

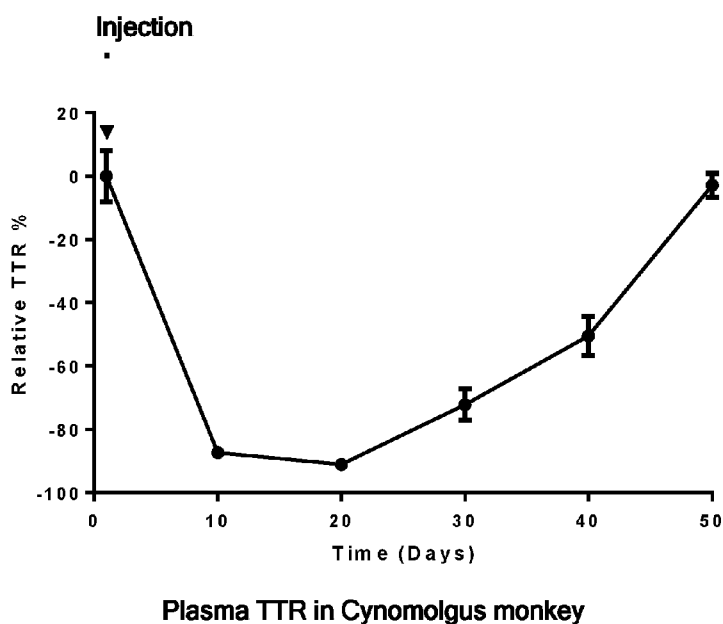


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(54) Title: COMPOSITIONS FOR TREATING AMYLOIDOSIS

FIG. 11



(57) Abstract: This invention encompasses compounds and compositions useful in methods for medical therapy, in general, for inhibiting expression of a TTR gene in a subject. The compounds have 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, and the compounds are targeted to a sequence of a TTR gene.



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COMPOSITIONS FOR TREATING AMYLOIDOSIS

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent Application No. 14/807,223, filed July 23, 2015, the contents of which is herein incorporated by reference in its entirety.

REFERENCE TO THE SEQUENCE LISTING

[0002] This application includes a Sequence Listing submitted electronically as an ASCII file, named ARC1241WO_SL.txt.

FIELD OF THE INVENTION

[0003] This invention relates to the fields of biopharmaceuticals and therapeutics that are operable by gene silencing. More particularly, this invention relates to the structures, compositions and uses of active agents for inhibiting expression of a TTR gene in a subject. The active agents are UNA oligomers that can be used for gene silencing, and among other things, in methods for treating transthyretin-related amyloidosis.

BACKGROUND OF THE INVENTION

[0004] 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.

[0005] 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.

[0006] 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.

[0007] 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.

[0008] 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.

[0009] There is currently no pharmacological therapy that can undo the formation of TTR amyloid.

[0010] There is a continuing need for therapeutics for ATTR and other amyloid-related diseases.

[0011] There is a long-standing need for gene silencing agents that can selectively downregulate a disease-related allele.

[0012] There is also a need for active agents that can provide efficient and specific knockdown of TTR.

BRIEF SUMMARY

[0013] 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.

[0014] 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.

[0015] Embodiments of this invention include the following:

[0016] 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.

[0017] 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.

[0018] The UNA oligomer above, wherein the oligomer has an IC₅₀ for reducing V30M TTR expression of less than 20 pM.

[0019] 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.

[0020] 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.

[0021] The UNA oligomer above, comprising at least one nucleic acid monomer that is base-modified, sugar-modified, or linkage modified.

[0022] A compound 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 compound has a duplex region of from 14 to 29 contiguous monomers in length, wherein the first strand is a passenger strand for RNA interference and the second strand is a guide strand for RNA interference, and wherein the compound comprises a sequence of bases targeted to inhibit expression of a TTR gene. In some embodiments, the compound may contain from one to seven UNA monomers.

[0023] In certain embodiments, the compound may contain a UNA monomer at the 1-end (5' end for non-UNA) of the first strand, a UNA monomer at the second position from the 3' end of the first strand, and a UNA monomer at the second position from the 3' end of the second strand.

[0024] In further embodiments, the compound may contain a UNA monomer at any one or more of positions 2 to 8 from the 5' end of the second strand.

[0025] In additional embodiments, the compound may have a 3' overhang comprising one or more UNA monomers, natural nucleotides, non-natural nucleotides, modified nucleotides, or chemically-modified nucleotides, or combinations thereof. The compound may have a 3' overhang comprising one or more deoxythymidine nucleotides, 2'-O-methyl nucleotides, inverted abasic monomers, inverted thymidine monomers, L-thymidine monomers, or glyceryl nucleotides.

[0026] Embodiments of this invention contemplate compounds in which one or more of the nucleic acid monomers may be a non-natural nucleotide, a modified nucleotide, or a chemically-modified nucleotide. A nucleic acid monomer may have a 2'-O-methyl group, a 2'-methoxyethoxy, or a 2'-deoxy-2'-fluoro ribonucleotide.

[0027] In some embodiments, the compound may not contain fluorine.

[0028] A compound may have one or more of three monomers at each end of each strand being connected by a phosphorothioate, a chiral phosphorothioate, or a phosphorodithioate linkage.

[0029] This invention further contemplates a lipid nanoparticle-oligomer compound comprising one or more compounds above attached to the lipid nanoparticle, as well as compositions containing one or more compounds above and a

pharmaceutically acceptable carrier. In certain embodiments, the carrier may include lipid nanoparticles or liposomes.

[0030] A composition of this disclosure, upon administering a single intravenous dose to a subject, may reduce TTR protein in the subject by at least 80% after 10 days, or at least 70% after 20 days, or at least 50% after 30 days.

[0031] Embodiments of this invention further include methods for treating or preventing TTR-related amyloidosis in a subject in need, by administering to the subject an effective amount of a composition above to the subject. The TTR-related amyloidosis can be ATTR. The administering can be intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, or dermal. The effective amount can be a dose of from 0.001 to 10.0 mg/kg.

[0032] In some embodiments, upon administering a single dose to a subject, the composition may reduce TTR protein in the subject by at least 70% after 20 days, or by at least 50% after 30 days.

[0033] This invention includes methods for inhibiting expression of a TTR gene in a subject, by administering to the subject a composition above. Further aspects include the use of a composition above for preventing, ameliorating or treating a disease or condition associated with TTR-related amyloidosis in a subject in need, or for use in medical therapy, or for use in the treatment of the human or animal body.

[0034] This invention further includes compositions above for use in preparing or manufacturing a medicament for preventing, ameliorating or treating a disease or condition associated with TTR-related amyloidosis in a subject in need.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] FIG. 11: Fig. 11 shows knockdown of Transthyretin protein in vivo as a result of treatment with UNA oligomers targeting Transthyretin TTR mRNA, delivered in

a liposomal formulation to male Cynomolgus monkeys. Measurements were made following a single intravenous (IV) dose administration. TTR protein concentration in plasma samples was assessed by LC-MS/MS at various time points. Animals were administered test doses on Day 1 at 0.3 mg/kg. Doses were administered as a 15-minute intravenous (IV) infusion, $\pm$ 1 minute, using a pump and disposable syringe.

DETAILED DESCRIPTION OF THE INVENTION

[0046] 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.

[0047] 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 compositions and methods for treating or preventing TTR-related amyloidosis by administering a UNA oligomer to a subject.

[0048] The UNA oligomers of this invention are capable of allele-specific knockdown of transthyretin.

[0049] 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.

[0050] In certain aspects, this invention provides therapeutics for ATTR and related amyloid-related diseases.

[0051] Aspects of this invention include UNA oligomers that can be used for treating clinical features of ATTR amyloidosis, including neuropathy and/or cardiomyopathy.

[0052] In some embodiments, UNA oligomers of this invention are targeted to one mutation Val-30-Met TTR.

[0053] This invention can provide a pharmacological therapy that can undo the formation of TTR amyloid.

[0054] Homo sapiens transthyretin (TTR) mRNA is found at NCBI Reference Sequence: NM_000371.3.

[0055] In some aspects, a composition of this invention can provide an unexpectedly advantageous duration of action. In certain embodiments, upon administering a single dose of a composition containing a UNA oligomer of this invention, the TTR protein in a subject may be reduced by at least 90% after 20 days.

[0056] In further embodiments, upon administering a single dose of a pharmaceutical composition containing a UNA oligomer of this invention, the TTR protein in a subject may be reduced by at least 70% for a period of at least 20 days.

[0057] In additional embodiments, upon administering a single dose of a pharmaceutical composition containing a UNA oligomer of this invention, the TTR protein in a subject may be reduced by at least 50% for a period of at least 30 days.

[0058] A subject can be a primate, a human, or other mammal.

[0059] UNA oligomers

[0060] The UNA oligomers of this invention can be used for inhibiting V30M TTR expression.

[0061] 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.

[0062] The monomers of a UNA oligomer can include UNA monomers and nucleic acid monomers

[0063] A UNA oligomer can be a duplex structure of from 14 to 29 monomers in length.

[0064] 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.

[0065] A UNA oligomer of this invention may have any number of UNA monomers within its total length.

[0066] A UNA oligomer can include a nucleic acid monomer that is base-modified, sugar-modified, or linkage modified.

[0067] Embodiments of this invention further provide UNA oligomers that selectively inhibit V30M TTR expression.

[0068] In certain embodiments, a UNA oligomer has an IC₅₀ for reducing V30M TTR expression in vitro of less than 20 pM.

[0069] 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.

