Abnormal amyloidogenic proteins may be either inherited or acquired through somatic mutations. Guan, J. *et al.* (Nov. 4, 2011) Current perspectives on cardiac amyloidosis, *Am J Physiol Heart Circ Physiol*, doi:10.1152/ajpheart.00815.2011. Transthyretin associated ATTR is the most frequent form of hereditary systemic amyloidosis. Lobato, L. (2003) *J. Nephrol.*, 16:438-442. TTR mutations accelerate the process of TTR amyloid formation and are the most important risk factor for the development of ATTR. More than 85 amyloidogenic TTR variants are known to cause systemic familial amyloidosis. TTR mutations usually give rise to systemic amyloid deposition, with particular involvement of the peripheral nervous system, although some mutations are associated with cardiomyopathy or vitreous opacities. *Ibid.*

The V30M mutation is the most prevalent TTR mutation. See, *e.g.*, Lobato, L. (2003) *J Nephrol*, 16:438-442. The V122I mutation is carried by 3.9% of the African American population and is the most common cause of FAC. Jacobson, D.R. et al. (1997) *N. Engl. J. Med.* 336 (7): 466–73. It is estimated that SSA affects more than 25% of the population over age 80. Westermark, P. et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87 (7): 2843–5.

Accordingly, there is a need in the art for effective treatments for TTR-associated diseases.

Summary

In one aspect, the present invention refers to a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of transthyretin (TTR) in a cell, wherein said RNAi agent comprises a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region complementary to SEQ ID NO:2,

wherein each strand is about 14 to about 30 nucleotides in length,

wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides,

wherein the sense strand comprises no more than 8 2′-fluoro modifications;

wherein the antisense strand comprises no more than 6 2′-fluoro modifications;

wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and

wherein the sense strand is conjugated to at least one ligand.

In another aspect, the present invention refers to a pharmaceutical composition comprising the double stranded RNAi agent of the present invention. In yet another aspect, the present invention refers to an *in vitro* method of inhibiting transthyretin (TTR) expression in a cell, the method comprising: contacting the cell with the double stranded RNAi agent of the present invention, or the pharmaceutical composition of the

present invention, thereby inhibiting expression of the TTR gene in the cell. In still another aspect, the present invention refers to the use of the double stranded RNAi agent of the present invention, or the pharmaceutical composition of the present invention in the manufacture of a medicament for treating a subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease, wherein said TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, hereditary transthyretin-mediated amyloidosis, and hyperthyroxinemia. In yet another aspect, the present invention refers to a double stranded RNAi agent of the present invention, or the pharmaceutical composition of the present invention for use in therapy.

Also described herein are RNAi agents, *e.g.*, double stranded RNAi agents, and compositions targeting the Transthyretin (TTR) gene. The present disclosure also provides methods of inhibiting expression of TTR and methods of treating or preventing a TTR-associated disease in a subject using the RNAi agents, *e.g.*, double stranded RNAi agents, of the invention. The present disclosure is based, at least in part, on the discovery that RNAi agents in which substantially all of the nucleotides on the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides and that comprise no more than 8 2'-fluoro modifications on the sense strand, no more than 6 2'-fluoro modifications on the antisense strand, two phosphorothioate linkages at the 5'-end of the sense strand, two phosphorothioate linkages at the 5'-end of the antisense strand, and a ligand, *e.g.*, a $GalNAc_3$ ligand, are shown herein to be effective in silencing the activity of the TTR gene. These agents show surprisingly enhanced TTR gene silencing activity. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing modifications and the specific target sites in these RNAi agents confer to the RNAi agents of the invention improved efficacy, stability, potency, and durability.

Accordingly, in one example, the present disclosure provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of transthyretin (TTR) in a cell, wherein the RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2'-fluoro modification wherein the antisense strand comprises no more than 6 2'-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, and wherein the double stranded RNAi agent is represented by formula (IIIe):

sense: 5' - N_a -Y Y Y – N_b - 3'

antisense: 3' - n_p'-N_a'- Y'Y'Y'- N_b'- 5' (IIIe)

wherein:

n_p' is a 2 nucleotide overhang and each nucleotide within n_p' is linked to a neighboring nucleotide *via* a phosphorothioate linkage;

each N_a, N_b, N_a' and N_b' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y'Y'Y' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one example, the YYY motif occurs at or near the cleavage site of the sense strand. In one example, the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

In one example, the Y nucleotides contain a 2'-fluoro modification.

In one example, the Y' nucleotides contain a 2'-O-methyl modification.

