



- (51) **International Patent Classification:**
A61K 31/713 (2006.01)
- (21) **International Application Number:**
PCT/US2015/022345
- (22) **International Filing Date:**
24 March 2015 (24.03.2015)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/970,319 25 March 2014 (25.03.2014) US
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- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) **Title:** TRANSTHYRETIN ALLELE SELECTIVE UNA OLIGOMERS FOR GENE SILENCING

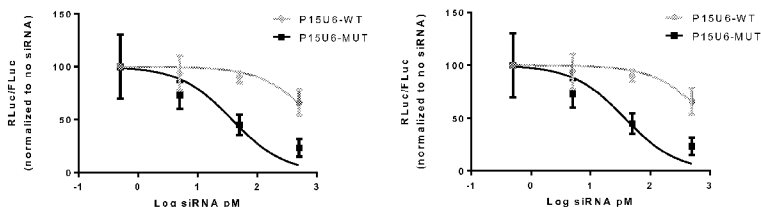


FIG. 10

(57) **Abstract:** This invention provides UNA oligomers for selectively inhibiting V30M TTR expression, which can be used in treating amyloidosis. The UNA oligomers can have a first strand and a second strand, each of the strands being 19-29 monomers in length, the monomers being UNA monomers and nucleic acid monomers. Embodiments include pharmaceutical compositions and methods for treating or preventing TTR-related amyloidosis by administering a UNA oligomer to a subject.

TRANSTHYRETIN ALLELE SELECTIVE
UNA OLIGOMERS FOR GENE SILENCING

TECHNICAL FIELD

[0001] This invention relates to the fields of biopharmaceuticals and therapeutics based on allele selective gene silencing. More particularly, this invention relates to methods for treating transthyretin-related amyloidosis with UNA oligomers capable of allele-selective knockdown of transthyretin.

BACKGROUND OF THE INVENTION

[0002] The presence of certain diseases appears to correlate with expression of a mutant allele. For example, amyloidosis can be correlated to certain transthyretin (TTR) mutations. In such cases, it is desirable to selectively silence expression of the mutant allele, while maintaining expression of the wild-type variant.

[0003] Amyloidosis related to transthyretin (ATTR) involves the depositing of amyloid fibril proteins in various organs and tissues, including the peripheral, autonomic, and central nervous systems. Transthyretin (TTR) is a secreted thyroid hormone-binding protein that binds and transports retinol binding protein, and serum thyroxine in plasma and cerebrospinal fluid.

[0004] The pathology of ATTR may include many TTR mutations. Symptoms of ATTR often include neuropathy and/or cardiomyopathy. Peripheral neuropathy can begin in the lower extremities, with sensory and motor neuropathy, and can progress to the upper extremities. Autonomic neuropathy can be manifest by gastrointestinal symptoms and orthostatic hypotension.

[0005] Patients with TTR gene Val-30-Met, the most common mutation, have normal echocardiograms. However, they may have conduction system irregularities and need a pacemaker. The ATTR V30M variant can cause lower extremity weakness, pain, and impaired sensation, as well as autonomic dysfunction. Vitreous and opaque amyloid deposits can be characteristic of ATTR.

[0006] Survival upon onset of ATTR may be from five to fifteen years. The major treatment for ATTR amyloidosis is liver transplantation, which removes the major source of variant TTR production and replaces it with normal TTR. Liver transplantation slows disease progression and some improvement in autonomic and peripheral neuropathy can occur.

[0007] There is currently no pharmacological therapy that can undo the formation of TTR amyloid.

[0008] There is a continuing need for therapeutics for ATTR and other amyloid-related diseases.

[0009] There is a long-standing need for gene silencing agents that can selectively downregulate a disease-related allele.

[0010] There is also a need for active agents that can provide efficient and specific knockdown of TTR.

BRIEF SUMMARY

[0011] This invention provides UNA oligomers for selectively inhibiting V30M TTR expression, which can be used in treating amyloidosis. The UNA oligomers can have a first strand and a second strand, each of the strands being 19-29 monomers in length, the monomers being UNA monomers and nucleic acid monomers. Embodiments include pharmaceutical compositions and methods for treating or preventing TTR-related amyloidosis by administering a UNA oligomer to a subject.

[0012] Embodiments of this invention include the following:

[0013] A UNA oligomer for selectively inhibiting V30M TTR expression, the oligomer comprising a first strand and a second strand, each of the strands being 19-29 monomers in length, the monomers comprising UNA monomers and nucleic acid monomers, wherein the oligomer has a duplex structure of from 14 to 29 monomers in length.

[0014] The UNA oligomer above, wherein the second strand has at least one UNA monomer in the duplex region. The UNA oligomer above, wherein the at least one

UNA monomer in the second strand is at any one of positions 2-8 from the 5' end. The UNA oligomer above, wherein the at least one UNA monomer in the second strand is at any one of positions 9-18 from the 5' end. The UNA oligomer above, wherein the at least one UNA monomer in the second strand is at position 6, 7, 15, 16 or 17 from the 5' end.

[0015] The UNA oligomer above, wherein the oligomer has an IC₅₀ for reducing V30M TTR expression of less than 20 pM.

[0016] The UNA oligomer above, wherein the oligomer has a selectivity ratio of at least 10, wherein the selectivity ratio is the ratio of the IC₅₀ for reducing wild type TTR expression to the IC₅₀ for reducing V30M TTR expression. The UNA oligomer above, wherein the oligomer has a selectivity ratio of at least 20. The UNA oligomer above, wherein the oligomer has a selectivity ratio in vitro of at least 50.

[0017] The UNA oligomer above, wherein the oligomer selectively inhibits V30M TTR expression in vivo. The UNA oligomer above, wherein the oligomer selectively inhibits V30M TTR expression ex vivo.

[0018] The UNA oligomer above, comprising at least one nucleic acid monomer that is base-modified, sugar-modified, or linkage modified.

[0019] A pharmaceutical composition comprising a UNA oligomer above and a pharmaceutically acceptable carrier. The pharmaceutical composition above, comprising a lipid formulation. The pharmaceutical composition above, comprising one or more lipids selected from cationic lipids, anionic lipids, sterols, pegylated lipids, and any combination of the foregoing. The pharmaceutical composition above, wherein the composition is substantially free of liposomes. The pharmaceutical composition above, wherein the composition contains liposomes.

[0020] A method for treating or preventing TTR-related amyloidosis, comprising administering to a subject in need an effective amount of a UNA oligomer above. The method above, wherein the TTR-related amyloidosis is ATTR. The method above, wherein the subject is human. The method above, wherein the subject comprises a V30M gene. The method above, wherein the method selectively reduces V30M TTR in the

subject. The method above, wherein the administering is local or systemic. The method above, wherein the administering is intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, or oral. The method above, wherein the method selectively reduces V30M TTR in the subject by at least 10% greater than control. The method above, wherein the effective amount is a dose of from 0.001 to 50.0 mg/kg.

[0021] The method above, wherein TTR mRNA expression is reduced for at least 5 days. The method above, wherein the method reduces peripheral neuropathy or autonomic neuropathy in the subject. The method above, wherein the administration does not result in an inflammatory response.

[0022] A method for inhibiting expression of a TTR gene in a cell, comprising treating the cell with a UNA oligomer above.

[0023] A method for inhibiting expression of a TTR gene in a mammal, comprising administering to the mammal a UNA oligomer above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIG. 1: Fig. 1 shows the single nucleotide polymorph (SNP) that exists at position 284 in the V30M mutation mRNA, as compared to the wild type (WT) TTR mRNA. Conventional siRNAs that are complementary to the WT mRNA can be tiled around position 284.

[0025] FIG. 2: Fig. 2 shows that the conventional siRNAs complementary to the WT mRNA have limited activity in silencing the WT TTR gene, as measured by TTR knockdown in HepG2 cells. Positions 5, 9, 14 and 15, indicated by arrows, appear to be more accessible to silencing than other positions.

[0026] FIG. 3: Fig. 3 shows that conventional siRNAs that are complementary to the V30M mRNA can be tiled around position 284. Four conventional siRNA variations, namely V30M-P5, V30M-P9, V30M-P14, and V30M-P15, were prepared. Also, as shown in Fig. 3, two reporter variants, V30V and V30M, each bearing nucleotide sequence 264 to 304 of human TTR, V30V being without the point mutation at position 284, and V30M containing the point mutation at position 284, were prepared and used in the PSICHECK reporter system in the 3'-UTR region of Luciferase gene.

[0027] FIG. 4: Fig. 4 shows the activity of the four conventional siRNA variations V30M-P5, V30M-P9, V30M-P14, and V30M-P15 measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants. The conventional siRNA variations V30M-P5, V30M-P9, and V30M-P15 were more effective against V30M than V30V. The conventional siRNA variation V30M-P14 was not more effective against V30M than V30V.

[0028] FIG. 5: Fig. 5 shows IC₅₀ analysis for the four conventional siRNA variations V30M-P5, V30M-P9, V30M-P14, and V30M-P15 measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants. The conventional V30M-P15 variant was 5.6 times more effective against V30M than V30V. Thus, the selectivity of the conventional siRNAs against V30M over V30V was no more than 5.6.

[0029] FIG. 6: Fig. 6 shows the structure of UNA oligomers that were effective in silencing V30M TTR, as measured in the PSICHECK reporter assay. Each of the UNA oligomer embodiments, P15U6, P15U7, P15U14, P15U15, and P15U16, contained four UNA monomers. In each UNA oligomer, a first UNA monomer was located at the 5' end of the first strand, also called the passenger strand. In each UNA oligomer, the second strand, also called the guide strand, formed a duplex region of 19 monomers length with the first strand. Each UNA oligomer had a duplex region of 19 monomers, and a two-monomer overhang at each end. In each UNA oligomer, a second UNA monomer was located at the 3' end of the first strand, in the 20th position, which is in an overhang portion. In each UNA oligomer, a third UNA monomer was located at the 3' end of the second strand, in the 20th position, which is in an overhang portion. In the UNA oligomer embodiments, P15U6, P15U7, P15U14, P15U15, and P15U16, a fourth UNA monomer was located in the second strand at positions 6, 7, 14, 15 and 16, respectively, counting from the 5' end of the second strand.