[0070] In some aspects, a UNA oligomer of this invention can selectively inhibit V30M TTR expression in vivo.

[0071] In certain aspects, a UNA oligomer of this invention can selectively inhibit V30M TTR expression ex vivo.

[0072] A UNA oligomer can be 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.

[0073] The UNA monomers are novel, synthetic molecules that can be attached in a chain to form an oligomer.

[0074] The nucleic acid monomers can be naturally-occurring nucleotides, modified naturally-occurring nucleotides, or certain non-naturally- occurring nucleotides.

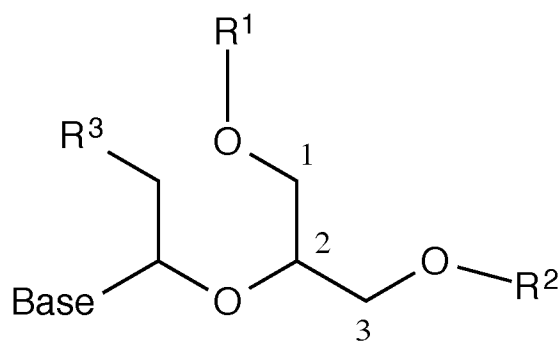
[0075] A UNA oligomer of this invention is a synthetic, pharmacologically active molecule and can be used in the treatment of a condition or disease.

[0076] 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.

[0077] 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.

[0078] UNA monomers

[0079] In some embodiments, linker group monomers can be unlocked nucleomonomers (UNA monomers), which are small organic molecules based on a propane-1,2,3-tri-yl-trisoxy structure as shown below:

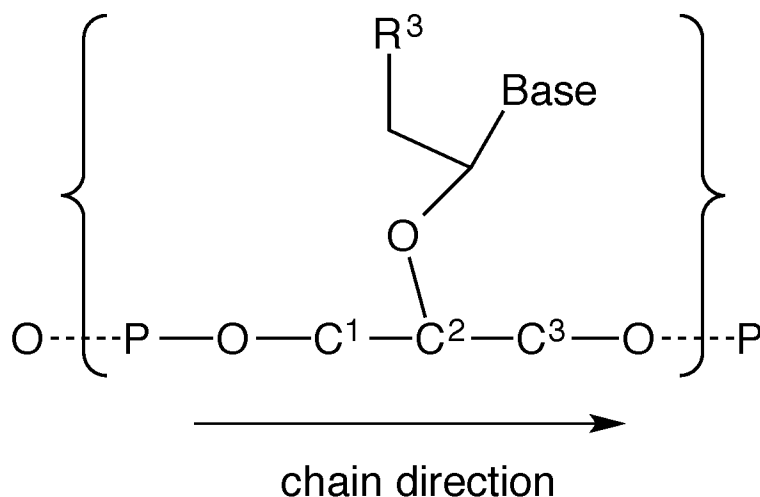


UNA MONOMER

where R¹ and R² are H, and R¹ and R² can be phosphodiester linkages, Base can be a nucleobase, and R³ is a functional group described below.

[0080] In another view, the UNA monomer main atoms can be drawn in IUPAC notation as follows:

UNA monomer unit

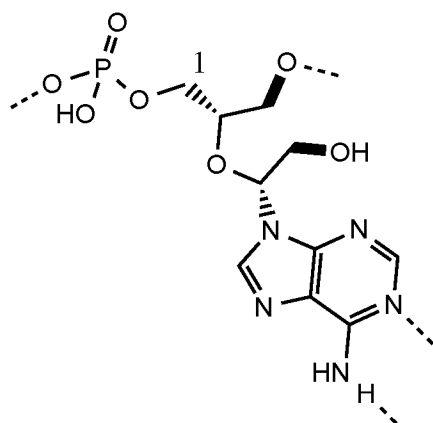


where the direction of progress of the oligomer chain is from the 1-end to the 3-end of the propane residue.

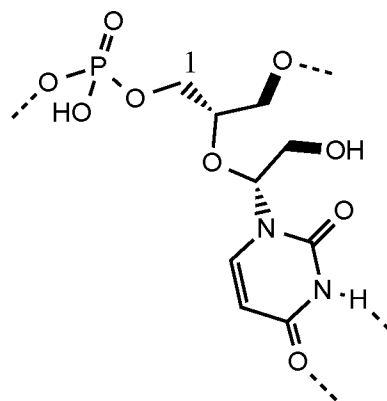
[0081] Examples of a nucleobase include uracil, thymine, cytosine, 5-methylcytosine, adenine, guanine, inosine, and natural and non-natural nucleobase analogues.

[0082] 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.

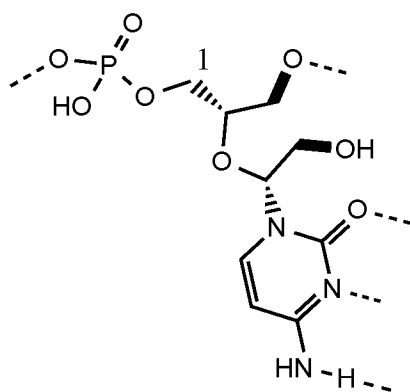
[0083] Examples of UNA monomer as internal monomers flanked at both the propane-1-yl position and the propane-3-yl position, where R^3 is -OH, are shown below.



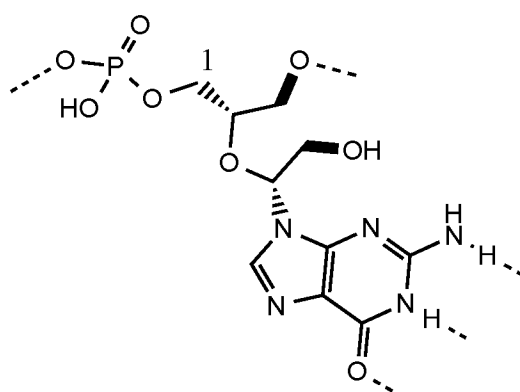
UNA-A



UNA-U



UNA-C



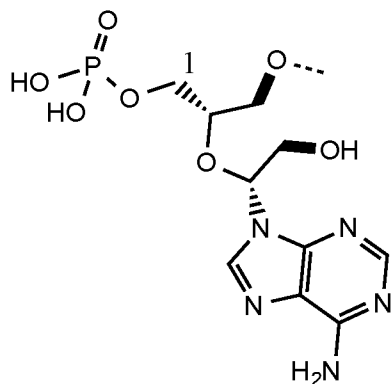
UNA-G

[0084] 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.

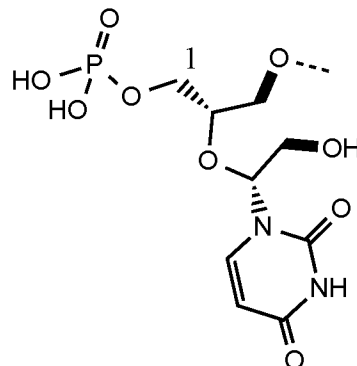
[0085] A UNA monomer can be a terminal monomer in an overhang of an oligomer, where the UNA monomer is attached to only one monomer at either the propane-1-yl position or the propane-3-yl position. In this form, the UNA monomer does not participate in base pairing. Because the UNA monomers are flexible organic

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

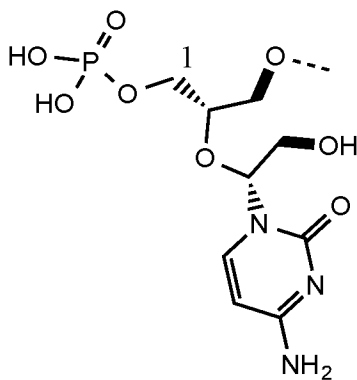
[0086] Examples of a UNA monomer as a terminal monomer attached at the propane-3-yl position are shown below.



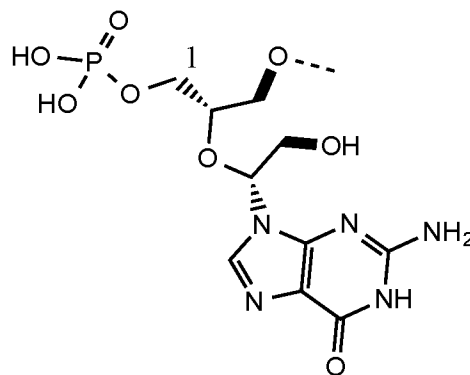
terminal UNA-A



terminal UNA-U

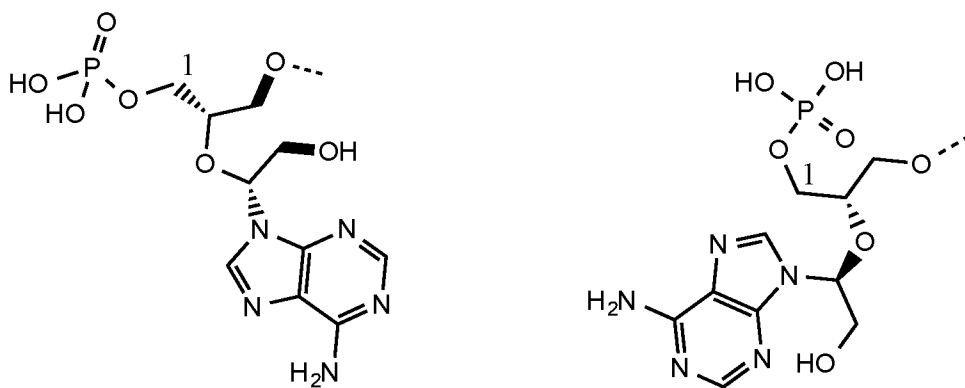


terminal UNA-C



terminal UNA-G

[0087] 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.