The double stranded region may be 15-30 nucleotide pairs in length, 17-23 nucleotide pairs in length,17-25 nucleotide pairs in length, 23-27 nucleotide pairs in length, 19-21 nucleotide pairs in length, or 21-23 nucleotide pairs in length.

Each strand of the double stranded RNAi agent may have 15-30 nucleotides or 19-30 nucleotides.

In one example, the modifications on the nucleotides are selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified

nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic, and combinations thereof.

In one example, the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro modifications.

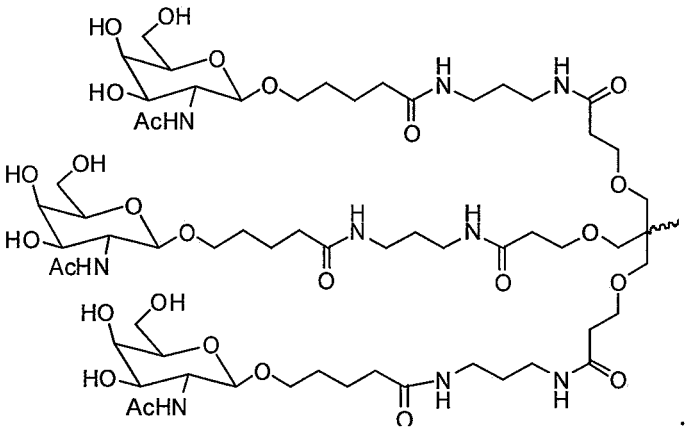
The sense strand may comprise no more than 7 2′-fluoro modifications, no more than 6 2′-fluoro modifications, no more than 5 2′-fluoro modification, no more than 4 2′-fluoro modifications, no more than 3 2′-fluoro modifications, or no more than 2 2′-fluoro modifications.

The antisense strand may comprise no more than 5 2′-fluoro modifications, no more than 4 2′-fluoro modifications, no more than 3 2′-fluoro modifications, or no more than 2 2′-fluoro modifications.

In one example, the double stranded RNAi agent further comprises a 5'-phosphate or a 5'-phosphate mimic at the 5' nucleotide of the antisense strand. In another example, the double stranded RNAi agent further comprises a 5'-phosphate mimic at the 5' nucleotide of the antisense strand.

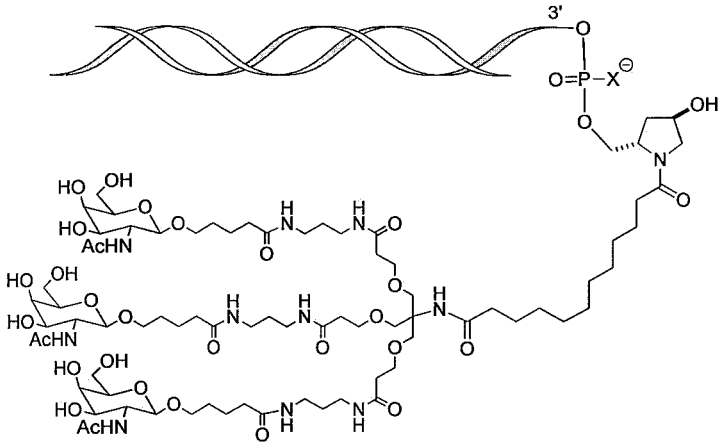
In one example, the 5'-phosphate mimic is a 5'-vinyl phosphate (5'-VP).

In one example, the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. In another example, the ligand is



In one example, the ligand is attached to the 3′ end of the sense strand.

In one example, the double stranded RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In one example, the antisense strands comprise a nucleotide sequence selected from the group consisting of

5'- usCfsuugguuacaugAfaaucccasusc – 3' (SEQ ID NO: 6),

5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO:7),

5'- UfsCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 8), and

5'- VPusCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 9), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; and s is a phosphorothioate linkage; and VP is a 5'-phosphate mimic.

In one example, the sense and antisense strands comprise nucleotide sequences selected from the group consisting of

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- usCfsuugguuacaugAfaaucccasusc – 3' (SEQ ID NO: 6);

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7);

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- UfsCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 8); and

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- VPusCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 9), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U;

and s is a phosphorothioate linkage; and VP is a 5'-phosphate mimic. In another example, the sense and antisense strands comprise the nucleotide sequences 5'-usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and 5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7), wherein a, c, g, and u are 2'-O-methyl (2'-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2'-fluoro A, C, G, or U; and s is a phosphorothioate linkage. In yet another example, the RNAi agent is selected from the group of any one of the RNAi agents listed in any one of Tables 1 and 3. In yet another example, the RNAi agent is AD-65492.