[0030] FIG. 7: Fig. 7 shows the activity of UNA oligomers P15U6, P15U7, P15U15, and P15U16, measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants, as compared to the conventional siRNA V30M-P15. For each of the UNA oligomer embodiments, P15U6, P15U7, P15U15, and P15U16, the

UNA oligomers were more effective against V30M than V30V. Surprisingly, the activity of the UNA oligomer P15U6 was substantially and advantageously superior to the activity of the conventional siRNA V30M-P15, where each is targeted to V30M. Furthermore, Fig. 7 shows the surprising and unexpected result that the selectivity of the UNA oligomers, P15U6, P15U7, P15U15, and P15U16, against V30M over V30V was substantially greater than for the conventional siRNA V30M-P15. In particular, the selectivity of UNA oligomer P15U6 against V30M over V30V was 24, meaning that the IC₅₀ of UNA oligomer P15U6 against V30M (37.6 pM) was 24 times lower than the IC₅₀ of UNA oligomer P15U6 against V30V (919.9 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown above in Fig. 5 for the conventional siRNA.

[0031] FIG. 8: Fig. 8 shows the structure of UNA oligomers that were effective in silencing V30M TTR, as measured in the PSICHECK reporter assay. Each of the UNA oligomer embodiments, P16U6, P16U7, P16U15, P16U16, and P16U17, contained four UNA monomers. In each UNA oligomer, a first UNA monomer was located at the 5' end of the first strand, also called the passenger strand. In each UNA oligomer, the second strand, also called the guide strand, formed a duplex region of 19 monomers length with the first strand. Each UNA oligomer had a duplex region of 19 monomers, and a two-monomer overhang at each end. In each UNA oligomer, a second UNA monomer was located at the 3' end of the first strand, in the 20th position, which is in an overhang portion. In each UNA oligomer, a third UNA monomer was located at the 3' end of the second strand, in the 20th position, which is in an overhang portion. In the UNA oligomer embodiments, P16U6, P16U7, P16U15, P16U16, and P16U17, a fourth UNA monomer was located in the second strand at positions 6, 7, 15, 16 and 17, respectively, counting from the 5' end of the second strand.

[0032] FIG. 9: Fig. 9 shows the activity of UNA oligomers P16U6, P16U7, P16U15, and P16U16, measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants, as compared to the conventional siRNA V30M-P16. For each of the UNA oligomer embodiments, P16U6, P16U7, P16U15, and P16U16, the UNA oligomers were more effective against V30M than V30V. Surprisingly, the activity

of each of the UNA oligomers P16U6, P16U7, P16U15, and P16U16, was substantially and advantageously superior to the activity of the conventional siRNA V30M-P16, where each is targeted to V30M. Furthermore, Fig. 9 shows the surprising and unexpected result that the selectivity of the UNA oligomers, P16U6, P16U7, P16U15, and P16U16, against V30M over V30V was substantially greater than for the conventional siRNA V30M-P16. In particular, the selectivity of UNA oligomer P16U6 against V30M over V30V was 23, meaning that the IC₅₀ of UNA oligomer P16U6 against V30M (92.4 pM) was 23 times lower than the IC₅₀ of UNA oligomer P16U6 against V30V (2119 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown above in Fig. 5 for the conventional siRNA.

[0033] FIG. 10: Fig. 10 (left) shows the selectivity of UNA oligomer P15U6 against V30M over V30V. The IC₅₀ of UNA oligomer P15U6 against V30M (37.6 pM) was 24 times lower than the IC₅₀ of UNA oligomer P15U6 against V30V (919.9 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown in Fig. 5 above for the conventional siRNA. Fig. 10 (right) shows the surprising and unexpected result that the IC₅₀ of UNA oligomer P16U6 against V30M (92.4 pM) was 23 times lower than the IC₅₀ of UNA oligomer P16U6 against V30V (2119 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown above in Fig. 5 for the conventional siRNA.

DETAILED DESCRIPTION OF THE INVENTION

[0034] This invention provides UNA oligomers for selectively inhibiting V30M TTR expression. The UNA oligomers of this invention can be used as therapeutics for treating amyloidosis. In particular, this invention provides UNA oligomers, compositions and methods for treating transthyretin-related amyloidosis.

[0035] The UNA oligomers can have a first strand and a second strand, each of the strands being 19-29 monomers in length, the monomers being UNA monomers and nucleic acid monomers. Embodiments of this invention include pharmaceutical compositions and methods for treating or preventing TTR-related amyloidosis by administering a UNA oligomer to a subject.

[0036] The UNA oligomers of this invention are capable of allele-specific knockdown of transthyretin.

[0037] In some embodiments, UNA oligomers are provided for treating amyloidosis related to transthyretin (ATTR). The UNA oligomers of this invention can reduce the depositing of amyloid fibril proteins in various organs and tissues, including the peripheral, autonomic, and central nervous systems.

[0038] In certain aspects, this invention provides therapeutics for ATTR and related amyloid-related diseases.

[0039] Aspects of this invention include UNA oligomers that can be used for treating clinical features of ATTR amyloidosis, including neuropathy and/or cardiomyopathy.

[0040] In some embodiments, UNA oligomers of this invention are targeted to one mutation Val-30-Met TTR.

[0041] This invention can provide a pharmacological therapy that can undo the formation of TTR amyloid.

[0042] UNA oligomers

[0043] The UNA oligomers of this invention can be used for inhibiting V30M TTR expression.

[0044] A UNA oligomer of this invention the oligomer may have a first strand and a second strand, each of the strands being 19-29 monomers in length.

[0045] The monomers of a UNA oligomer can include UNA monomers and nucleic acid monomers

[0046] A UNA oligomer can be a duplex structure of from 14 to 29 monomers in length.

[0047] In some embodiments, the second strand of a UNA oligomer can have at least one UNA monomer in the duplex region. In certain embodiments, a UNA oligomer can have at least one UNA monomer in the second strand at any of positions 6, 7, 15, 16 or 17 from the 5' end in the duplex region.

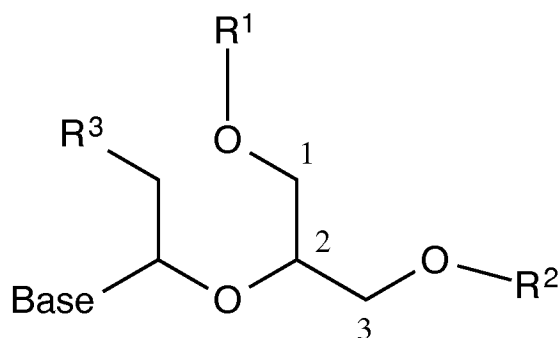
- [0048]** A UNA oligomer of this invention may have any number of UNA monomers within its total length.
- [0049]** A UNA oligomer can include a nucleic acid monomer that is base-modified, sugar-modified, or linkage modified.
- [0050]** Embodiments of this invention further provide UNA oligomers that selectively inhibit V30M TTR expression.
- [0051]** In certain embodiments, a UNA oligomer has an IC₅₀ for reducing V30M TTR expression in vitro of less than 20 pM.
- [0052]** In further embodiments, a UNA oligomer can have a selectivity ratio in vitro of at least 5. The selectivity ratio is the ratio of the IC₅₀ for reducing V30M TTR expression to the IC₅₀ for reducing wild type TTR expression. The selectivity ratio of a UNA oligomer of this invention can range from 2 to 1000. In certain embodiments, the selectivity ratio of a UNA oligomer is at least 2, or at least 5, or at least 10, or at least 30, or at least 30, or at least 50, or at least 100.
- [0053]** In some aspects, a UNA oligomer of this invention can selectively inhibit V30M TTR expression in vivo.
- [0054]** In certain aspects, a UNA oligomer of this invention can selectively inhibit V30M TTR expression ex vivo.
- [0055]** A UNA oligomer is an active pharmaceutical molecule being a chain composed of monomers, also called an oligomer. The monomers of the oligomer can include UNA monomers and other nucleic acid monomers.
- [0056]** The UNA monomers are novel, synthetic molecules that can be attached in a chain to form an oligomer.
- [0057]** The nucleic acid monomers can be naturally-occurring nucleotides, modified naturally-occurring nucleotides, or certain non-naturally- occurring nucleotides.
- [0058]** A UNA oligomer of this invention is a synthetic, pharmacologically active molecule and can be used in the treatment of a condition or disease.

[0059] A UNA oligomer of this disclosure can be a double stranded oligomer. Each strand of the double stranded oligomer can be composed of UNA monomers along with a number of nucleic acid monomers for a total length of 19 to 29 monomers.

[0060] A UNA oligomer of this invention can contain one or more UNA monomers in any strand. The UNA monomers can be in a single strand, or in either strand of a double stranded UNA oligomer, or in both strands of a double stranded UNA oligomer.

[0061] UNA monomers

[0062] UNA monomers are small organic molecules based on a propane-1,2,3-tri-yl-trisoxy structure as shown below:

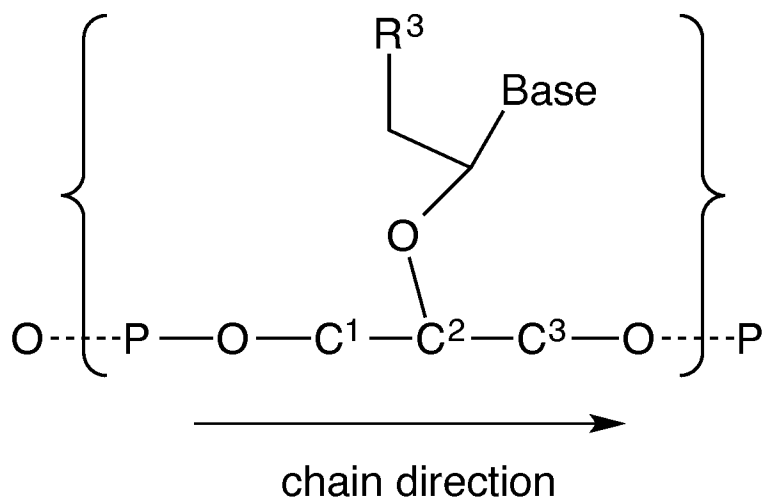


UNA MONOMER

where R^1 and R^2 are H, and R^1 and R^2 can be phosphodiester linkages, Base can be a nucleobase, and R^3 is a functional group described below.

[0063] In another view, the UNA monomer main atoms can be drawn in IUPAC notation as follows:

UNA monomer unit

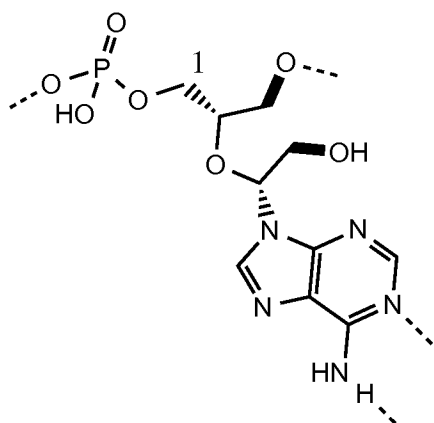


where the direction of progress of the oligomer chain is from the 1-end to the 3-end of the propane residue.