[0088] 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.

[0089] In some embodiments, the functional group R^3 of a UNA monomer can be $—OR^4$, $—SR^4$, $—NR^4$, $—NH(C=O)R^4$, 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.

[0090] 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.

[0091] 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.

[0092] 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 $\tilde{G}$).

[0093] Designations that may be used herein include mA, mG, mC, and mU, which refer to the 2'-O-Methyl modified ribonucleotides.

[0094] Designations that may be used herein include lower case c and u, which refer to the 2'-O-methyl modified ribonucleotides.

[0095] Designations that may be used herein include dT, which refers to a 2'-deoxy T nucleotide.

[0096] Monomers for UNA oligomers

[0097] As used herein, in the context of oligomer sequences, the symbol X represents a UNA monomer.

[0098] As used herein, in the context of oligomer sequences, the symbol N represents any natural nucleotide monomer, or a modified nucleotide monomer.

[0099] As used herein, in the context of oligomer sequences, the symbol Q represents a non-natural, modified, or chemically-modified nucleotide monomer. When a Q monomer appears in one strand of the oligomer, and is unpaired with the other strand, the monomer can have any base attached. When a Q monomer appears in one strand of the oligomer, and is paired with a monomer in the other strand, the Q monomer can have any base attached that would be complementary to the monomer in the corresponding paired position in the other strand.

[00100] Examples of nucleic acid monomers include non-natural, modified, and chemically-modified nucleotides, including any such nucleotides known in the art.

[00101] 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.

[00102] 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, and L-thymidine.

[00103] 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.

[00104] 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.

[00105] Examples of non-natural, modified, and chemically-modified nucleotide monomers include N⁶-methyladenosine nucleotides.

[00106] 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.

[00107] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-O-aminopropyl substituted nucleotides.

[00108] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-O-guanidinopropyl substituted nucleotides.

[00109] Examples of non-natural, modified, and chemically-modified nucleotide monomers include Pseudouridines.

[00110] 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, or a 2'-azido, where R can be H, alkyl, fluorine-substituted alkyl, alkenyl, or alkynyl.

[00111] Examples of non-natural, modified, and chemically-modified nucleotide monomers include replacing the 2'-OH group of a nucleotide with a 2'-R or 2'-OR, where R can be CN, CF₃, alkylamino, or aralkyl.

[00112] Examples of non-natural, modified, and chemically-modified nucleotide monomers include nucleotides with a modified sugar such as an F-HNA, an HNA, a CeNA, a bicyclic sugar, or an LNA.

[00113] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-oxa-3'-aza-4'a-carbanucleoside monomers, 3-hydroxymethyl-5-(1*H*-1,2,3-triazol)-isoxazolidine monomers, and 5'-triazolyl-2'-oxa-3'-aza-4'a-carbanucleoside monomers.

[00114] Some examples of modified nucleotides are given in Saenger, Principles of Nucleic Acid Structure, Springer-Verlag, 1984.

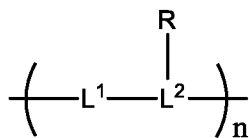
[00115] Details of UNA oligomer structure

[00116] Aspects of this invention can provide structures and compositions for UNA-containing oligomeric compounds. The oligomeric agents may incorporate one or more UNA monomers. Oligomeric molecules of this invention can be used as active agents in formulations for gene regulating or gene silencing therapeutics.

[00117] In some embodiments, this invention provides oligomeric compounds having a structure that incorporates novel combinations of UNA monomers with certain natural nucleotides, non-natural nucleotides, modified nucleotides, or chemically-modified nucleotides.

[00118] In further aspects, the oligomeric compounds can be pharmacologically active molecules. UNA oligomers of this invention can be used as active pharmaceutical ingredients for regulating gene expression, and in RNA interference methods, as well as antisense, RNA blocking, and micro-RNA strategies.

[00119] A UNA oligomer of this invention can have the structure of Formula I



Formula I

wherein L^1 is a linkage, n is from 19 to 29, and for each occurrence L^2 is a UNA linker group having the formula $\text{---C}^1\text{---C}^2\text{---C}^3\text{---}$, where R is attached to C^2 and has the formula $\text{---OCH}(\text{CH}_2\text{R}^3)\text{R}^5$, where R^3 is ---OR^4 , ---SR^4 , ---NR^4_2 , $\text{---NH}(\text{C=O})\text{R}^4$, 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 is H, alkyl, a cholesterol, a lipid molecule, a polyamine, an amino acid, or a polypeptide, and where R^5 is a nucleobase, or $\text{L}^2(\text{R})$ is a sugar such as a ribose and R is a nucleobase, or L^2 is a modified sugar such as a modified ribose and R is a nucleobase. In certain embodiments, a nucleobase can be a modified nucleobase. L^1 can be a phosphodiester linkage.

[00120] A UNA oligomer of this invention can be a short 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 with respect to the nucleobases, although up to three mismatches can occur. A UNA oligomer duplex can have overhangs.

[00121] Some UNA oligomers are discussed in US Patent No. 8,314,227, as well as US Patent Publication No. 20110313020 A1.

[00122] 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.

[00123] 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.

[00124] 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.

[00125] 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.

[00126] In some embodiments, any one or more of the intermonomer linkages of a UNA oligomer can be a phosphodiester, a phosphorothioate including dithioates, a chiral phosphorothioate, and other chemically modified forms.

[00127] 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.

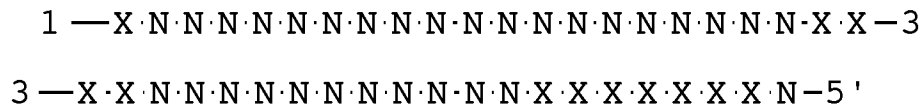
[00128] For example, the symbol “N” in the first strand can represent any nucleotide that is complementary to the monomer in the corresponding position in the second strand. Example UNA oligomers of this disclosure are shown with 2-monomer length overhangs, although overhangs of from 1 to 8 monomers, or longer, can be used.

[00129] The symbol “X” in a strand or oligomer represents a UNA monomer. When a UNA monomer appears in one strand of the oligomer, and is unpaired with the other strand, the monomer can have any base attached. When a UNA monomer appears in one strand of the oligomer, and is paired with a monomer in the other strand, the UNA monomer can have any base attached that would be complementary to the monomer in the corresponding paired position in the other strand.

[00130] 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

[00131]

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.

[00132] Complementarity of strands can involve mismatches. In certain embodiments, complementarity of strands can include one to three, or more, mismatches.

[00133] 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.

[00134] In further embodiments, a UNA oligomer of this invention can have one or more UNA monomers at the 3-end of the second strand.

[00135] 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.

[00136] 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.

[00137] In certain embodiments, a UNA oligomer of this invention may have a first strand that is 19-23 monomers in length.

[00138] In certain embodiments, a UNA oligomer of this invention may have a duplex region that is 19-21 monomers in length.

[00139] In further embodiments, a UNA oligomer of this invention may have a second strand that is 19-23 monomers in length.

[00140] 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.

[00141] 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.

[00142] 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.

[00143] 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.

[00144] A UNA oligomer of this invention for inhibiting gene expression can have a first strand and a second strand, each of the strands being 19-29 monomers in length. The monomers can be UNA monomers and nucleic acid nucleoside monomers. The oligomer can have a duplex structure of from 14 to 29 monomers in length. The UNA oligomer can be targeted to a target gene and can exhibit reduced off-target effects as compared to a conventional siRNA. In some embodiments, a UNA oligomer of this invention can have a first strand and a second strand, each of the strands being 19-23 monomers in length.

[00145] 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.

[00146] In certain embodiments, an overhang can be one or two monomers in length.

[00147] Examples of an overhang can contain one or more UNA monomers, natural nucleotides, non-natural nucleotides, modified nucleotides, or chemically-modified nucleotides, and combinations thereof.

[00148] Examples of an overhang can contain one or more deoxythymidine nucleotides, 2'-O-methyl nucleotides, inverted abasic monomers, inverted thymidine monomers, L-thymidine monomers, or glyceryl nucleotides.

[00149] 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.

[00150] The second strand can be incorporated into an RNA Induced Silencing Complex (RISC). In some embodiments, a UNA oligomer may have a strand that is a DICER substrate.

[00151] A UNA oligomer of this disclosure may comprise naturally-occurring nucleic acid nucleotides, and modifications thereof that are compatible with gene silencing activity.

[00152] In some aspects, a UNA oligomer is a double stranded construct molecule that is able to inhibit gene expression.

[00153] 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.

[00154] The monomers of a UNA oligomer may be attached via phosphodiester linkages, phosphorothioate linkages, gapped linkages, and other variations.

[00155] 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.

[00156] The target of a UNA oligomer can be a target nucleic acid of a target gene.

[00157] 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.

[00158] 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.

[00159] A UNA oligomer can be RISC length, which means that it has a duplex length of less than 25 base pairs.

[00160] 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 at the end of the double stranded region.

[00161] Examples of UNA oligomer structures of this invention are shown in Table 1.

[00162] 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
P15U15	SEQ ID NO:9 ĜGCCAUGCAUGUGUUCAGAŨmU	SEQ ID NO:10 UCUGAACACAUGCAŨGGCCŨmU
P15U16	SEQ ID NO:11 ĜGCCAUGCAUGUGUUCAGAŨmU	SEQ ID NO:12 UCUGAACACAUGCAUĜGCCŨmU

[00163] Examples of UNA oligomer structures of this invention are shown in Table 2.