In one example, the present disclosure provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of transthyretin (TTR) in a cell, wherein the RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region fully complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2'-fluoro modifications; wherein the antisense strand comprises no more than 6 2'-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, the double stranded RNAi agent is represented by formula (IIIe):

sense: 5' - N_a -Y Y Y – N_b - 3'

antisense: 3' - n_p'-N_a'- Y'Y'Y'- N_b'- 5' (IIIe)

wherein:

n_p' is a 2 nucleotide overhang and each nucleotide within n_p' is linked to a neighboring nucleotide *via* a phosphorothioate linkage;

each N_a, N_b, N_a' and N_b' independently represents an oligonucleotide sequence comprising 8-10 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y'Y'Y' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications.

The present disclosure also provides cells containing the double stranded RNAi agents of the disclosure, cells comprising the vectors of the disclosure, and pharmaceutical compositions comprising the double stranded RNAi agents of the disclosure or the vectors of the disclosure.

In one example, the double stranded RNAi agent is administered in an unbuffered solution, *e.g.,* saline or water.

In another example, the double stranded RNAi agent is administered with a buffer solution. In one example, the buffer solution comprises acetate, citrate,

prolamine, carbonate, or phosphate or any combination thereof. In another example, the buffer solution is phosphate buffered saline (PBS).

In another example, the present disclosure provides methods of inhibiting transthyretin (TTR) expression in a cell. The methods include (a) contacting the cell with the double stranded RNAi agents of the disclosure, the vectors of the disclosure, or the pharmaceutical compositions of the disclosure; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TTR gene, thereby inhibiting expression of the TTR gene in the cell.

In one example, the cell is within a subject.

In one example, the subject is a human.

In one example, the subject suffers from TTR-associated disease.

In one example, the TTR expression is inhibited by at least about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

In yet another example, the present disclosure provides methods of treating a subject having a transthyretin (TTR)-associated disorder, by administering to the subject a therapeutically effective amount of the double stranded RNAi agents of the disclosure, or the vectors of the disclosure, or the pharmaceutical compositions of the disclosure, thereby treating the subject.

In yet another example, the present disclosure provides methods of prophylactically treating a subject at risk of developing a transthyretin (TTR)-associated disorder, by administering to the subject a prophylactically effective amount of the double stranded RNAi agents of the disclosure, or the vectors of the disclosure, or the pharmaceutical compositions of the disclosure, thereby prophylactically treating the subject.

In a further example, the present disclosure provides methods of treating a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'- UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2'-fluoro modifications; wherein the antisense strand comprises no more than 6 2'-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In a further example, the present disclosure provides methods of prophylactically treating a subject at risk of developing a transthyretin (TTR)-associated disorder. The methods include administering to the subject a prophylactically effective amount of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the present disclosure provides methods of reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a therapeutically effective amount of the double stranded RNAi agents of the disclosure, or the vectors of the disclosure, or the pharmaceutical compositions of the disclosure, thereby reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in the subject.

In a further example, the present disclosure provides methods of reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region complementary to SEQ ID NO:2, wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the present disclosure provides methods of increasing a 6-minute walk test (6MWT) in a subject having a transthyretin (TTR)-associated disorder. The

methods include administering to the subject a therapeutically effective amount of the the double stranded RNAi agents of the disclosure, or the vectors of the disclosure, or the pharmaceutical compositions of the disclosure, thereby increasing a 6-minute walk test (6MWT) in the subject.

In a further example, the present disclosure provides methods of increasing a 6-minute walk test (6MWT) in a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region complementary to SEQ ID NO:2, wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the double stranded RNAi agent is represented by formula (IIIe):

sense: 5' - N_a -Y Y Y – N_b - 3'

antisense: 3' - n_p'-N_a'- Y′Y′Y′- N_b'- 5' (IIIe)

wherein:

n_p' is a 2 nucleotide overhang and each nucleotide within n_p' is linked to a neighboring nucleotide *via* a phosphorothioate linkage;

each N_a, N_b, N_b and N_b' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y′Y′Y′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one example, the subject is a human.

In one example, the subject is a subject suffering from a TTR-associated disease. In another example, the subject is a subject at risk for developing a TTR-associated disease. In one example, the subject at risk of developing a TTR-associated disease carries a TTR gene mutation that is associated with the development of a TTR associated disease, or a subject with a family history of TTR-associated disease, or a subject who has signs or symptoms suggesting the development of TTR amyloidosis.

In one example, the TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, and hyperthyroxinemia.

In one example, the subject has a TTR-associated amyloidosis and the method reduces an amyloid TTR deposit in the subject.