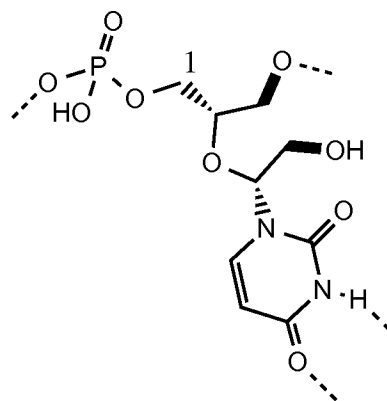
[0064] Examples of a nucleobase include uracil, thymine, cytosine, 5-methylcytosine, adenine, guanine, inosine, and natural and non-natural nucleobase analogues.

[0065] In general, because the UNA monomers are not nucleotides, they can exhibit at least four forms in an oligomer. First, a UNA monomer can be an internal monomer in an oligomer, where the UNA monomer is flanked by other monomers on both sides. In this form, the UNA monomer can participate in base pairing when the oligomer is a duplex, for example, and there are other monomers with nucleobases in the duplex.

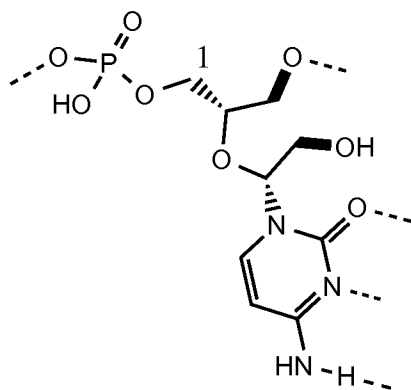
[0066] Examples of UNA monomer as internal monomers flanked at both the propane-1-yl position and the propane-3-yl position, where R^3 is -OH, are shown below.



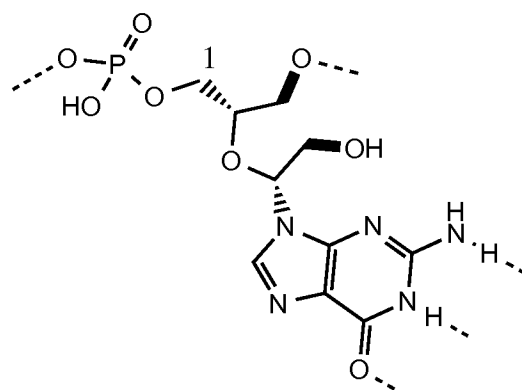
UNA-A



UNA-U



UNA-C



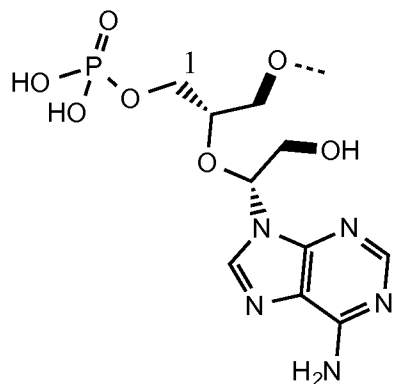
UNA-G

[0067] Second, a UNA monomer can be a monomer in an overhang of an oligomer duplex, where the UNA monomer is flanked by other monomers on both sides. In this form, the UNA monomer does not participate in base pairing. Because the UNA monomers are flexible organic structures, unlike nucleotides, the overhang containing a UNA monomer will be a flexible terminator for the oligomer.

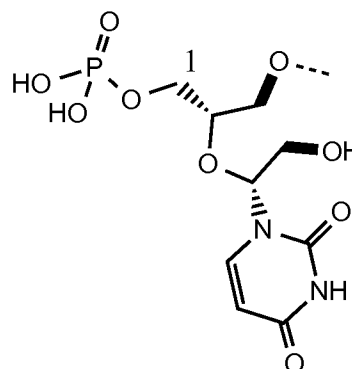
[0068] A UNA monomer can be a terminal monomer in an overhang of an oligomer, where the UNA monomer is attached to only one monomer at either the propane-1-yl position or the propane-3-yl position. In this form, the UNA monomer does not participate in base pairing. Because the UNA monomers are flexible organic

structures, unlike nucleotides, the overhang containing a UNA monomer can be a flexible terminator for the oligomer.

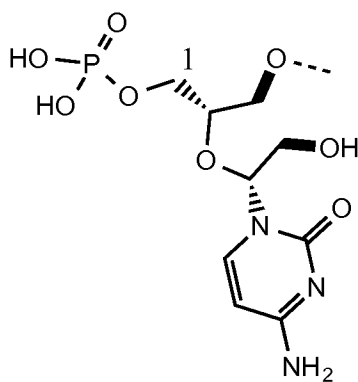
[0069] Examples of a UNA monomer as a terminal monomer attached at the propane-3-yl position are shown below.



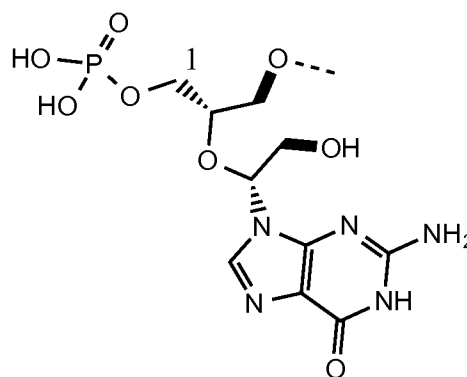
terminal UNA-A



terminal UNA-U

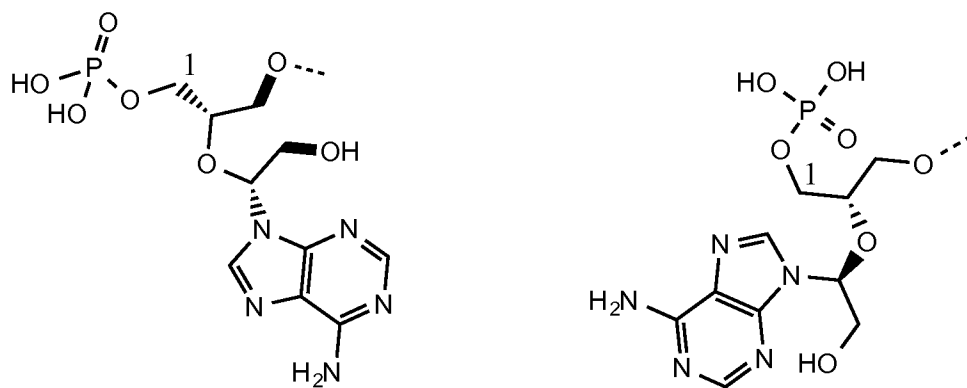


terminal UNA-C



terminal UNA-G

[0070] Because a UNA monomer can be a flexible molecule, a UNA monomer as a terminal monomer can assume widely differing conformations. An example of an energy minimized UNA monomer conformation as a terminal monomer attached at the propane-3-yl position is shown below.



UNA-A terminal forms: the dashed bond shows the propane-3-yl attachment

Thus, UNA oligomers having a terminal UNA monomer are significantly different in structure from conventional nucleic acid agents, such as siRNAs. For example, siRNAs may require that terminal monomers or overhangs in a duplex be stabilized. In contrast, the conformability of a terminal UNA monomer can provide UNA oligomers with different properties.

[0071] Among other things, the structure of the UNA monomer allows it to be attached to naturally-occurring nucleotides. A UNA oligomer can be a chain composed of UNA monomers, as well as various nucleotides that may be based on naturally-occurring nucleosides.

[0072] In some embodiments, the functional group R^3 of a UNA monomer can be $—OR^4$, $—SR^4$, $—NR^4_2$, $—NH(C=O)R^4$, morpholino, morpholin-1-yl, piperazin-1-yl, or 4-alkanoyl-piperazin-1-yl, where R^4 is the same or different for each occurrence, and can be H, alkyl, a cholesterol, a lipid molecule, a polyamine, an amino acid, or a polypeptide.

[0073] The UNA monomers are organic molecules. UNA monomers are not nucleic acid monomers or nucleotides, nor are they naturally-occurring nucleosides or modified naturally-occurring nucleosides.

[0074] A UNA oligomer of this invention is a synthetic chain molecule. A UNA oligomer of this invention is not a nucleic acid, nor an oligonucleotide.

[0075] In some embodiments, as shown above, a UNA monomer can be UNA-A (designated \tilde{A}), UNA-U (designated \tilde{U}), UNA-C (designated \tilde{C}), and UNA-G (designated \tilde{G}).

[0076] Designations that may be used herein include mA, mG, mC, and mU, which refer to the 2'-O-Methyl modified ribonucleotides.

[0077] Designations that may be used herein include lower case c and u, which refer to the 2'-O-methyl modified ribonucleotides.

[0078] Designations that may be used herein include dT, which refers to a 2'-deoxy T nucleotide.

[0079] Monomers for UNA oligomers

[0080] As used herein, in the context of oligomer sequences, the symbol X represents a UNA monomer.

[0081] As used herein, in the context of oligomer sequences, the symbol N represents any natural nucleotide monomer, or a modified nucleotide monomer.

[0082] As used herein, in the context of oligomer sequences, the symbol Q represents a non-natural, modified, or chemically-modified nucleotide monomer.

[0083] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-O-methyl ribonucleotides, 2'-O-methyl purine nucleotides, 2'-deoxy-2'-fluoro ribonucleotides, 2'-deoxy-2'-fluoro pyrimidine nucleotides, 2'-deoxy ribonucleotides, 2'-deoxy purine nucleotides, universal base nucleotides, 5-C-methyl-nucleotides, and inverted deoxyabasic monomer residues.

[0084] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 3'-end stabilized nucleotides, 3'-glyceryl nucleotides, 3'-inverted abasic nucleotides, and 3'-inverted thymidine.

[0085] Examples of non-natural, modified, and chemically-modified nucleotide monomers include locked nucleic acid nucleotides, 2'-O,4'-C-methylene-(D-

ribofuranosyl) nucleotides, 2'-methoxyethoxy (MOE) nucleotides, 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, and 2'-O-methyl nucleotides.

[0086] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-amino nucleotides, 2'-O-amino nucleotides, 2'-C-allyl nucleotides, and 2'-O-allyl nucleotides.

[0087] Examples of non-natural, modified, and chemically-modified nucleotide monomers include N⁶-methyladenosine nucleotides.