[00164] 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 UUCUGĀĀCACAUGCAUGGCŨmU
P16U15	SEQ ID NO:17 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:18 UUCUGAACACAUGCĀUGGCŨmU
P16U16	SEQ ID NO:19 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:20 UUCUGAACACAUGCAŨGGCŨmU
P16U17	SEQ ID NO:21 ĜCCAUGCAUGUGUUCAGAAŨmU	SEQ ID NO:22 UUCUGAACACAUGCAUĜGCŨmU

[00165] Examples of sequences of UNA oligomers of this invention targeted to a TTR component are shown in Table 3. “Ref Pos” refers to reference position, which is the numerical position of a reference nucleotide in a TTR mRNA. In Table 3, a UNA oligomer would be composed of pairs SEQ ID NOS:23 and 63, 24 and 64, etc.

Table 3: UNA oligomers for TTR component target sequences

REF POS	SEQ ID NO	Sense (5'-3') SEQ ID NOS:23 to 62	SEQ ID NO	Antisense (5'-3') SEQ ID NOS:63 to 102
626	23	AUGUAACCAAGAGUAUCCAUUUUUac	63	GUAAAAAUGGAAUACUCUUGGUUACAU
626	24	AUGUAACCAAGAGUAUCCAUUUUUac	64	GUAAAAAUGGAAUACUCUUGGUUACĀŨ
626	25	ĀŨGUAACCAAGAGUAUCCAUUUUUac	65	GUAAAAAUGGAAUACUCUUGGUUACAU
626	26	ĀŨGUAACCAAGAGUAUCCAUUUUUac	66	GUAAAAAUGGAAUACUCUUGGUUACĀŨ
626	27	AUGUAACCAAGAGUAUCCAUUUUUĀĈ	67	GUAAAAAUGGAAUACUCUUGGUUACĀŨ
626	28	AUGUAACCAAGAGUAUCCĀŨUUUUUac	68	GUAAAAAUGGAAŨACUCUUGGUUACĀŨ
628	29	GUAACCAAGAGUAUCCAUUUUUUActa	69	UAGUAAAAAUGGAAUACUCUUGGUUAC
628	30	ĜŨAACCAAGAGUAUCCAUUUUUUActa	70	UAGUAAAAAUGGAAUACUCUUGGUUAC
628	31	GUAACCAAGAGUAUCCAUUUUUUActa	71	UAGUAAAAAUGGAAUACUCUUGGUUĀĈ
628	32	ĜŨAACCAAGAGUAUCCAUUUUUUActa	72	UAGUAAAAAUGGAAUACUCUUGGUUĀĈ
628	33	GUAACCAAGAGUAUCCAUUUUUUACŨĀ	73	UAGUAAAAAUGGAAUACUCUUGGUUĀĈ

REF POS	SEQ ID NO	Sense (5'-3') SEQ ID NOS:23 to 62	SEQ ID NO	Antisense (5'-3') SEQ ID NOS:63 to 102
628	34	GUAACCAAGAGUAUCCA $\bar{U}\bar{U}\bar{U}\bar{U}$ ACUA	74	UAGUAAAAUGG $\bar{A}\bar{A}\bar{U}$ ACUCUUGGUU $\bar{A}\bar{C}$
628	35	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}t$	75	AUGGAAUACUCUUGGUUAC t
628	36	GUAACCAAGAGUAUCCA $\bar{U}t$	76	AUGGAAUACUCUUGGUUAC $\bar{U}$
628	37	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}t$	77	AUGGAAUACUCUUGGUUAC $\bar{U}$
628	38	GUAACCAAGAGUAUCCA $\bar{U}\bar{U}$	78	AUGGAAUACUCUUGGUUAC $\bar{U}\bar{U}$
628	39	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}\bar{U}$	79	AUGGAAUACUCUUGGUUAC $\bar{U}\bar{U}$
628	40	GUAACCAAGAGUAUCCA $\bar{U}\bar{U}$	80	AUGGAA $\bar{U}$ ACUCUUGGUUAC $\bar{U}\bar{U}$
628	41	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}\bar{U}$	81	AUGGAA $\bar{U}$ ACUCUUGGUUAC $\bar{U}\bar{U}$
628	42	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}\bar{U}$	82	AUGGAA $\bar{U}$ ACUCUUGGUUAC $\bar{A}\bar{U}$
628	43	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}\bar{m}$	83	AUGGAAUACUCUUGGUUAC $\bar{U}\bar{m}$
628	44	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}\bar{m}$	84	AUG $\bar{G}$ AAUACUCUUGGUUAC $\bar{U}\bar{m}$
628	45	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}\bar{m}$	85	AUGG $\bar{A}$ UACUCUUGGUUAC $\bar{U}\bar{m}$
628	46	$\bar{G}$ UAACCAAGAGUAUCCA $\bar{U}\bar{m}$	86	AUGGA $\bar{A}$ UACUCUUGGUUAC $\bar{U}\bar{m}$
626	47	$\bar{A}$ UGUAACCAAGAGUAUCC $\bar{U}\bar{m}$	87	GGAAUACUCUUGGUUACAU $\bar{U}\bar{m}$
626	48	$\bar{A}$ UGUAACCAAGAGUAUCC $\bar{U}\bar{m}$	88	GGA $\bar{A}$ UACUCUUGGUUACAU $\bar{U}\bar{m}$
626	49	$\bar{A}$ UGUAACCAAGAGUAUCC $\bar{U}\bar{m}$	89	GGAA $\bar{U}$ ACUCUUGGUUACAU $\bar{U}\bar{m}$
626	50	$\bar{A}$ UGUAACCAAGAGUAUCC $\bar{U}\bar{m}$	90	GGAAU $\bar{A}$ CUCUUGGUUACAU $\bar{U}\bar{m}$
635	51	$\bar{A}$ GAGUAUCCA $\bar{U}\bar{U}\bar{U}\bar{U}$ AC $\bar{U}\bar{m}$	91	AGUAAAAUGGAAUACUC $\bar{U}\bar{m}$
635	52	$\bar{A}$ GAGUAUCCA $\bar{U}\bar{U}\bar{U}\bar{U}$ AC $\bar{U}\bar{m}$	92	AGU $\bar{A}$ AAAAUGGAAUACUC $\bar{U}\bar{m}$
635	53	$\bar{A}$ GAGUAUCCA $\bar{U}\bar{U}\bar{U}\bar{U}$ AC $\bar{U}\bar{m}$	93	AGUA $\bar{A}$ AAAUGGAAUACUC $\bar{U}\bar{m}$
635	54	$\bar{A}$ GAGUAUCCA $\bar{U}\bar{U}\bar{U}\bar{U}$ AC $\bar{U}\bar{m}$	94	AGUAA $\bar{A}$ AAUGGAAUACUC $\bar{U}\bar{m}$
174	55	$\bar{G}$ ACUGGUAUUUGUGUCUGA $\bar{U}\bar{m}$	95	UCAGACACAAAUACCAGUC $\bar{C}\bar{m}$
174	56	$\bar{G}$ ACUGGUAUUUGUGUCUGA $\bar{U}\bar{m}$	96	UCA $\bar{G}$ ACACAAAUACCAGUC $\bar{C}\bar{m}$
174	57	$\bar{G}$ ACUGGUAUUUGUGUCUGA $\bar{U}\bar{m}$	97	UCAG $\bar{A}$ CACAAAUACCAGUC $\bar{C}\bar{m}$
174	58	$\bar{G}$ ACUGGUAUUUGUGUCUGA $\bar{U}\bar{m}$	98	UCAGA $\bar{C}$ ACAAAUACCAGUC $\bar{C}\bar{m}$
627	59	$\bar{U}$ GUAACCAAGAGUAUCCA $\bar{U}\bar{m}$	99	UGGAAUACUCUUGGUUACA $\bar{U}\bar{m}$
629	60	$\bar{U}$ AACCAAGAGUAUCCA $\bar{U}\bar{U}\bar{m}$	100	AAUGGAAUACUCUUGGUUA $\bar{U}\bar{m}$
632	61	$\bar{C}$ CAAGAGUAUCCA $\bar{U}\bar{U}\bar{U}\bar{U}$ $\bar{U}\bar{m}$	101	AAAAAUGGAAUACUCUUGG $\bar{U}\bar{m}$
632	62	$\bar{C}$ CAAGAGUAUCCA $\bar{U}\bar{U}\bar{U}\bar{U}$ $\bar{U}\bar{m}$	102	AAAAAUG $\bar{G}$ AAUACUCUUGG $\bar{U}\bar{m}$

[00166] In Table 3, lower case designates 2'-deoxyribo-N; upper case designates Ribo-N; underlined-lower case designates 2'-deoxy-N; mA, mG, mC, and mU designate 2'-O-Methyl RNA; and $\bar{A}$ $\bar{U}$ $\bar{G}$ and $\hat{C}$ designate UNA monomers.

[00167] 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.

[00168] 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.

[00169] 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.

[00170] 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.

[00171] Methods for treating amyloidosis

[00172] Methods of this invention include the treatment and prevention of TTR-related amyloidosis in human and mammalian subjects.

[00173] 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.

[00174] The subject may have TTR-related amyloidosis, also known as ATTR.

[00175] In particular, a subject can have a V30M gene. The methods of this invention can selectively reduce V30M TTR in the subject.

[00176] 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.

[00177] 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, or from 0.001 mg/kg to 10.0 mg/kg.

[00178] 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.