In one example, the double stranded RNAi agent is administered to the subject by an administration means selected from the group consisting of subcutaneous, intravenous, intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof. In another example, the double stranded RNAi agent is administered to the subject via subcutaneous, intramuscular or intravenous administration. In yet another example, the double stranded RNAi agent is administered to the subject via subcutaneous administration.

In one example, the methods further comprise assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the subject.

In one example, administering the double stranded RNAi agent does not result in an inflammatory response in the subject as assessed based on the level of a cytokine or chemokine selected from the group consisting of G-CSF, IFN-γ, IL-10, IL-12 (p70), IL1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and any combinations thereof, in a sample from the subject.

In one example, the present disclosure provides methods of treating a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a fixed dose of about 12.5 mg to about 200 mg (*e.g.,* about 12.5 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 125 mg, about 150 mg, about 175 mg, or about 200 mg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In another example, the present disclosure provides methods of prophylactically treating a subject at risk of devloping a transthyretin (TTR)-associated disorder. The

methods include administering to the subject a fixed dose of about 12.5 mg to about 200 mg (*e.g.,* about 12.5 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 125 mg, about 150 mg, about 175 mg, or about 200 mg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'- UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the present disclosure provides methods of treating a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a dose of about 0.15 mg/kg to about 2.5 mg/kg (*e.g.,* about 0.15 mg/kg, about 0.3 mg/kg, about 0.6 mg/kg, about 1 mg/kg, about 1.25 mg/kg, about 2 mg/kg, about 2.5 mg/kg, or about 3 mg/kg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'- UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the present disclosure provides methods of prophylactically treating a subject at risk of developing a transthyretin (TTR)-associated disorder. The methods include administering to the subject a dose of about 0.15 mg/kg to about 2.5 mg/kg (*e.g.,* about 0.15 mg/kg, about 0.3 mg/kg, about 0.6 mg/kg, about 1 mg/kg, about 1.25 mg/kg, about 2 mg/kg, about 2.5 mg/kg, or about 3 mg/kg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'- UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of

the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In another example, the present disclosure provides methods of reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a dose of about 0.15 mg/kg to about 2.5 mg/kg (*e.g.,* about 0.15 mg/kg, about 0.3 mg/kg, about 0.6 mg/kg, about 1 mg/kg, about 1.25 mg/kg, about 2 mg/kg, about 2.5 mg/kg, or about 3 mg/kg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'- UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In yet another example, the present disclosure provides methods of increasing a 6-minute walk test (6MWT) in a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a dose of about 0.15 mg/kg to about 2.5 mg/kg (*e.g.,* about 0.15 mg/kg, about 0.3 mg/kg, about 0.6 mg/kg, about 1 mg/kg, about 1.25 mg/kg, about 2 mg/kg, about 2.5 mg/kg, or about 3 mg/kg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the present disclosure provides methods of treating a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a fixed dose of about 10 mg to about 600 mg, about 25 mg to about 500 mg, about 50 mg to about 500 mg, or about 80 mg to about 500 mg, about 25 mg to about 300 mg, about 50 mg to about 300 mg, or about 80 mg to about 300 mg (*e.g.*, about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, about 125, about 130, about 140, about 150, about 160, about 170, about 175, about 180, about 190, about 200, about 210, about 220, about 225, about 230, about 240, about 250 mg, about 260, about 270, about 275, about 280, about 290, about 300, about 310, about 320, about 325, about 330, about 340, about 350, about 360, about 370, about 375, about 380, about 390, about 400, about 410, about 420, about 425, about 430, about 440, about 450 mg, about 460, about 470, about 475, about 480, about 490, about 500, about 510, about 520, about 525, about 530, about 540, about 550, about 560, about 570, about 575, about 580, about 590, or about 600 mg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'- UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the present disclosure provides methods of prophylactically treating a subject at risk of developing a transthyretin (TTR)-associated disorder. The methods include administering to the subject a fixed dose of about 10 mg to about 600 mg, about 25 mg to about 500 mg, about 50 mg to about 500 mg, or about 80 mg to about 500 mg, about 25 mg to about 300 mg, about 50 mg to about 300 mg, or about 80 mg to about 300 mg (*e.g.*, about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, about 125, about 130, about 140, about 150, about 160, about 170, about 175, about 180, about 190, about 200, about 210, about 220, about 225, about 230, about 240, about 250 mg, about 260, about 270, about 275, about 280, about 290, about 300, about 310, about 320, about 325, about 330, about 340, about 350, about 360, about 370, about 375, about 380, about 390, about 400, about 410, about 420, about 425, about 430, about 440, about 450 mg, about 460, about 470, about 475, about 480, about 490, about 500, about 510, about 520, about 525, about