[0088] Examples of non-natural, modified, and chemically-modified nucleotide monomers include nucleotide monomers with modified bases 5-(3-amino)propyluridine, 5-(2-mercapto)ethyluridine, 5-bromouridine; 8-bromoguanosine, or 7-deazaadenosine.

[0089] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-O-aminopropyl substituted nucleotides.

[0090] Examples of non-natural, modified, and chemically-modified nucleotide monomers include replacing the 2'-OH group of a nucleotide with a 2'-R, a 2'-OR, a 2'-halogen, a 2'-SR, or a 2'-amino, where R can be H, alkyl, alkenyl, or alkynyl.

[0091] Some examples of modified nucleotides are given in Saenger, Principles of Nucleic Acid Structure, Springer-Verlag, 1984.

[0092] Details of UNA oligomer structure

[0093] A UNA oligomer of this invention is a chain molecule. A UNA oligomer can be a duplex pair. Thus, a UNA oligomer can have a first strand of the duplex and a second strand of the duplex, which is complementary to the first strand, although up to three mismatches can occur. A UNA oligomer duplex can have overhangs.

[0094] Some UNA oligomers are discussed in US Patent No. 8,314,227, as well as US Patent Publication No. 20110313020 A1.

[0095] The target of a UNA oligomer can be a target nucleic acid. In some embodiments, the target can be any TTR mRNA of a subject. A UNA oligomer can be active for gene silencing in RNA interference.

[0096] A UNA oligomer may comprise two strands that together provide a duplex. The duplex may be composed of a first strand, which may also be referred to as a passenger strand or sense strand, and a second strand, which may also be referred to as a guide strand or antisense strand.

[0097] In some aspects, a UNA oligomer of this invention can have any number of phosphorothioate intermonomer linkages in any position in any strand, or in both strands of a duplex structure.

[0098] In certain embodiments, a UNA oligomer of this invention can have a phosphorothioate intermonomer linkage between the last one or two monomers at either end of any strand.

[0099] Examples of UNA oligomers of this invention include duplex pairs, which are in general complementary. Thus, for example, SEQ ID NO:1 can represent a first strand of a duplex and SEQ ID NO:2 can represent a second strand of the duplex, which is complementary to the first strand.

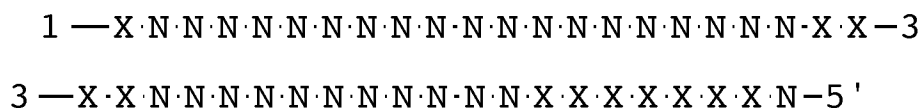
[00100] For example, the symbol “N” in the first strand can represent any nucleotide that is complementary to the monomer in the corresponding position in the second strand. Example UNA oligomers of this disclosure are shown with 2-monomer length overhangs, although overhangs of from 1 to 8 monomers, or longer, can be used.

[00101] The symbol “X” in a strand or oligomer represents a UNA monomer.

[00102] Further, when the oligomer terminates in a UNA monomer, the terminal position has a 1-end, according to the positional numbering shown above, instead of a 5'-end as for a nucleotide, or the terminal position has a 3-end, according to the positional numbering shown above, instead of a 3'-end as for a nucleotide. For example, the UNA oligomer

[00103]

SEQ ID NO:1



SEQ ID NO:2

has a UNA monomer 1-end on the first strand, a UNA monomer 3-end on the first strand, a UNA monomer 3-end on the second strand, and a nucleotide 5'-end on the second strand.

[00104] In some embodiments, a UNA oligomer of this invention can have one or more UNA monomers at the 1-end of the first strand, and one or more UNA monomers at the 3-end of the first strand.

[00105] In further embodiments, a UNA oligomer of this invention can have one or more UNA monomers at the 3-end of the second strand.

[00106] In certain embodiments, a duplex UNA oligomer of this invention can have one or more UNA monomers at the 1-end of the first strand, one or more UNA monomers at the 3-end of the first strand, and one or more UNA monomers at the 3-end of the second strand.

[00107] A UNA oligomer of this invention the oligomer may have a first strand and a second strand, each of the strands independently being 19-23 monomers in length.

[00108] In certain embodiments, a UNA oligomer of this invention may have a first strand that is 19-23 monomers in length.

[00109] In certain embodiments, a UNA oligomer of this invention may have a duplex region that is 19-21 monomers in length.

[00110] In further embodiments, a UNA oligomer of this invention may have a second strand that is 19-23 monomers in length.

[00111] In certain embodiments, a UNA oligomer of this invention may have a first strand that is 19 monomers in length, and a second strand that is 21 monomers in length.

[00112] In certain embodiments, a UNA oligomer of this invention may have a first strand that is 20 monomers in length, and a second strand that is 21 monomers in length.

[00113] In certain embodiments, a UNA oligomer of this invention may have a first strand that is 21 monomers in length, and a second strand that is 21 monomers in length.

[00114] In certain embodiments, a UNA oligomer of this invention may have a first strand that is 22 monomers in length, and a second strand that is 21 monomers in length.

[00115] In another aspect, the UNA oligomer may have a blunt end, or may have one or more overhangs. In some embodiments, the first and second strands may be connected with a connecting oligomer in between the strands, and form a duplex region with a connecting loop at one end.

[00116] In certain embodiments, an overhang can be one or two monomers in length.

[00117] A UNA oligomer can mediate cleavage of a target nucleic acid in a cell. In some processes, the second strand of the UNA oligomer, at least a portion of which can be complementary to the target nucleic acid, can act as a guide strand that can hybridize to the target nucleic acid.

[00118] The second strand can be incorporated into an RNA Induced Silencing Complex (RISC).

[00119] A UNA oligomer of this disclosure may comprise naturally-occurring nucleic acid nucleotides, and modifications thereof that are compatible with gene silencing activity.

[00120] In some aspects, a UNA oligomer is a double stranded construct molecule that is able to inhibit gene expression.

[00121] As used herein, the term strand refers to a single, contiguous chain of monomers, the chain having any number of internal monomers and two end monomers, where each end monomer is attached to one internal monomer on one side, and is not attached to a monomer on the other side, so that it ends the chain.

[00122] The monomers of a UNA oligomer may be attached via phosphodiester linkages, phosphorothioate linkages, gapped linkages, and other variations.

[00123] In some embodiments, a UNA oligomer can include mismatches in complementarity between the first and second strands. In other embodiments, a UNA oligomer may have 1, or 2, or 3 mismatches. The mismatches may occur at any position in the duplex region.

[00124] The target of a UNA oligomer can be a target nucleic acid of a target gene.

[00125] A UNA oligomer may have one or two overhangs outside the duplex region. The overhangs can be an unpaired portion at the end of the first strand or second strand. The lengths of the overhang portions of the first and second strands can be the same or different.

[00126] A UNA oligomer may have at least one blunt end. A blunt end does not have an overhang portion, and the duplex region at a blunt end terminates at the same position for both the first and second strands.

[00127] A UNA oligomer can be RISC length, which means that it has a duplex length of less than 25 base pairs.

[00128] In certain embodiments, a UNA oligomer can be a single strand that folds upon itself and hybridizes to itself to form a double stranded region having a connecting loop.

[00129] Examples of UNA oligomer structures of this invention are shown in Table 1.

[00130] Table 1: Examples of UNA oligomer structures.

Oligomer	First strand 1 to 3'	Second strand 5' to 3'
P15U6	SEQ ID NO:3 ĜGCCAUGCAUGUGUUCAGAŨmU	SEQ ID NO:4 UCUGAĀCACAUGCAUGGCCŨmU
P15U7	SEQ ID NO:5 ĜGCCAUGCAUGUGUUCAGAŨmU	SEQ ID NO:6 UCUGAAĈACAUGCAUGGCCŨmU
P15U14	SEQ ID NO:7 ĜGCCAUGCAUGUGUUCAGAŨmU	SEQ ID NO:8 UCUGAACACAUGCĀUGGCCŨmU

Oligomer	First strand 1 to 3'	Second strand 5' to 3'
P15U15	SEQ ID NO:9 ĜCCAUGCAUGUGUUCAGAŨmU	SEQ ID NO:10 UCUGAACACAUGCAŨGGCCŨmU
P15U16	SEQ ID NO:11 ĜCCAUGCAUGUGUUCAGAŨmU	SEQ ID NO:12 UCUGAACACAUGCAUĜGCCŨmU

[00131] Examples of UNA oligomer structures of this invention are shown in Table 2.

[00132] Table 2: Examples of UNA oligomer structures.

Oligomer	First strand 1 to 3'	Second strand 5' to 3'
P16U6	SEQ ID NO:13 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:14 UUCUGĀACACAUGCAUGGCŨmU
P16U7	SEQ ID NO:15 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:16 UUCUGAĀCACAUGCAUGGCŨmU
P16U15	SEQ ID NO:17 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:18 UUCUGAACACAUGCĀUGGCŨmU
P16U16	SEQ ID NO:19 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:20 UUCUGAACACAUGCAŨGGCCŨmU
P16U17	SEQ ID NO:21 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:22 UUCUGAACACAUGCAUĜGCŨmU

[00133] In certain embodiments, a UNA oligomer may have a duplex region, and have a UNA monomer in the second strand within the duplex region, where the UNA monomer in the second strand is present in any of positions 1 through 19, counting from the 5' end of the second strand.

[00134] In certain embodiments, a UNA oligomer may have a duplex region, and have a UNA monomer in the second strand within the duplex region, where the UNA monomer in the second strand is present in any of positions 6, 7, 14, 15 and 16, counting from the 5' end of the second strand.

[00135] In some embodiments, a UNA oligomer may comprise an overhang portion of two monomers in length, or longer, at the 3' end of the first strand, wherein the overhang monomer immediately flanking the duplex portion is a UNA monomer.

[00136] In some embodiments, a UNA oligomer may comprise an overhang portion of two monomers in length, or longer, at the 3' end of the second strand, wherein the overhang monomer immediately flanking the duplex portion is a UNA monomer.

[00137] Methods for treating amyloidosis

[00138] Methods of this invention include the treatment and prevention of TTR-related amyloidosis in human and mammalian subjects.

[00139] In the methods of this invention, a subject in need of treatment or prevention can be administered an effective amount of a UNA oligomer. Administration can be performed for 1, 2, or up to 7 days, or 1, 2, 3, or up to 4 weeks, or longer.

[00140] The subject may have TTR-related amyloidosis, also known as ATTR.

[00141] In particular, a subject can have a V30M gene. The methods of this invention can selectively reduce V30M TTR in the subject.