[00179] In the methods of this invention, peripheral neuropathy or autonomic neuropathy in the subject can be reduced.

[00180] 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.

[00181] In the methods of this disclosure, the administration of a UNA oligomer may not result in an inflammatory response.

[00182] 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.

[00183] 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.

[00184] Pharmaceutical compositions

[00185] In some aspects, this invention provides compositions containing a UNA oligomer and a pharmaceutically acceptable carrier.

[00186] In further aspects, this invention includes nanoparticle compositions that can encapsulate and deliver a UNA oligomer to cells with surprisingly advantageous potency. The nanoparticles can be formed with lipid molecules, for example, any one or more of the compounds ATX-001 to ATX-032 disclosed in WO/2015/074085. In certain embodiments, lipid nanoparticles of this invention can be formed with compound ATX-

002, as disclosed in WO/2015/074085, and the nanoparticles can encapsulate the UNA oligomer.

[00187] A 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.

[00188] Embodiments of this invention include pharmaceutical compositions containing a UNA oligomer in a lipid formulation.

[00189] In some embodiments, a composition may comprise one or more lipids selected from cationic lipids, anionic lipids, sterols, pegylated lipids, and any combination of the foregoing.

[00190] In certain embodiments, a composition can be substantially free of liposomes.

[00191] In further embodiments, a composition can include liposomes.

[00192] In additional embodiments, a composition can contain a UNA oligomer within a viral or bacterial vector.

[00193] 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).

[00194] Examples of excipients for a pharmaceutical composition include antioxidants, suspending agents, dispersing agents, preservatives, buffering agents, tonicity agents, and surfactants.

EXAMPLES

[00195] **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.

[00196] 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.

[00197] 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.

[00198] 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.

[00199] 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.

[00200] **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.

[00201] 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.

[00202] **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.

[00203] 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.

[00204] 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.

[00205] 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.

[00206] 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.

[00207] **Example 8: UNA oligomers reduce TTR protein in vivo primate.**

[00208] A single-dose, pharmacokinetic and pharmacodynamic study of liposomally formulated UNA Oligomers targeting transthyretin (TTR) mRNA following intravenous infusion in Cynomolgus monkeys was performed.

[00209] Cynomolgus monkeys (*Macaca fascicularis*) were selected for this study because the TTR mRNA of Cynomolgus monkeys is homologous to human TTR mRNA, making them a good model for screening the potency of UNA oligomers targeting to TTR mRNA.

[00210] In this study, the knockdown of Transthyretin protein was evaluated as a result of treatment with UNA oligomers targeting Transthyretin TTR mRNA, based on SEQ ID NOs:46/86, 59/99, and 60/100, delivered in a liposomal formulation to male Cynomolgus monkeys. Measurements were made following a single intravenous (IV) dose administration. TTR protein concentration in plasma samples was assessed by LC-MS/MS at various time points.

[00211] Twenty-four (24) Cynomolgus non-naïve and naïve monkeys were used in this study. There were eight (8) groups of animals in the study with three (3) per group and three (3) extra animals. The animals weighed 2.4 to 6.8 kg and were 3 to 6 years old at pre-study physical examination. Animals were housed in a temperature- and humidity-monitored environment. The targeted range of temperature and relative humidity was from 18°C to 29°C, and from 35% to 70%, respectively. An automatic lighting system provided a 12-hour light/dark cycle. Previously quarantined animals were acclimated to the study room for a minimum of 14 days prior to initiation of dosing. Acclimation phase data was collected from all animals.

[00212] Animals in Groups 1 through 8 were administered the test articles on Day 1 at 0.3 mg/kg, dose volume 6.25 mL/kg, concentration 0.048 mg/mL. Doses were administered as a 15-minute intravenous (IV) infusion, $\pm$ 1 minute, using a pump and disposable syringe.

[00213] For each animal, infusion start and end times were recorded; and subsequent sample collection target times were determined based on infusion end time ($t = 0$). All blood specimens, approximately 1 mL, were collected by venipuncture from a peripheral vein from restrained, conscious animals.

[00214] Knockdown of TTR was observed in all treated groups with observation at Days 10 and 20 after dosing. The study period was lengthened to 50 days for one group to observe long-term effects.

[00215] Referring to Fig. 11, knockdown of Transthyretin protein in vivo was achieved as a result of treatment with UNA oligomer SEQ ID NO:60/100 targeting Transthyretin TTR mRNA, delivered in a liposomal formulation to male Cynomolgus monkeys. Measurements were made following a single intravenous (IV) dose administration. TTR protein concentration in plasma samples was assessed by LC-MS/MS at various time points.

[00216] As shown in Fig. 11, a single injection in the primates of a UNA oligomer targeted to TTR surprisingly knocked down TTR protein levels to below 50% with a continuous duration of more than one month (30 days).

[00217] Fig. 11 shows the unexpectedly advantageous result of over 91% knockdown of TTR in the primates at 20 days, and more than 70% knockdown with a continuous duration of more than 20 days.

[00218] The Monkey TTR SRM assay was used, which is a high throughput absolute protein quantitation assay using Selected Reaction Monitoring (SRM) technology platform. In a single SRM assay, the panel can multiplex up to 200 proteins. The assay was performed on a targeted absolute protein quantitation platform, where the key technology was SRM, sometimes also referred to as Multiple Reaction Monitoring (MRM). In a triple-quadrupole (QQQ) mass spectrometer, SRM/MRM is a tandem selection process where selecting peptides by precursor m/z is followed by selecting fragmentations (transitions) of the targeted peptide. Further, coupled with liquid chromatography and the addition of heavy labeled internal standards, SRM/MRM is a highly selective, sensitive technology to quantify designated protein with precision.

[00219] All publications, patents and literature specifically mentioned herein are incorporated by reference for all purposes.

[00220] 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.

[00221] 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.

[00222] 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.

[00223] 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 compound 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 compound has a duplex region of from 14 to 29 contiguous monomers in length, wherein the first strand is a passenger strand for RNA interference and the second strand is a guide strand for RNA interference, and wherein the compound comprises a sequence of bases targeted to inhibit expression of a TTR gene.
2. The compound of claim 1, wherein the compound contains one to seven UNA monomers.
3. The compound of claim 1, wherein the compound contains a UNA monomer at the 1-end (5' end for non-UNA) of the first strand, a UNA monomer at the second position from the 3' end of the first strand, and a UNA monomer at the second position from the 3' end of the second strand.
4. The compound of claim 1, wherein the compound contains a UNA monomer at any one or more of positions 2 to 8 from the 5' end of the second strand.
5. The compound of claim 1, wherein the compound comprises SEQ ID NOs:60/100, SEQ ID NOs:59/99, SEQ ID NOs:43/83, or SEQ ID NOs:61/101.
6. The compound of claim 1, wherein the compound has a 3' overhang comprising one or more UNA monomers, natural nucleotides, non-natural nucleotides, modified nucleotides, or chemically-modified nucleotides, or combinations thereof.
7. The compound of claim 1, wherein the compound has a 3' overhang comprising one or more deoxythymidine nucleotides, 2'-O-methyl nucleotides, inverted abasic monomers, inverted thymidine monomers, L-thymidine monomers, or glyceryl nucleotides.
8. The compound of claim 1, wherein one or more of the nucleic acid monomers is a non-natural nucleotide, a modified nucleotide, or a chemically-modified nucleotide.
9. The compound of claim 1, wherein each nucleic acid monomer has a 2'-O-methyl group.
10. The compound of claim 1, wherein the compound contains one or more 2'-methoxyethoxy nucleotides.

11. The compound of claim 1, wherein the compound contains one or more 2'-deoxy-2'-fluoro ribonucleotides.
12. The compound of claim 1, wherein the compound does not contain fluorine.
13. The compound of claim 1, wherein one or more of three monomers at each end of each strand is connected by a phosphorothioate, a chiral phosphorothioate, or a phosphorodithioate linkage.
14. A lipid nanoparticle-oligomer compound comprising one or more compounds of any of claims 1-13 attached to the lipid nanoparticle.
15. A composition comprising one or more compounds of any of claims 1-13 and a pharmaceutically acceptable carrier.
16. The composition of claim 15, wherein the carrier comprises lipid nanoparticles or liposomes.
17. The composition of claim 15, wherein the carrier is a lipid formulation comprising any one or more of the compounds ATX-001 to ATX-032.
18. The composition of claim 15, wherein upon administering a single intravenous dose to a subject, the composition reduces TTR protein in the subject by at least 80% after 10 days.
19. The composition of claim 15, wherein upon administering a single intravenous dose to a subject, the composition reduces TTR protein in the subject by at least 70% after 20 days.
20. The composition of claim 15, wherein upon administering a single intravenous dose to a subject, the composition reduces TTR protein in the subject by at least 50% after 30 days.
21. A method for treating or preventing TTR-related amyloidosis in a subject in need, the method comprising administering to the subject an effective amount of a composition of claim 15 to the subject.
22. The method of claim 21, wherein the TTR-related amyloidosis is ATTR.
23. The method of claim 21, wherein the administering is intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, or dermal.
24. The method of claim 21, wherein the effective amount is a dose of from 0.001 to 10.0 mg/kg.