530, about 540, about 550, about 560, about 570, about 575, about 580, about 590, or about 600 mg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In another example, the present disclosure provides methods of reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject a fixed dose of about 10 mg to about 600 mg, about 25 mg to about 500 mg, about 50 mg to about 500 mg, or about 80 mg to about 500 mg, about 25 mg to about 300 mg, about 50 mg to about 300 mg, or about 80 mg to about 300 mg (*e.g.*, about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, about 125, about 130, about 140, about 150, about 160, about 170, about 175, about 180, about 190, about 200, about 210, about 220, about 225, about 230, about 240, about 250, about 260, about 270, about 275, about 280, about 290, about 300, about 310, about 320, about 325, about 330, about 340, about 350, about 360, about 370, about 375, about 380, about 390, about 400, about 410, about 420, about 425, about 430, about 440, about 450, about 460, about 470, about 475, about 480, about 490, about 500, about 510, about 520, about 525, about 530, about 540, about 550, about 560, about 570, about 575, about 580, about 590, or about 600 mg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate

linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In yet another example, the present disclosure provides methods of increasing a 6-minute walk test (6MWT) in a subject having a transthyretin (TTR)-associated disorder. The methods include administering to the subject afixed dose of about 10 mg to about 600 mg, about 25 mg to about 500 mg, about 50 mg to about 500 mg, or about 80 mg to about 500 mg, about 25 mg to about 300 mg, about 50 mg to about 300 mg, or about 80 mg to about 300 mg (*e.g.,* about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 75, about 80, about 90, about 100, about 110, about 120, about 125, about 130, about 140, about 150, about 160, about 170, about 175, about 180, about 190, about 200, about 210, about 220, about 225, about 230, about 240, about 250, about 260, about 270, about 275, about 280, about 290, about 300, about 310, about 320, about 325, about 330, about 340, about 350, about 360, about 370, about 375, about 380, about 390, about 400, about 410, about 420, about 425, about 430, about 440, about 450, about 460, about 470, about 475, about 480, about 490, about 500, about 510, about 520, about 525, about 530, about 540, about 550, about 560, about 570, about 575, about 580, about 590, or about 600 mg) of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to SEQ ID NO:2 (5'-UGGGAUUUCAUGUAACCAAGA -3'), wherein each strand is about 14 to about 30 nucleotides in length, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, wherein the sense strand comprises no more than 8 2′-fluoro modifications; wherein the antisense strand comprises no more than 6 2′-fluoro modifications; wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and wherein the sense strand is conjugated to at least one ligand.

In one example, the double stranded RNAi agent is represented by formula (IIIe):

sense: 5' - N_a -Y Y Y – N_b - 3'

antisense: 3' - n_p'-N_a'- Y′Y′Y′- N_b'- 5' (IIIe)

wherein:

n_p' is a 2 nucleotide overhang and each nucleotide within n_p' is linked to a neighboring nucleotide *via* a phosphorothioate linkage;

each N_a, N_b, N_b and N_b' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y′Y′Y′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one example, the antisense strand comprises a nucleotide sequence selected from the group consisting of

5'- usCfsuugguuacaugAfaaucccasusc – 3' (SEQ ID NO: 6),

5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3'(SEQ ID NO: 7),

5'- UfsCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 8), and

5'- VPusCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 9), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; s is a phosphorothioate linkage; and VP is a 5'-phosphate mimic.

In one example, the sense and antisense strands comprise nucleotide sequences selected from the group consisting of

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- usCfsuugguuacaugAfaaucccasusc – 3' (SEQ ID NO: 6);

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7);

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- UfsCfsuugGfuuAfcaugAfaAfucccasusc – 3'(SEQ ID NO: 8); and

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- VPusCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 9), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; and s is a phosphorothioate linkage; and VP is a 5'-phosphate mimic.

In one example, the sense and antisense strands comprise the nucleotide sequences

5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7),

wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; and s is a phosphorothioate linkage.

The fixed dose of the double stranded RNAi agent may be administered to the subject once about every 4 weeks, every 5 weeks, every six weeks, every eight weeks or quarterly.

The dose of the double stranded RNAi agent may be administered to the subject once about every 4 weeks, every 5 weeks, every six weeks, every eight weeks or quarterly.

In one example, the double stranded RNAi agent is administered to the subject about once every quarter.

In one example, the double stranded RNAi agent is chronically administered to the subject.

In one example, the subject is a human.

