, familial ischemic cerebral small-vessel disease, Alzhiemer’s disease and Parkinson's disease.

In another aspect, the present disclosure relates to the oligonucleotide of the disclosure or the conjugate of the disclosure or the pharmaceutical composition of the disclosure, for use in the treatment of macular degeneration. This may be wet or dry age related macular degeneration (e.g. wAMD, dAMD, geographic atrophy, early AMD, intermediate AMD) or diabetic retinopathy.

The disclosure provides for the use of the oligonucleotide, conjugate or composition of the disclosure, for the manufacture of a medicament for the treatment of macular degeneration, such as wet or dry age related macular degeneration (*e.g.* wAMD, dAMD, geographic atrophy, intermediate dAMD) or diabetic retinopathy.

In a further aspect, the present disclosure refers to a use of an oligonucleotide defined herein or the conjugate defined herein or the pharmaceutical composition defined herein in the manufacture of a medicament for the treatment or prevention of a macular degeneration in a subject.

The disclosure provides for the use of the oligonucleotide, conjugate or composition of the disclosure, for the manufacture of a medicament for the treatment of a disease or disorder selected from the group consisting of Duchenne muscular dystrophy, arthritis, such as osteoarthritis, familial ischemic cerebral small-vessel disease, Alzhiemer’s disease and Parkinson's disease.

The disclosure provides for a method of treatment of a subject suffering from a disease or disorder selected from the group consisting of Duchenne muscular dystrophy, arthritis, such as osteoarthritis, familial ischemic cerebral small-vessel disease, Alzhiemer’s disease and Parkinson's disease, said method comprising the step of administering an effective amount of the oligonucleotide, conjugate or composition of the disclosure to the subject.

The disclosure provides for a method of treatment of a subject suffering from an ocular disease, such as macular degeneration, such as wet or dry age related macular degeneration (*e.g.* wAMD, dAMD, geographic atrophy, intermediate dAMD) or diabetic retinopathy, said method comprising the step of administering an effective amount of the oligonucleotide, conjugate or composition of the disclosure to the subject.

The disclosure provides for a method of treatment of a subject suffering from an ocular disease, such as macular degeneration, such as wet or dry age related macular degeneration (*e.g.* wAMD, dAMD, geographic atrophy, intermediate AMD) or diabetic retinopathy, said method comprising administering at least two dosages of the oligonucleotide of the

disclosure, or pharmaceutically acceptable salt thereof, in an intraocular injection in a dosage of from about 10µg - 200 µg, wherein the dosage interval between administration

consecutive is at least 4 weeks (*i.e.* a dosage interval is ≥ 4 weeks), or at least monthly (*i.e.* a dosage interval is ≥ 1 month).

BRIEF DESCRIPTION OF FIGURES

Figure 1. A library of n=231 HTRA1 LNA oligonucleotides were screened in U251 cell lines at 5 µM. The residual HTRA1 mRNA expression level was measured by qPCR and is shown as % of