25. The method of claim 21, wherein upon administering a single dose to the subject, the composition reduces TTR protein in the subject by at least 70% after 20 days.
26. The method of claim 21, wherein upon administering a single dose to the subject, the composition reduces TTR protein in the subject by at least 50% after 30 days.
27. A method for inhibiting expression of a TTR gene in a subject, the method comprising administering to the subject a composition of claim 15.
28. The use of a composition of claim 15 for preventing, ameliorating or treating a disease or condition associated with TTR-related amyloidosis in a subject in need.
29. A composition of claim 15 for use in medical therapy.
30. A composition of claim 15 for use in the treatment of the human or animal body.
31. The use of a composition of claim 15 for preparing or manufacturing a medicament for preventing, ameliorating or treating a disease or condition associated with TTR-related amyloidosis in a subject in need.

284

SEQ ID NOS: 103-118

V30M TTR mRNA

3'-304-UCGGAAGACUUGUGUACGUACCGGUGUAAACUACCGUCCUG-264
3'-304-UCGGAAGACUUGUGUACGUACCGGUGUAAACUACCGUCCUG-264

wt TTR mRNA

V30V-P2: ACGGCCACAUAUGAUGGCAGdTdT
V30V-P3: CACGGCCACAUAUGAUGGCAdTdT
V30V-P4: GCACGGCCACAUAUGAUGGCdTdT
V30V-P5: UGCACGGCCACAUAUGAUGGdTdT
V30V-P6: AUGCACGGCCACAUAUGAUGdTdT
V30V-P7: CAUGCACGGCCACAUAUGAdTdT
V30V-P8: ACAUGCACGGCCACAUAUGAdTdT
V30V-P9: CACAUGCACGGCCACAUAUGdTdT
V30V-P10: ACACAUGCACGGCCACAUAUdTdT
V30V-P11: AACACAUGCACGGCCACAAdTdT
V30V-P12: GAACACAUGCACGGCCACAdTdT
V30V-P13: UGAACACAUGCACGGCCACdTdT
V30V-P14: CUGAACACAUGCACGGCCAdTdT
V30V-P15: UCUGAACACAUGCACGGCCdTdT

FIG. 1

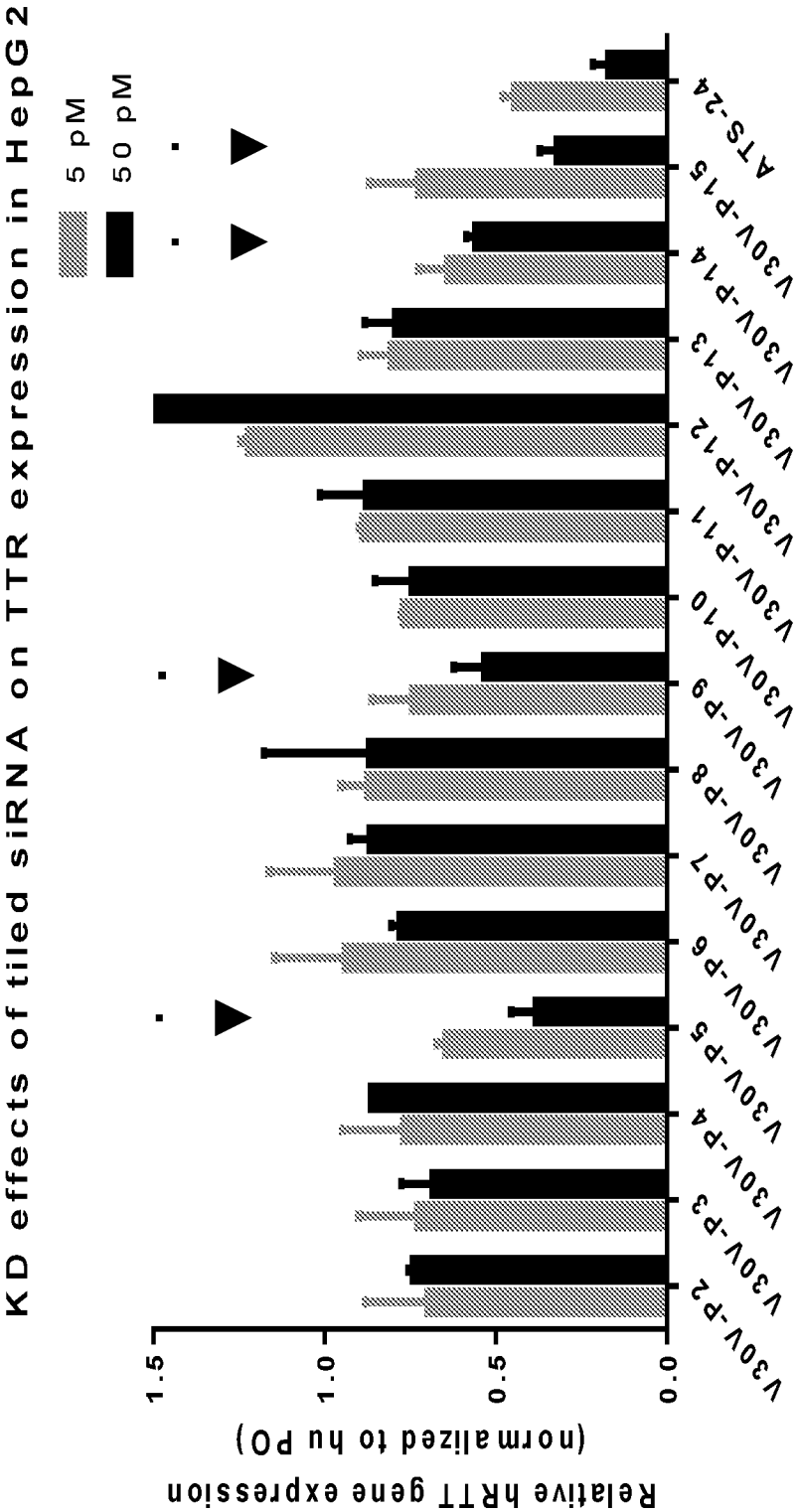


FIG. 2

284 SEQ ID NOS: 103-104,
8 108, 112, and 117-118

V30M TTR mRNA

3'-304-UCGGAAAGACUUGUGUACGU**A**CCGGUGUAACUACCGUCCUG-264

3'-304-UCGGAAAGACUUGUGUACGU**G**CCGGUGUAACUACCGUCCUG-264

wt TTR mRNA V30V-P5: UGC**A**CGGCCACAUAUGGdTdT

V30V-P9: CACAUGC**A**CGGCCACAUAUGdTdT

V30V-P14: CUGAAACAUGC**A**CGGCCAdTdT

V30V-P15: UCUGAAACAUGC**A**CGGCCdTdT

.