In one example, the subject is a subject suffering from a TTR-associated disease. In another example, the subject is a subject at risk for developing a TTR-associated disease. In one example, the subject at risk of developing a TTR-associated disease carries a TTR gene mutation that is associated with the development of a TTR associated disease, or a subject with a family history of TTR-associated disease, or a subject who has signs or symptoms suggesting the development of TTR amyloidosis.

In one example, the TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, and hyperthyroxinemia.

In one example, the subject has a TTR-associated amyloidosis and the method reduces an amyloid TTR deposit in the subject.

In one example, the double stranded RNAi agent is administered to the subject by an administration means selected from the group consisting of subcutaneous, intravenous, intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof. In another example, the double stranded RNAi agent is administered to the subject via subcutaneous, intramuscular or intravenous administration. In yet another example, the double stranded RNAi agent is administered to the subject *via* subcutaneous administration, *e.g., via* self administration, *e.g., via* a pre-filled syringe or auto-injector syringe.

In one example, the methods further comprise assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the subject.

In one example, administering the double stranded RNAi agent does not result in an inflammatory response in the subject as assessed based on the level of a cytokine or chemokine selected from the group consisting of G-CSF, IFN-γ, IL-10, IL-12 (p70), IL1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and any combinations thereof, in a sample from the subject.

In one example, the agent suitable for use in the methods of the disclosure is AD-65492. AD-65492 may be chronically administered to the subject every 4 weeks, every 5 weeks, or every six weeks, or every quarter.

In one example, the present disclosure provides double stranded ribonucleic acid (RNAi) agents for use in inhibiting expression of transthyretin (TTR) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the sense

and antisense strands comprise nucleotide sequences selected from the group consisting of any of the nucleotide sequnces in Table 5.

In another example, the present disclosure provides double stranded ribonucleic acid (RNAi) agents for use in inhibiting expression of transthyretin (TTR) in a cell. The agents include a sense strand complementary to an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing no more than 3 nucleotides from any one of the antisense sequences in Table 6, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides; and wherein the sense strand is conjugated to at least one ligand.

The sense and antisense strand may comprise nucleotide sequences selected from the group consisting of any of the nucleotide sequnces in Table 6 or Table 7.

In one example the present disclosure provides double stranded ribonucleic acid (RNAi) agents for use in inhibiting expression of transthyretin (TTR) in a cell, wherein the RNAi agents comprise a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and the antisense strand comprises the nucleotide sequence 5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7), wherein a, c, g, and u are 2'-O-methyl (2'-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2'-fluoro A, C, G, or U; and s is a phosphorothioate linkage.

In another example, the present disclosure provides methods of treating a subject suffering from a TTR-associated disease. The methods include administering to the subject a dose of about 50 mg to about 300 mg of a double stranded RNAi agent, wherein the RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and the antisense strand comprises the nucleotide sequence 5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7), wherein a, c, g, and u are 2'-O-methyl (2'-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2'-fluoro A, C, G, or U; and s is a phosphorothioate linkage, thereby treating the subject suffering from a TTR-associated disease.

In yet another example, the present disclosure provides methods of prophylactically treating a subject at risk for developing a TTR-associated disease. The methods include administering to the subject a dose of about 50 mg to about 300 mg of a double stranded RNAi agent, wherein the RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and the antisense strand comprises the nucleotide sequence 5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ

ID NO: 7), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; and s is a phosphorothioate linkage, thereby prophylactically treating the subject at risk for developing a TTR-associated disease.

In one example, the present disclosure provides methods of reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in a subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the subject a dose of about 50 mg to about 300 mg of a double stranded RNAi agent, wherein the RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and the antisense strand comprises the nucleotide sequence 5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; and s is a phosphorothioate linkage, thereby reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in the subject.

In another example, the present disclosure provides methods of increasing a 6-minute walk test (6MWT) in a subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the subject a dose of about 50 mg to about 300 mg of of a double stranded RNAi agent, wherein the RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and the antisense strand comprises the nucleotide sequence 5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; and s is a phosphorothioate linkage, thereby increasing a 6-minute walk test (6MWT) in a subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

The present invention is further illustrated by the following detailed description and drawins.

Brief Description of the Drawings

Figure 1 is a graph depicting the stability of the indicated RNAi agents in a twenty-four hour tristosome stability assay.

Figure 2A is a graph depicting the stability of the indicated RNAi agents in a twenty-four hour rat cytosol stability assay and Figure 2B is a graph depicting the stability of the indicated RNAi agents in a twenty-four hour tristosome stability assay.