[00142] In some embodiments, a method of this invention can selectively reduce V30M TTR in the subject by at least 10%, as compared to control. In certain embodiments, V30M TTR in the subject can be reduced by at least 20%, or 30%, or 50%, as compared to control.

[00143] An effective amount of a UNA oligomer of this invention can be a dose ranging from 0.001 mg/kg to 50.0 mg/kg.

[00144] In the methods of this invention, TTR mRNA expression can be reduced in a subject for at least 5 days. In certain embodiments, TTR mRNA expression can be reduced in a subject for at least 10 days, or 15 days.

[00145] In the methods of this invention, peripheral neuropathy or autonomic neuropathy in the subject can be reduced.

[00146] In the methods of this invention, peripheral neuropathy or autonomic neuropathy in the subject can be reduced. In some embodiments, a subject may undergo

reduced lower extremity weakness, reduced pain, or improved sensation. Methods of this invention can reduce occurrence of vitreous opacities in the subject.

[00147] In the methods of this disclosure, the administration of a UNA oligomer may not result in an inflammatory response.

[00148] In further embodiments, this invention includes methods for inhibiting expression of a TTR gene in a cell, by treating the cell with a UNA oligomer.

[00149] In additional embodiments, this invention includes methods for inhibiting expression of a TTR gene in a mammal, by administering to the mammal a composition containing a UNA oligomer.

[00150] Pharmaceutical compositions

[00151] In some aspects, this invention provides pharmaceutical compositions containing a UNA oligomer and a pharmaceutically acceptable carrier.

[00152] A pharmaceutical composition can be capable of local or systemic administration. In some aspects, a pharmaceutical composition can be capable of any modality of administration. In certain aspects, the administration can be intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, or nasal administration.

[00153] Embodiments of this invention include pharmaceutical compositions containing a UNA oligomer in a lipid formulation.

[00154] In some embodiments, a pharmaceutical composition may comprise one or more lipids selected from cationic lipids, anionic lipids, sterols, pegylated lipids, and any combination of the foregoing.

[00155] In certain embodiments, a pharmaceutical composition can be substantially free of liposomes.

[00156] In further embodiments, a pharmaceutical composition can include liposomes.

[00157] In additional embodiments, a pharmaceutical composition can contain a UNA oligomer within a viral or bacterial vector.

[00158] A pharmaceutical composition of this disclosure may include carriers, diluents or excipients as are known in the art. Examples of pharmaceutical compositions are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro ed. 1985).

[00159] Examples of excipients for a pharmaceutical composition include antioxidants, suspending agents, dispersing agents, preservatives, buffering agents, tonicity agents, and surfactants.

[00160] EXAMPLES

[00161] **Example 1:** Fig. 1 shows the single nucleotide polymorph (SNP) that exists at position 284 in the V30M mutation of the human TTR mRNA, as compared to the wild type (WT) TTR mRNA. Conventional siRNAs that are complementary to the WT mRNA were tiled around position 284. Fig. 2 shows that the conventional siRNAs complementary to the WT mRNA have limited activity in silencing the WT TTR gene, as measured by TTR knockdown in HepG2 cells. Positions 5, 9, 14 and 15 appear to be more accessible to silencing than other positions. Fig. 3 shows conventional siRNAs that were complementary to the V30M mRNA were tiled around position 284. Four conventional siRNA variations, namely V30M-P5, V30M-P9, V30M-P14, and V30M-P15, were prepared. Also, as shown in Fig. 3, two gene reporter variants, V30V and V30M, each bearing nucleotide sequence 264 to 304 of human TTR, V30V being without the point mutation at position 284, and V30M containing the point mutation at position 284, were prepared and used in the PSICHECK reporter system in the 3'-UTR region of Luciferase gene.

[00162] **Example 2:** Fig. 4 shows the activity of the four conventional siRNA variations V30M-P5, V30M-P9, V30M-P14, and V30M-P15 as measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants. The

conventional siRNA variations V30M-P5, V30M-P9, and V30M-P15 were more effective against V30M than V30V. The conventional siRNA variation V30M-P14 was not more effective against V30M than V30V. Fig. 5 shows IC₅₀ analysis for the four conventional siRNA variations V30M-P5, V30M-P9, V30M-P14, and V30M-P15 measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants. The conventional V30M-P15 variant was 5.6 times more effective against V30M than V30V. Thus, the selectivity of the conventional siRNAs against V30M over V30V was no more than 5.6.

[00163] **Example 3:** Fig. 6 shows the structure of UNA oligomers that were effective in silencing V30M TTR, as measured in the PSICHECK reporter assay. Each of the UNA oligomer embodiments, P15U6, P15U7, P15U14, P15U15, and P15U16, contained four UNA monomers. In each UNA oligomer, a first UNA monomer was located at the 5' end of the first strand, also called the passenger strand. In each UNA oligomer, the second strand, also called the guide strand, formed a duplex region of 19 monomers length with the first strand. Each UNA oligomer had a duplex region of 19 monomers, and a two-monomer overhang at each end. In each UNA oligomer, a second UNA monomer was located at the 3' end of the first strand, in the 20th position, which is in an overhang portion. In each UNA oligomer, a third UNA monomer was located at the 3' end of the second strand, in the 20th position, which is in an overhang portion. In the UNA oligomer embodiments, P15U6, P15U7, P15U14, P15U15, and P15U16, a fourth UNA monomer was located in the second strand at positions 6, 7, 14, 15 and 16, respectively, counting from the 5' end of the second strand.

[00164] **Example 4:** Fig. 7 shows the activity of UNA oligomers P15U6, P15U7, P15U15, and P15U16, measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants, as compared to the conventional siRNA V30M-P15. For each of the UNA oligomer embodiments, P15U6, P15U7, P15U15, and P15U16, the UNA oligomers were more effective against V30M than V30V. Surprisingly, the activity of the UNA oligomer P15U6 was substantially and advantageously superior to the

activity of the conventional siRNA V30M-P15, where each is targeted to V30M. Furthermore, Fig. 7 shows the surprising and unexpected result that the selectivity of the UNA oligomers, P15U6, P15U7, P15U15, and P15U16, against V30M over V30V was substantially greater than for the conventional siRNA V30M-P15. In particular, the selectivity of UNA oligomer P15U6 against V30M over V30V was 24, meaning that the IC₅₀ of UNA oligomer P15U6 against V30M (37.6 pM) was 24 times lower than the IC₅₀ of UNA oligomer P15U6 against V30V (919.9 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown by the conventional siRNA.

[00165] Example 5: Fig. 8 shows the structure of UNA oligomers that were effective in silencing V30M TTR, as measured in the PSICHECK reporter assay. Each of the UNA oligomer embodiments, P16U6, P16U7, P16U15, P16U16, and P16U17, contained four UNA monomers. In each UNA oligomer, a first UNA monomer was located at the 5' end of the first strand, also called the passenger strand. In each UNA oligomer, the second strand, also called the guide strand, formed a duplex region of 19 monomers length with the first strand. Each UNA oligomer had a duplex region of 19 monomers, and a two-monomer overhang at each end. In each UNA oligomer, a second UNA monomer was located at the 3' end of the first strand, in the 20th position, which is in an overhang portion. In each UNA oligomer, a third UNA monomer was located at the 3' end of the second strand, in the 20th position, which is in an overhang portion. In the UNA oligomer embodiments, P16U6, P16U7, P16U15, P16U16, and P16U17, a fourth UNA monomer was located in the second strand at positions 6, 7, 15, 16 and 17, respectively, counting from the 5' end of the second strand.

[00166] Example 6: Fig. 9 shows the activity of UNA oligomers P16U6, P16U7, P16U15, and P16U16, measured in the PSICHECK reporter assay against V30V and V30M gene reporter variants, as compared to the conventional siRNA V30M-P16. For each of the UNA oligomer embodiments, P16U6, P16U7, P16U15, and P16U16, the UNA oligomers were more effective against V30M than V30V. Surprisingly, the activity

of each of the UNA oligomers P16U6, P16U7, P16U15, and P16U16, was substantially and advantageously superior to the activity of the conventional siRNA V30M-P16, where each is targeted to V30M. Furthermore, Fig. 9 shows the surprising and unexpected result that the selectivity of the UNA oligomers, P16U6, P16U7, P16U15, and P16U16, against V30M over V30V was substantially greater than for the conventional siRNA V30M-P16. In particular, the selectivity of UNA oligomer P16U6 against V30M over V30V was 23, meaning that the IC₅₀ of UNA oligomer P16U6 against V30M (92.4 pM) was 23 times lower than the IC₅₀ of UNA oligomer P16U6 against V30V (2119 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown by the conventional siRNA.

[00167] Fig. 10 (left) shows the selectivity of UNA oligomer P15U6 against V30M over V30V. The IC₅₀ of UNA oligomer P15U6 against V30M (37.6 pM) was 24 times lower than the IC₅₀ of UNA oligomer P15U6 against V30V (919.9 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown by the conventional siRNA. Fig. 10 (right) shows the surprising and unexpected result that the IC₅₀ of UNA oligomer P16U6 against V30M (92.4 pM) was 23 times lower than the IC₅₀ of UNA oligomer P16U6 against V30V (2119 pM). This selectivity was advantageously 4-fold superior to the selectivity of 5.6 shown by the conventional siRNA.

[00168] Example 7: UNA oligomers reduce V30M TTR deposits in vivo.

Transgenic mice for human TTR V30M overexpression are used at 6 months age. TTR wild-type and TTR knockout mice are used as controls. Animals are housed in controlled environment, and euthanized with ketamine and medetomidine.

[00169] For TTR gene silencing, the TTR UNA oligomer, as well as controls, are delivered in liposome formulations. Mice are injected in the tail vein with TTR UNA oligomer ($n = 6$), at a concentration of 1 mg/kg. Untreated age-matched controls are treated with blank formulation. One injection is given per week for 4 weeks, and animals are sacrificed 48 h after last injection. Liver and colon are removed and collected to 10% formalin and frozen.

[00170] Liver and colon mRNA are isolated using phenol extraction (Invitrogen). Sciatic nerve from V30M mice is dissected from other tissue, and mRNA is extracted with a RNeasy Mini column (Qiagen). cDNA is synthesized with a SuperScript double-stranded cDNA Kit (Invitrogen). Extracted RNA is validated with Experion RNA StdSens Analysis Kit (Bio-Rad). qPCR is performed with primers and iQ Syber Green Super Mix (Bio-Rad). Double immunofluorescence analysis is performed with sciatic nerve, dorsal root ganglia, and colon from V30M animals that is removed and treated as above. Comparisons are performed with Student T-test or One-way ANOVA. Data are expressed as mean values \pm standard error (SEM). *p*-values less than 0.05 are considered significant.