SEQ ID NOS: 119-122

▼

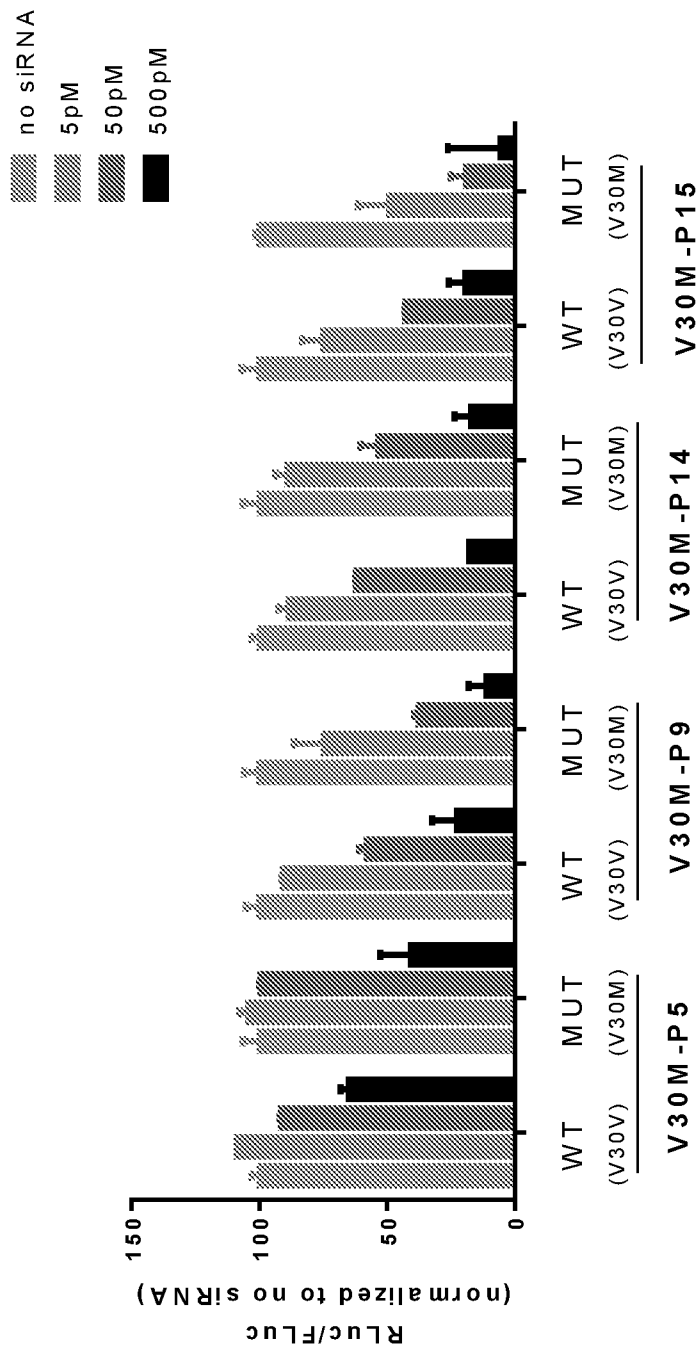
V30M-P5: UGC**A**UGGCCACAUAUGGdTdT

V30M-P9: CACAUGC**A**UGGCCACAUAUGdTdT

V30M-P14: CUGAAACAUGC**A**UGGCCAdTdT

V30M-P15: UCUGAAACAUGC**A**UGGCCdTdT

FIG. 3



siRNA ID

FIG. 4

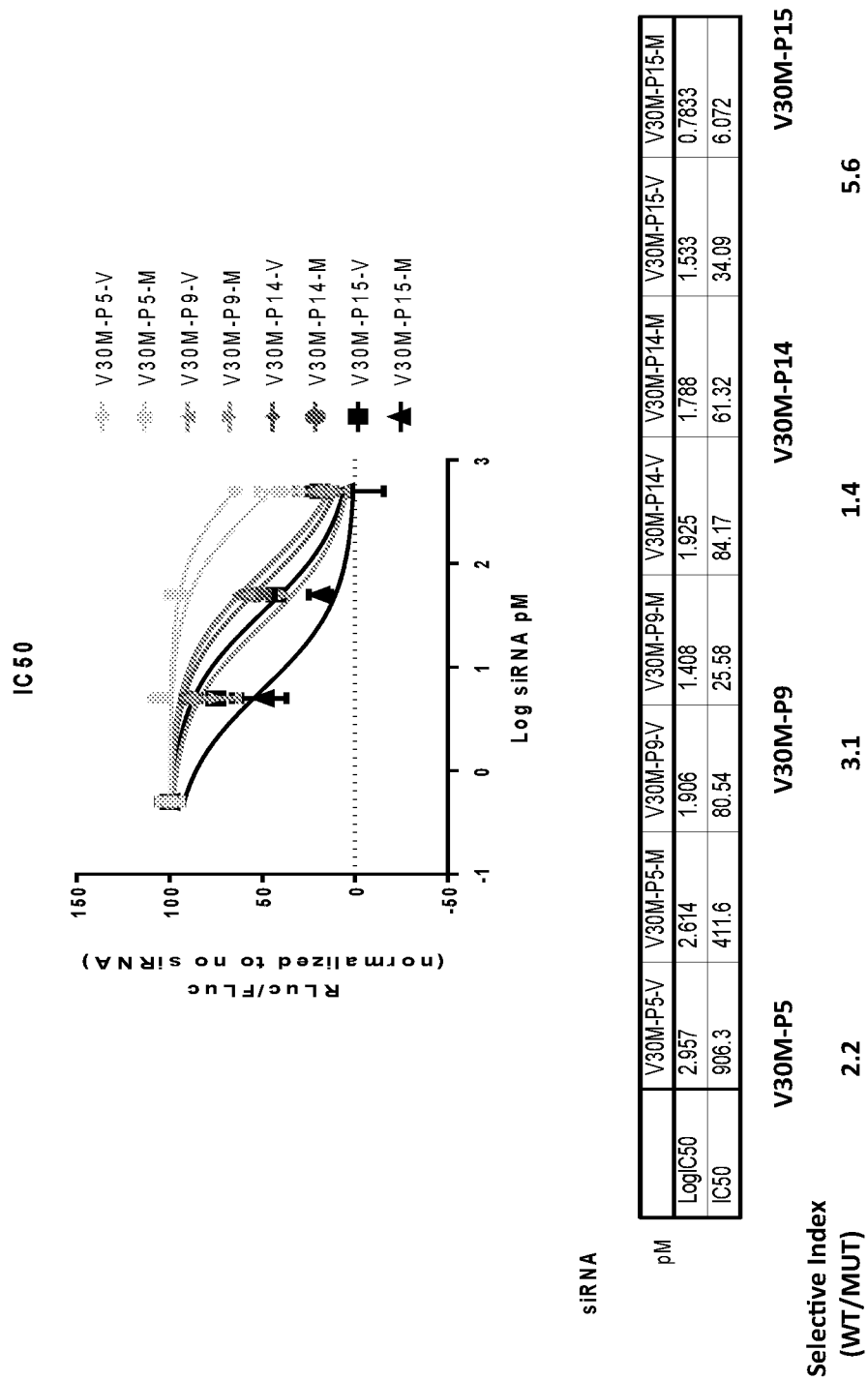
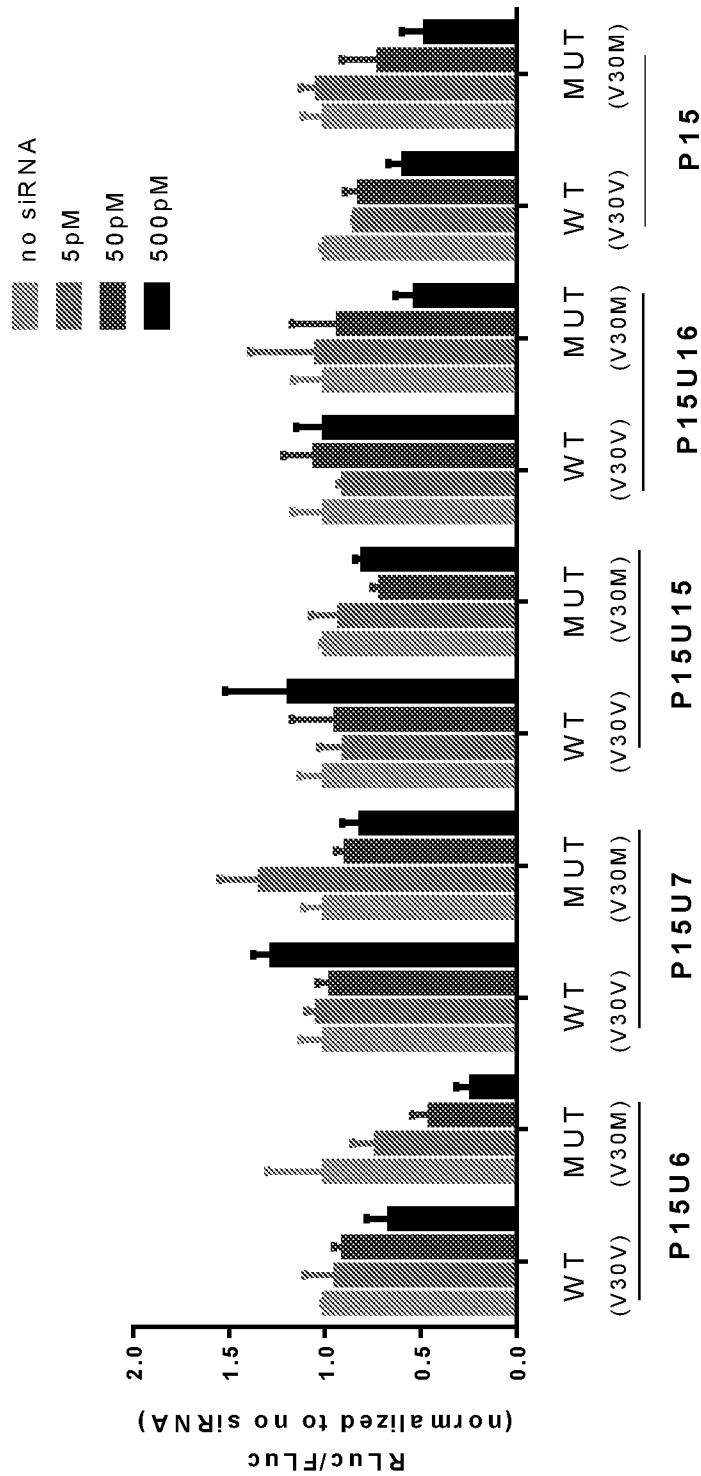


FIG. 5

siRNA ID	SS ID	SEQ ID NOS: 123-128		SEQ ID NOS: 129-134	
		seq. (5' -> 3')		AS ID	
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P15U6	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U6AS	
P15U7	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U7AS	
P15U14	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U14AS	
P15U15	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U15AS	
P15U16	P15USS	ĜGCCAUGCAUGUGUUCAGAUmU		P15U16AS	
					UCUGAACACACAUUGGCCdTdT
					UCUGAĀCACACAUUGGCCŨmU
					UCUGAAĈCACACAUUGGCCŨmU
					UCUGAACACACAUĜGCCŨmU
					UCUGAACACACAUĜGCCŨmU
					UCUGAACACACAUĜGCCŨmU