We claim:

1. A double stranded ribonucleic acid (RNAi) agent for inhibiting expression of transthyretin (TTR) in a cell, wherein said RNAi agent comprises a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region complementary to SEQ ID NO:2,

wherein each strand is about 14 to about 30 nucleotides in length,

wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides,

wherein the sense strand comprises no more than 8 2′-fluoro modifications;

wherein the antisense strand comprises no more than 6 2′-fluoro modifications;

wherein the sense strand and the antisense strand each independently comprise two phosphorothioate linkages at the 5'-terminus; and

wherein the sense strand is conjugated to at least one ligand.

2. The double stranded RNAi agent of claim 1, wherein said double stranded RNAi agent is represented by formula (IIIe):

sense: 5' - N_a -Y Y Y – N_b - 3'

antisense: 3' - n_p'-N_a'- Y′Y′Y′- N_b'- 5' (IIIe)

wherein:

n_p' is a 2 nucleotide overhang and each nucleotide within n_p' is linked to a neighboring nucleotide *via* a phosphorothioate linkage;

each N_a, N_b, N_a' and N_b' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof,;

YYY and Y′Y′Y′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

3. The double stranded RNAi agent of claim 2, wherein the YYY motif occurs at or near the cleavage site of the sense strand.

4. The double stranded RNAi agent of claim 1, wherein the double stranded region is 15-30 nucleotide pairs in length.

5. The double stranded RNAi agent of claim 4, wherein the double stranded region is 17-23 nucleotide pairs in length.

6. The double stranded RNAi agent of claim 4, wherein the double stranded region is 19-21 nucleotide pairs in length.

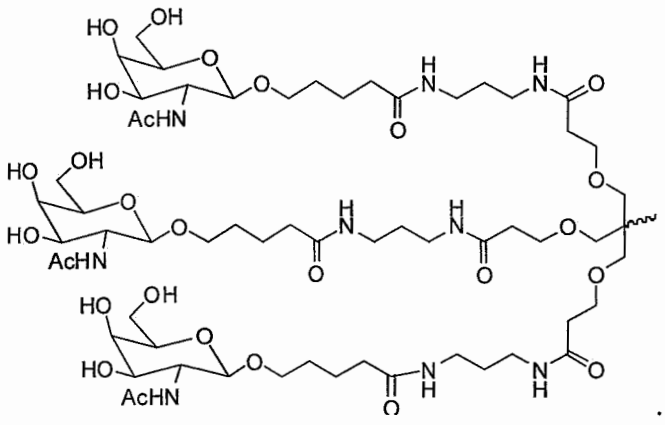
7. The double stranded RNAi agent of claim 1, wherein the double stranded region is 21-23 nucleotide pairs in length.

8. The double stranded RNAi agent of claim 1, wherein each strand has 19-30 nucleotides.

9. The double stranded RNAi agent of claim 1, wherein the modifications on the nucleotides are selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic, and combinations thereof.

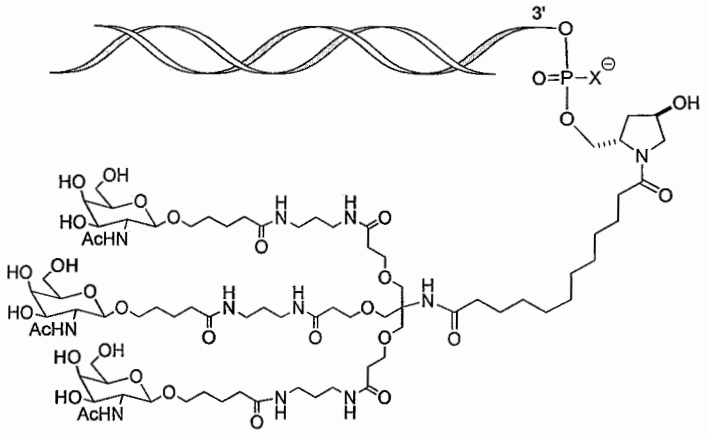
10. The double stranded RNAi agent of claim 1, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

11. The double stranded RNAi agent of claim 1, wherein the ligand is



12. The double stranded RNAi agent of claim 1, wherein the ligand is attached to the 3′ end of the sense strand.

13. The double stranded RNAi agent of claim 12, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

14. The double stranded RNAi agent of claim 1, wherein the antisense strand comprise a nucleotide sequence selected from the group consisting of
5'- usCfsuugguuacaugAfaaucccasusc – 3' (SEQ ID NO: 6),
5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7),
5'- UfsCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 8), and
5'- VPusCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 9), wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, or U; s is a phosphorothioate linkage; and VP is a 5'-phosphate mimic.

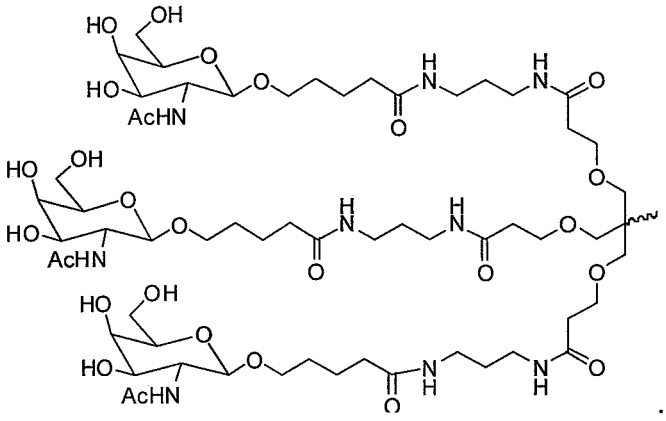
15. The double stranded RNAi agent of claim 1, wherein the sense and antisense strands comprise nucleotide sequences selected from the group consisting of
5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and
5'- usCfsuugguuacaugAfaaucccasusc – 3' (SEQ ID NO: 6);
5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and
5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7);
5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and
5'- UfsCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 8); and
5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and

5'- VPusCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 9), wherein a, c, g, and u are 2'-O-methyl (2'-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2'-fluoro A, C, G, or U; and s is a phosphorothioate linkage; and VP is a 5'-phosphate mimic.

16. The double stranded RNAi agent of claim 15, wherein the sense and antisense strands comprise the nucleotide sequences
5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and
5'- usCfsuugGfuuAfcaugAfaAfucccasusc – 3' (SEQ ID NO: 7),
wherein a, c, g, and u are 2'-O-methyl (2'-OMe) A, C, G, or U; Af, Cf, Gf, and Uf are 2'-fluoro A, C, G, or U; and s is a phosphorothioate linkage.

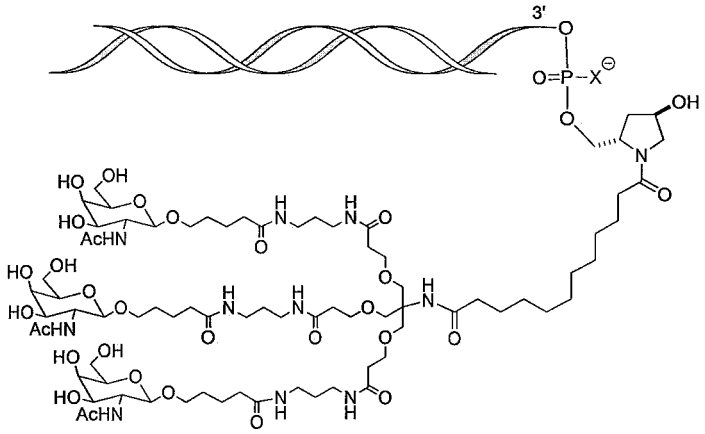
17. The double stranded RNAi agent of any one of claims 14 to 16, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

18. The double stranded RNAi agent of any one of claims 14 to 16, wherein the ligand is



19. The double stranded RNAi agent of any one of claims 14 to 16, wherein the ligand is attached to the 3' end of the sense strand.

20. The double stranded RNAi agent of claim 19, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

21. A pharmaceutical composition comprising the double stranded RNAi agent of any one of claims 1 to 20.

22. An *in vitro* method of inhibiting transthyretin (TTR) expression in a cell, the method comprising:

contacting the cell with the double stranded RNAi agent of any one of claims 1 to 20, or the pharmaceutical composition of claim 21, thereby inhibiting expression of the TTR gene in the cell.

23. Use of the double stranded RNAi agent of any one of claims 1 to 20, or the pharmaceutical composition of claim 44 in the manufacture of a medicament for treating a subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease, wherein said TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, hereditary transthyretin-mediated amyloidosis, and hyperthyroxinemia..

24. The use of claim 23, wherein the subject is a human.

25. The use of claim 23, wherein said subject is a subject suffering from a TTR-associated disease.

26. The use of claim 23, wherein said subject is a subject at risk for developing the TTR-associated disease.

27. The use of claim 23, wherein said subject carries a TTR gene mutation that is associated with the development of the TTR-associated disease.

28. The use of claim 23, wherein said subject has a TTR-associated amyloidosis and said method reduces an amyloid TTR deposit in said subject.

29. The use of claim 23, wherein the double stranded RNAi agent is administered to the subject at a fixed dose of about 50 mg.

30. A double stranded RNAi agent of any one of claims 1 to 20, or the pharmaceutical composition of claim 21 for use in therapy.