[00171] Injection of a composition containing one or more UNA oligomers in V30M mice reduces the V30M TTR deposits in sciatic nerve, dorsal root ganglia, and colon by at least 90% over controls.

[00172] Injection of a composition containing any one of UNA oligomers P15U6, P15U7, P15U15, or P15U16, or any combination of these UNA oligomers, in V30M mice reduces the V30M TTR deposits in sciatic nerve, dorsal root ganglia, and colon by at least 90% over controls.

[00173] All publications, patents and literature specifically mentioned herein are incorporated by reference for all purposes.

[00174] It is understood that this invention is not limited to the particular methodology, protocols, materials, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which will be encompassed by the appended claims.

[00175] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. As well, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprises," "comprising", "containing," "including", and "having" can be used interchangeably.

[00176] Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

[00177] All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose.

WHAT IS CLAIMED IS:

1. A UNA oligomer for selectively inhibiting V30M TTR expression, the oligomer comprising a first strand and a second strand, each of the strands being 19-29 monomers in length, the monomers comprising UNA monomers and nucleic acid monomers, wherein the oligomer has a duplex structure of from 14 to 29 monomers in length.
2. The UNA oligomer of claim 1, wherein the second strand has at least one UNA monomer in the duplex region.
3. The UNA oligomer of claim 2, wherein the at least one UNA monomer in the second strand is at any one of positions 2-8 from the 5' end.
4. The UNA oligomer of claim 2, wherein the at least one UNA monomer in the second strand is at any one of positions 9-18 from the 5' end.
5. The UNA oligomer of claim 2, wherein the at least one UNA monomer in the second strand is at position 6, 7, 15, 16 or 17 from the 5' end.
6. The UNA oligomer of claim 1, wherein the oligomer has an IC50 for reducing V30M TTR expression of less than 20 pM.
7. The UNA oligomer of claim 1, wherein the oligomer has a selectivity ratio of at least 10, wherein the selectivity ratio is the ratio of the IC50 for reducing wild type TTR expression to the IC50 for reducing V30M TTR expression.
8. The UNA oligomer of claim 7, wherein the oligomer has a selectivity ratio of at least 20.
9. The UNA oligomer of claim 7, wherein the oligomer has a selectivity ratio in vitro of at least 50.
10. The UNA oligomer of claim 1, wherein the oligomer selectively inhibits V30M TTR expression in vivo.

11. The UNA oligomer of claim 1, wherein the oligomer selectively inhibits V30M TTR expression *ex vivo*.
12. The UNA oligomer of claim 1, comprising at least one nucleic acid monomer that is base-modified, sugar-modified, or linkage modified.
13. The UNA oligomer of claim 1, wherein the oligomer has a blunt end, or has one or more overhangs.
14. The UNA oligomer of claim 1, wherein the first and second strands are connected and form a duplex region with a loop at one end.
15. A pharmaceutical composition comprising a UNA oligomer of any one of claims 1-14 and a pharmaceutically acceptable carrier.
16. The pharmaceutical composition of claim 15, comprising a lipid formulation.
17. The pharmaceutical composition of claim 15, comprising one or more lipids selected from cationic lipids, anionic lipids, sterols, pegylated lipids, and any combination of the foregoing.
18. The pharmaceutical composition of claim 15, wherein the composition is substantially free of liposomes.
19. The pharmaceutical composition of claim 15, wherein the composition contains liposomes.
20. A method for treating or preventing TTR-related amyloidosis, comprising administering to a subject in need an effective amount of a UNA oligomer of any one of claims 1-14.
21. The method of claim 20, wherein the TTR-related amyloidosis is ATTR.
22. The method of claim 20, wherein the subject is human.
23. The method of claim 20, wherein the subject comprises a V30M gene.

24. The method of claim 20, wherein the method selectively reduces V30M TTR in the subject.
25. The method of claim 20, wherein the administering is local or systemic.
26. The method of claim 20, wherein the administering is intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, or oral.
27. The method of claim 20, wherein the method selectively reduces V30M TTR in the subject by at least 10% greater than control.
28. The method of claim 20, wherein the effective amount is a dose of from 0.001 to 50.0 mg/kg.
29. The method of claim 20, wherein TTR mRNA expression is reduced for at least 5 days.
30. The method of claim 20, wherein the method reduces peripheral neuropathy or autonomic neuropathy in the subject.
31. The method of claim 20, wherein the administration does not result in an inflammatory response.
32. A method for inhibiting expression of a TTR gene in a cell, comprising treating the cell with a UNA oligomer of any one of claims 1-14.
33. A method for inhibiting expression of a TTR gene in a mammal, comprising administering to the mammal a UNA oligomer of any one of claims 1-14.

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SEQ ID NOS: 23-38

V30M TTR mRNA

3'- 304-UCGGAAAGACUUGUGUACGUACCGGUGUAACUACCGUCCUG-264
 3'- 304-UCGGAAAGACUUGUGUACGUACCGGUGUAACUACCGUCCUG-264

wt TTR mRNA

V30V-P2: ACGGCCACAUUGAUUGGCAGdTdT

V30V-P3: CACGGCCACAUUGAUUGGCAdTdT

V30V-P4: GCACGGCCACAUUGAUUGGCdTdT

V30V-P5: UGCACGGCCACAUUGAUUGGdTdT

V30V-P6: AUGCACGGCCACAUUGAUUGdTdT

V30V-P7: CAUGCACGGCCACAUUGAUdTdT

V30V-P8: ACAUGCACGGCCACAUUGAdTdT

V30V-P9: CACAUGCACGGCCACAUUUGdTdT

V30V-P10: ACACAUGCACGGCCACAUUdTdT

V30V-P11: AACACAUGCACGGCCACAUDTdT

V30V-P12: GAACACAUGCACGGCCACAdTdT

V30V-P13: UGAACACAUGCACGGCCACdTdT

V30V-P14: CUGAACACAUGCACGGCCAdTdT

V30V-P15: UCUGAACACAUGCACGGCCdTdT

FIG. 1

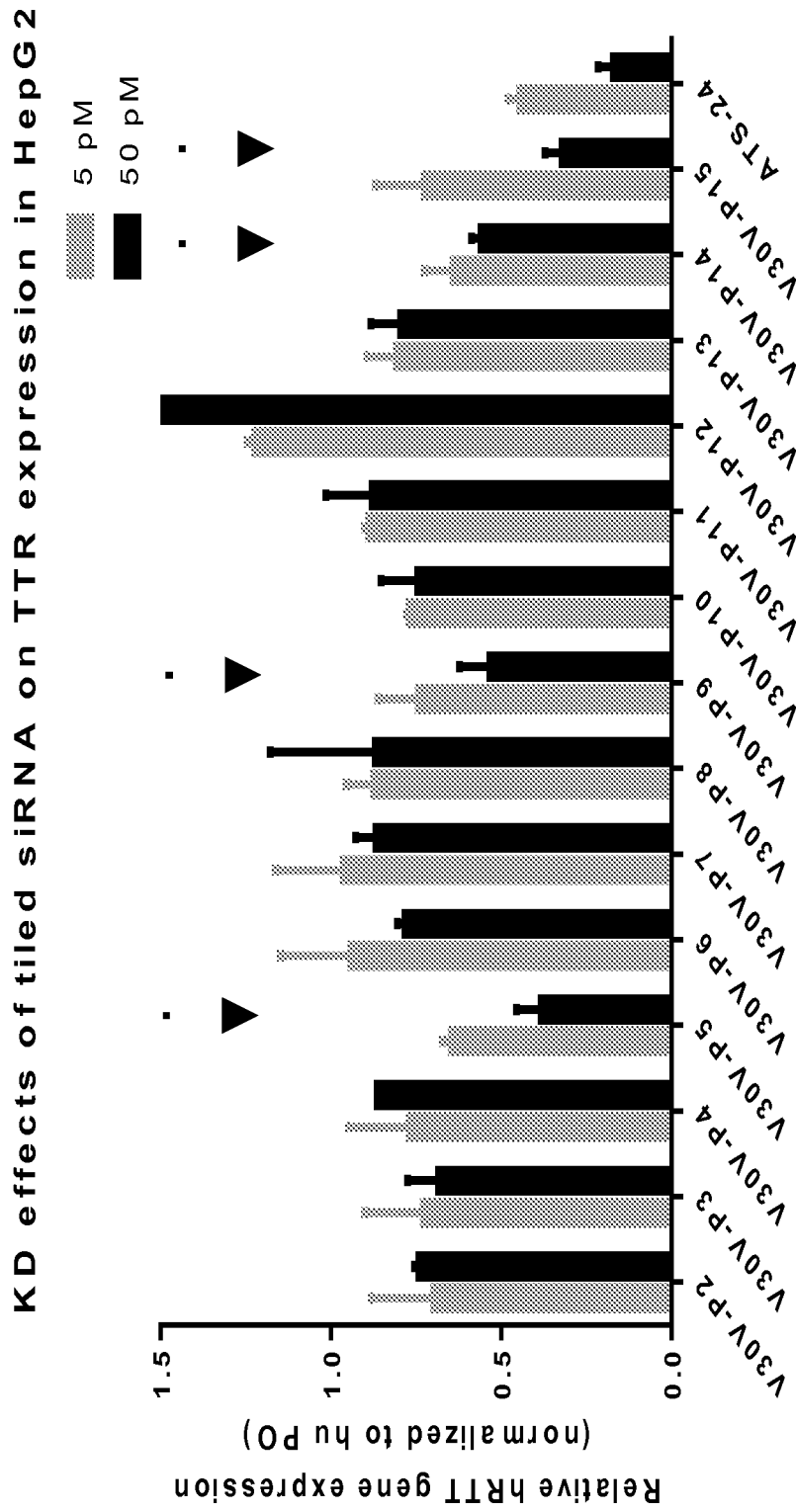
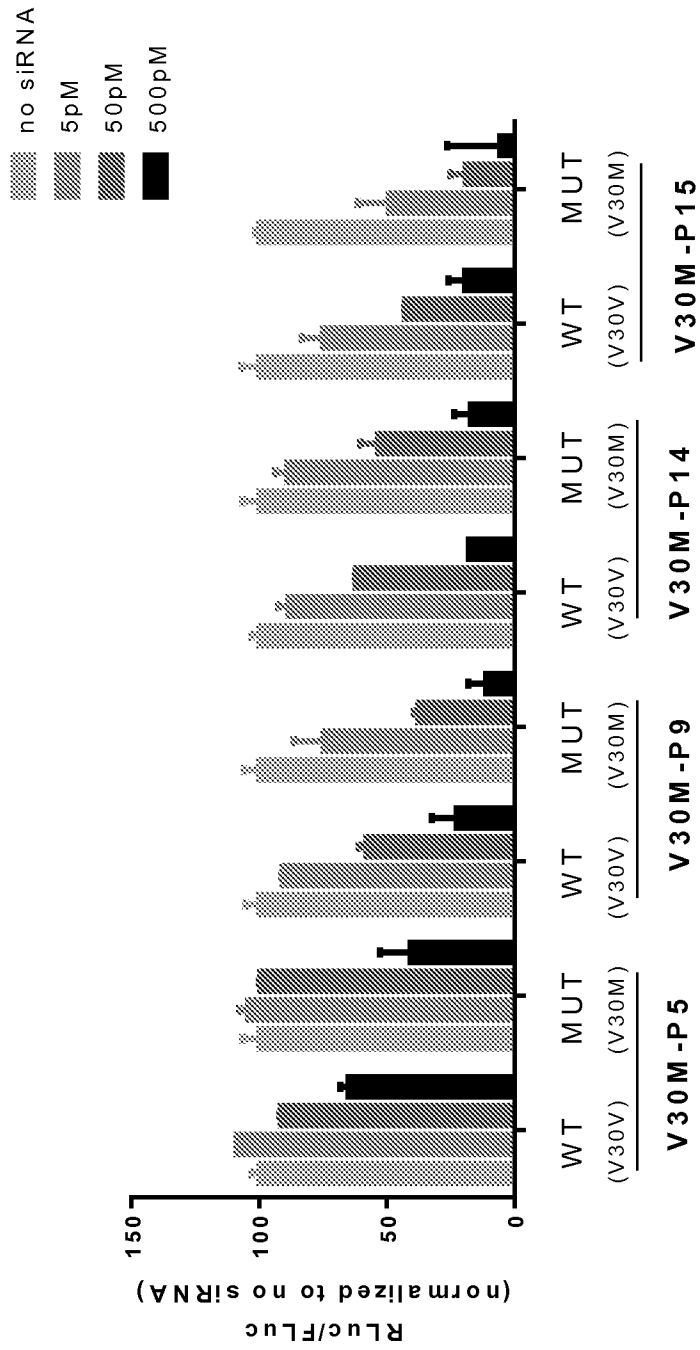


FIG. 2

284 SEQ ID NOS: 39-48
*

V30M TTR mRNA
 3'-304-UCGGAAAGACUUGUGUACGU**AC**CGGUGUAACUACCGUCCUG-264
 3'-304-UCGGAAAGACUUGUGUACGU**G**CCGGUGUAACUACCGUCCUG-264
 wt TTR mRNA V30V-P5 : UGC**A**CGGCCACAUUGAUGGdTdT
 V30V-P9 : CACAUGC**A**CGGCCACAUUGdTdT
 V30V-P14 : CUGAACACAUGC**A**CGGCCAdTdT
 V30V-P15 : UCUGAACACAUGC**A**CGGCCdTdT
 .
 ▼
 V30M-P5 : UGC**A**UGGCCACAUUGAUGGdTdT
 V30M-P9 : CACAUGC**A**UGGCCACAUUGdTdT
 V30M-P14 : CUGAACACAUGC**A**UGGCCAdTdT
 V30M-P15 : UCUGAACACAUGC**A**UGGCCdTdT

FIG. 3



siRNA ID

FIG. 4

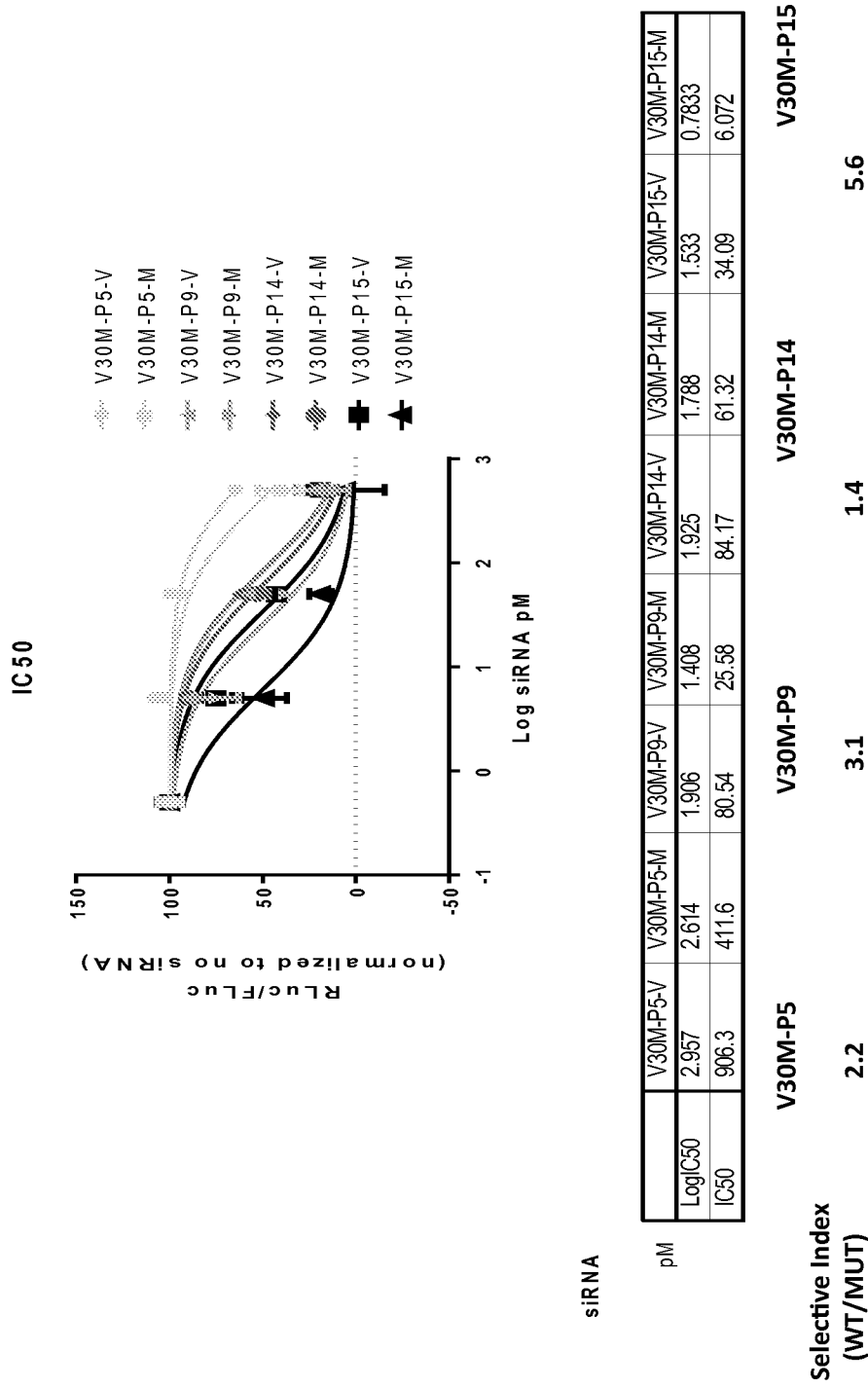
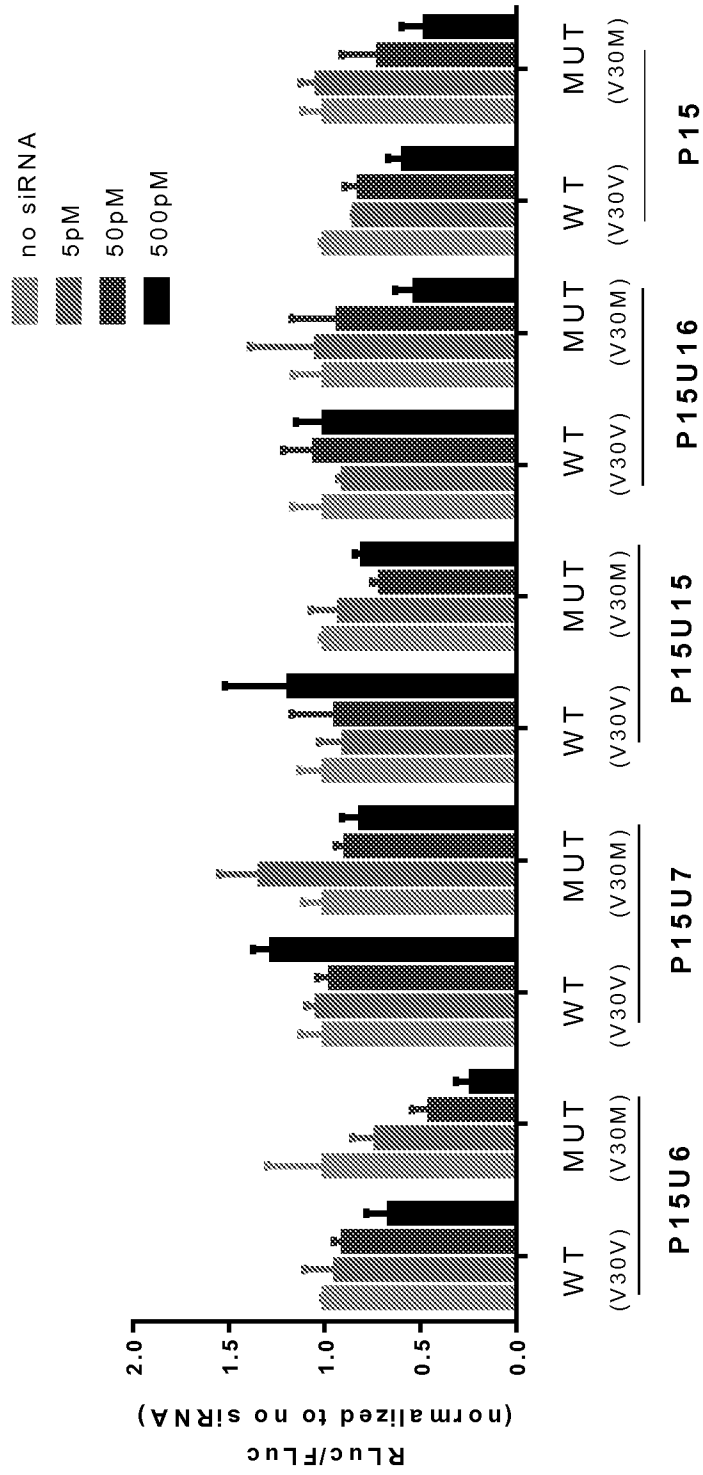


FIG. 5

siRNA ID	SS ID	SEQ ID NOS: 49-54		AS ID	SEQ ID NOS: 55-60	
		seq. (5' -> 3')	seq. (5' -> 3')		seq. (5' -> 3')	seq. (5' -> 3')
P15	P15SS	GGCCAUGCAUGUGUUCAGAdTdT		P15AS	UCUGAACACACAUGCAUGGCCdTdT	
P15U6	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U6AS	UCUGAĀCACACAUGCAUGGCCŪmL	
P15U7	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U7AS	UCUGAAĀCACACAUGCAUGGCCŪmL	
P15U14	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U14AS	UCUGAACACACAUGCĀUGGCCŪmL	
P15U15	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U15AS	UCUGAACACACAUGCĀUGGCCŪmL	
P15U16	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U16AS	UCUGAACACACAUGCĀUGGCCŪmL	

FIG. 6



siRNA ID

FIG. 7

siRNA ID	SS ID	SEQ ID NOS: 61-66		AS ID	SEQ ID NOS: 67-72	
		seq. (5' -> 3')	seq. (5' -> 3')		seq. (5' -> 3')	seq. (5' -> 3')
P16	P16SS	GCCAUGCAUGUGUUUCAGAAAdTdT		P16AS	UUCUGAACACACAUGCAUGGCdTdT	
P16U6	P16USS	ĜCCAUGCAUGUGUUUCAGAAŪmU		P16U6AS	UUCUGĀACACAUGCAUGGCŪmU	
P16U7	P16USS	ĜCCAUGCAUGUGUUUCAGAAŪmU		P16U7AS	UUCUGAĀĀCACAUGCAUGGCŪmU	
P16U15	P16USS	ĜCCAUGCAUGUGUUUCAGAAŪmU		P16U15AS	UUCUGAACACACAUGCĀUGGCŪmU	
P16U16	P16USS	ĜCCAUGCAUGUGUUUCAGAAŪmU		P16U16AS	UUCUGAACACACAUGCAŪGGCŪmU	
P16U17	P16USS	ĜCCAUGCAUGUGUUUCAGAAŪmU		P16U17AS	UUCUGAACACACAUGCAUGĜGCŪmU	

FIG. 8

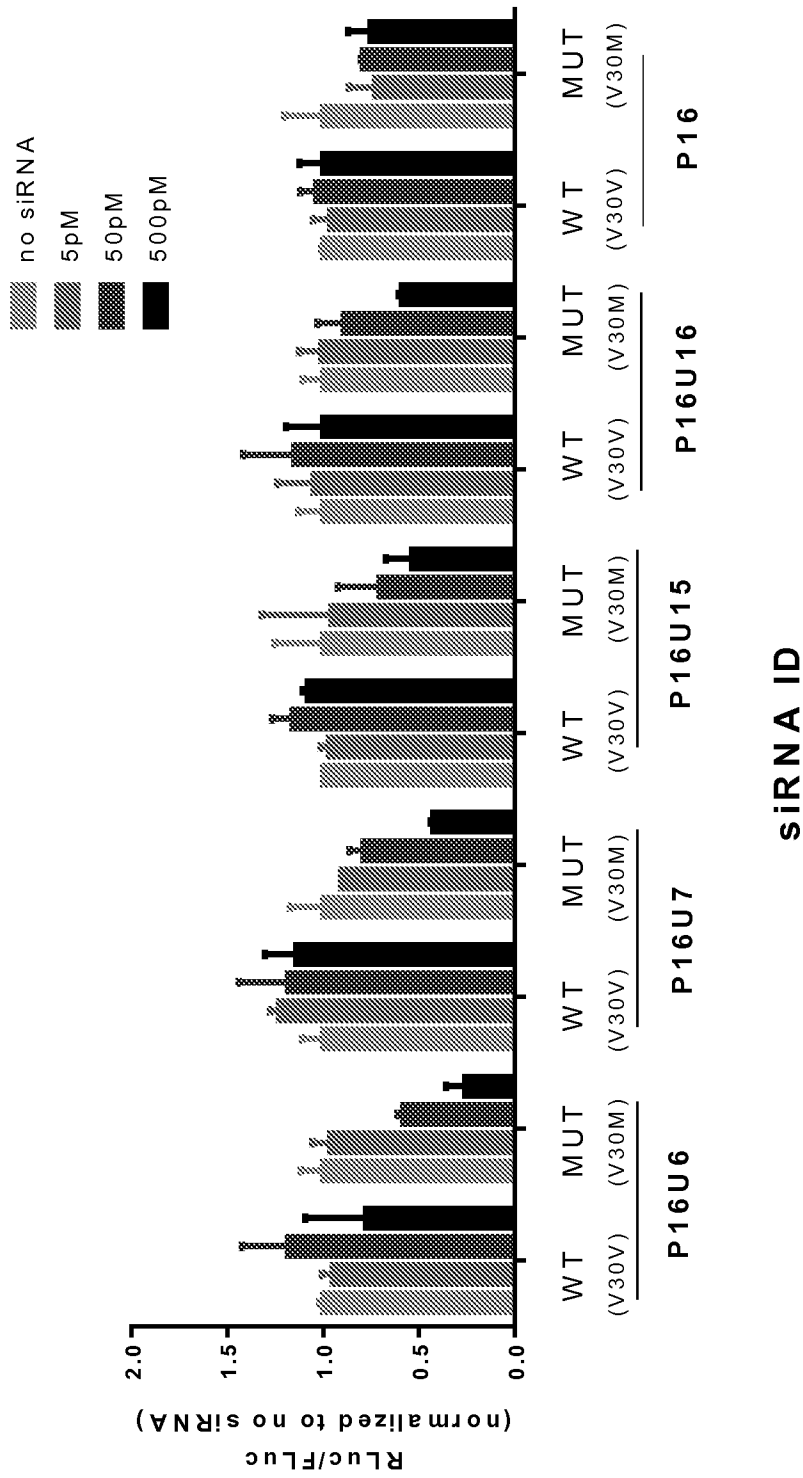


FIG. 9

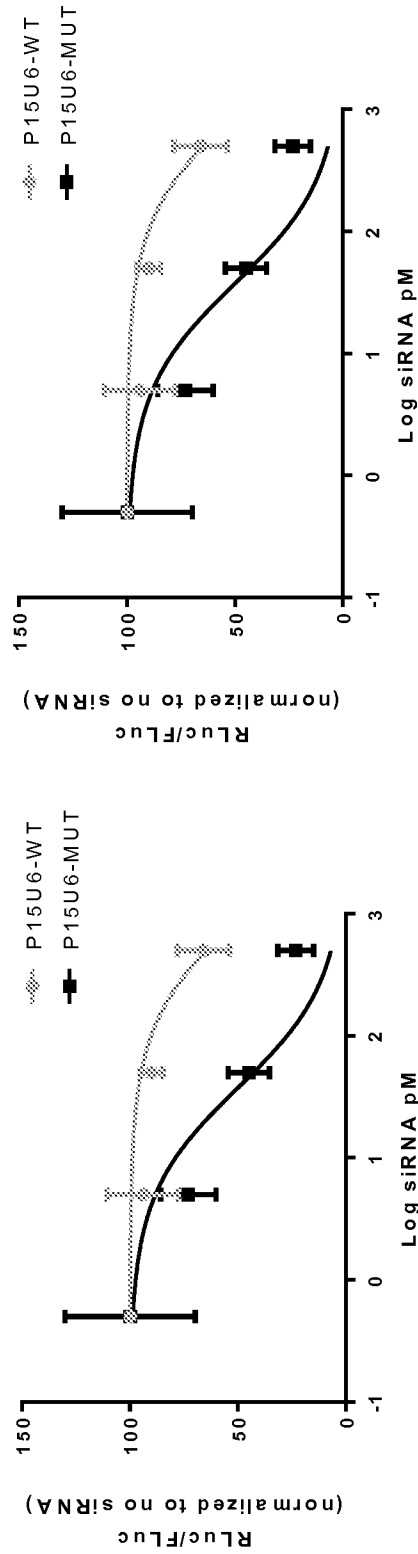


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/022345

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 31/713 (2015.01) CPC - C12N 15/113 (2015.04) 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) IPC(8) - A61K 31/7088, 31/713; A61P 25/00, 25/02; C12N 5/071, 15/11, 15/113 (2015.01) CPC - A61K 31/713; C12N 15/11, 15/111, 15/113, 2310/10, 2310/32, 2310/3231 (2015.04)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched CPC - A61K 31/713; C12N 15/11, 15/111, 15/113, 2310/10, 2310/32, 2310/3231 (2015.04) (keyword delimited) USPC - 424/179.1; 435/255.1, 255.2, 255.5, 256.1, 348, 358, 363, 366, 375, 410; 514/21.2; 530/358		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatBase, PubMed, Google Patents, Google Search terms used: transthyretin TTR ATTR V30M CTS CTS1 HEL111 HsT2651 PALB TBPA UNA monomer* acyclic derivatives UNA oligomer RNA mimics lock* unlock*		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document; with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/0190383 A1 (VAISH et al) 25 July 2013 (25.07.2013) entire document	1-13
Y		14-33
Y	WO 2004/108897 A2 (CYTOKINETICS, INC) 16 December 2004 (16.12.2004) entire document	14
Y	WO 2014/037436 A1 (BIOMEDICAL RESEARCH FOUNDATION OF THE ACADEMY OF ATHENS et al) 13 March 2014 (13.03.2014) entire document	15-19
Y	US 2005/0129778 A1 (MULYE) 16 June 2005 (16.06.2005) entire document	18
Y	US 2012/0225927 A1 (SAH et al) 06 September 2012 (06.09.2012) entire document	20-33
Y	WO 2011/123468 A1 (ALNYLAM PHARMACEUTICALS, INC) 06 October 2011 (06.10.2011) entire document	31
P, Y	"Arctus to present gene knockdown data in non-human primates, showing up to 94% reduction in gene expression with single low dose," Pharmabiz.com, 14 October 2014 (14.10.2014), Pg. 1. Retrieved from the Internet: <www.pharmabiz.com/PrintArticle.aspx?aid=84566&sid=2> on 08 June 2015 (08.06.2015). entire document	1-33
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* 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 08 June 2015		Date of mailing of the international search report 02 JUL 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/022345

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 filed or furnished:

a. (means)

on paper

in electronic form

b. (time)

in the international application as filed

together with the international application in electronic form

subsequently to this Authority for the purposes of search

2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 3-12 were searched.