FIG. 6



siRNA ID

FIG. 7

Fig. 8

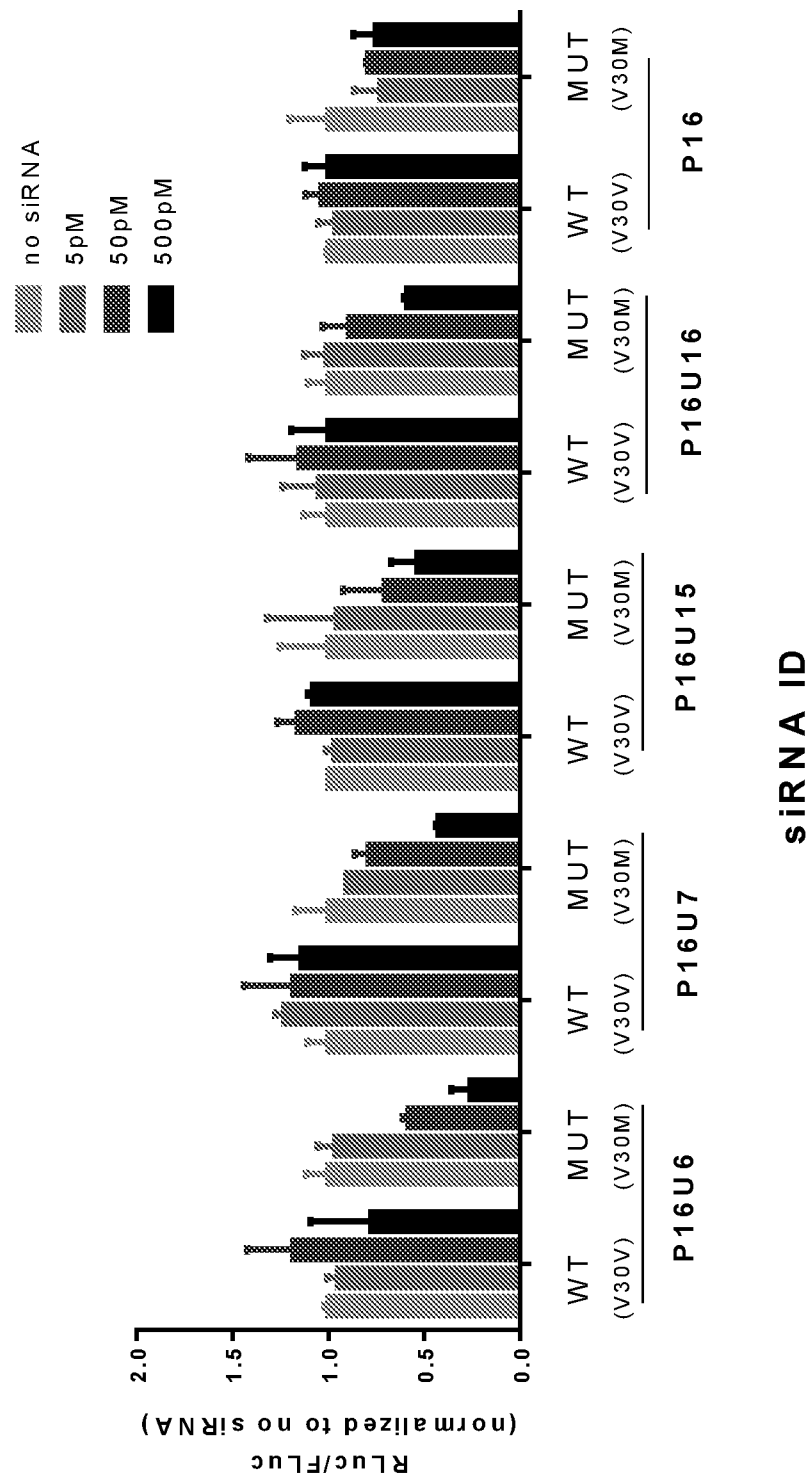


FIG. 9

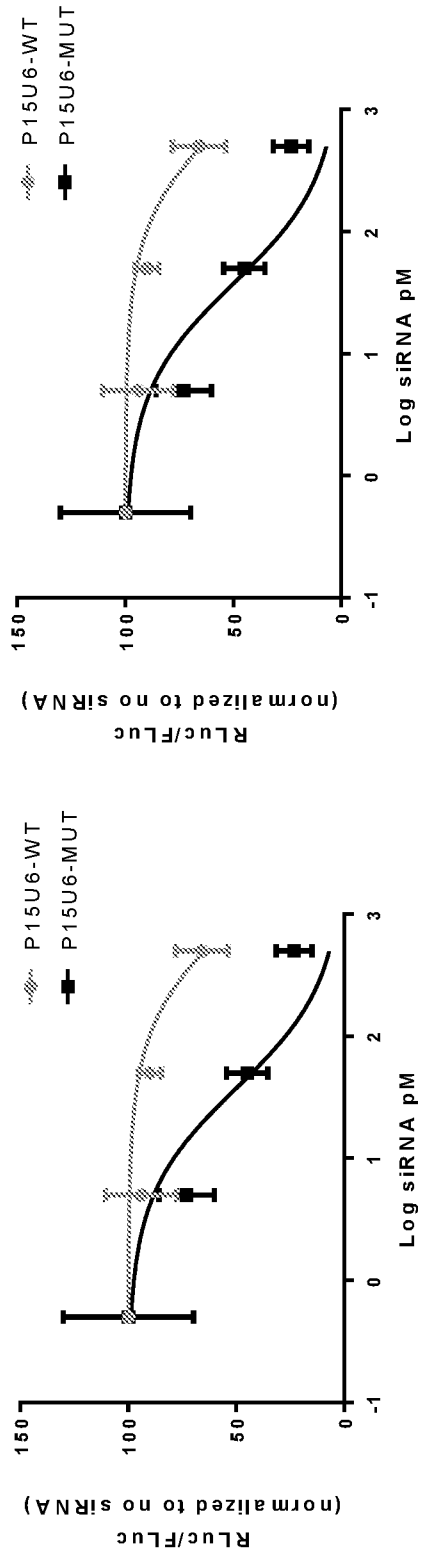
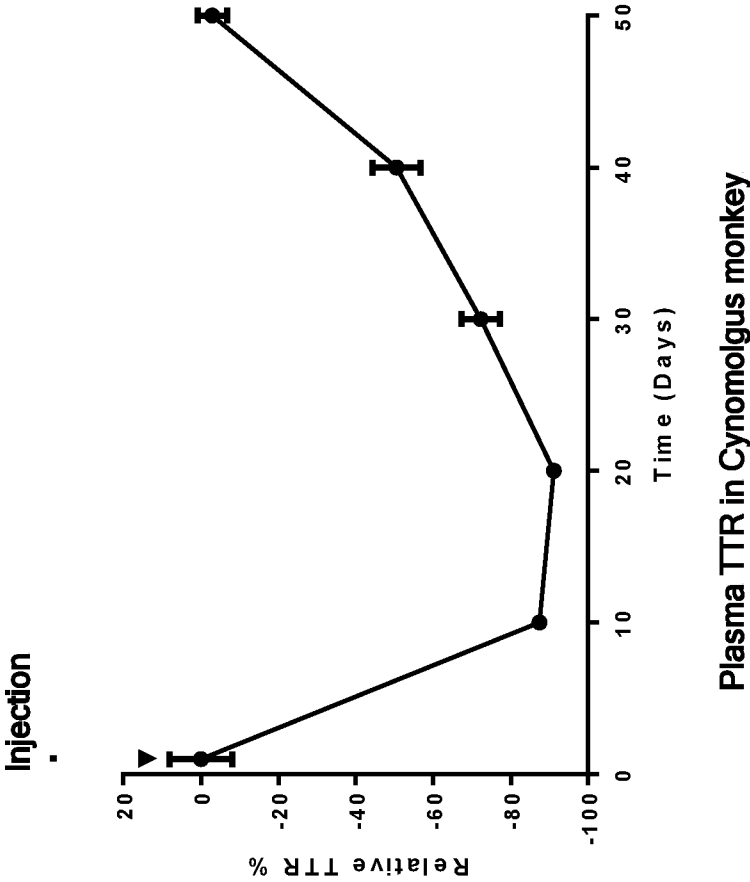


FIG. 10

FIG. 11



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43927

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/713, C12N 15/113, A61K 9/127 (2016.01)

CPC - A61K 31/713, C12N 2310/14, C12N 15/113, A61K 9/127

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 31/713, C12N 15/113, A61K 9/127 (2016.01)

CPC - A61K 31/713, C12N 2310/14, C12N 15/113, A61K 9/127

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

CPC - C12N 2320/32, C12N 15/111

(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar

Search terms: TTR, transthyretin, UNA, unlocked nucleic acid, un-locked nucleic acid, amyloidosis, monomer, duplex, overhang, lipid nanoparticle, lipid nanosphere, liposome, inhibit, block, RNAi, silencing, ATX-001, ATX-032

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2015/042564 A1 (ALNYLAM PHARMACEUTICALS INC.) 26 March 2015 (26.03.2015) p 3, ln 31 to p 4, ln 4; p 4, ln 4-13; p 5, ln 13-16; p 5, ln 29-33; p 6, ln 31-32; p 8, ln 6; p 13, ln 26-32; p 23, ln 17-20; p 27, ln 28 to p 28, ln 7; p 28, ln 3-7; p 33, ln 23-24; p 52, ln 12-15; p 53, ln 23-24; p 54, ln 7-9; p 54, ln 26-30; p 56, ln 28-29; p 58, ln 6-7; p 63, ln 26-29; p 68, ln 25-29; p 70, ln 13-14; p 72, ln 35-36	1-4, 6-9, 11-13, (14-16, 18-31)/(1-4,6-9,11-13) ----- 5, 10, (14-16)/(5,10), 17, (18-31)/(5,10)
Y	US 2010/0120893 A1 (SAH et al.) 13 May 2010 (13.05.2010) para [0042], [0090], [0097], [0352]; SEQ ID NO: 1352, SEQ ID NO: 1353	5, 10, (14-31)/(5,10)
Y	US 2015/0141678 A1 (ARCTURUS THERAPEUTICS, INC.) 21 May 2015 (21.05.2015) para [0034], [0084]	17
X, P	US 2015/0307881 A1 (ARCTURUS THERAPEUTICS, INC.) 29 October 2015 (29.10.2015) entire document	1-31
X, P	US 2015/0307880 A1 (ARCTURUS THERAPEUTICS, INC.) 29 October 2015 (29.10.2015) entire document	1-31

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

* 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

19 October 2016

Date of mailing of the international search report

31 OCT 2016

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/43927

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. ☐ forming part of the international application as filed:
- ☐ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
- ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
- ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments: