thyroxine with TTR due to a mutant TTR molecule with increased affinity for thyroxine. See, *e.g.*, Moses *et al.* (1982) *J. Clin. Invest.*, 86, 2025-2033.

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 ~~of the Invention~~

The present ~~invention~~disclosure provides methods of inhibiting expression of TTR and methods of treating or preventing a Transthyretin- (TTR-) associated disease in a human subject using RNAi agents, *e.g.*, double stranded RNAi agents, targeting the TTR gene. The present ~~invention~~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~~disclosure improved efficacy, stability, potency, and durability.

In one aspect, the present disclosure refers to the use of a pharmaceutical composition comprising a double stranded RNAi agent in the manufacture of a medicament for treating a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease, wherein the pharmaceutical composition is to be administered at a fixed dose of about 25 mg, wherein 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, amyloidotic vitreous opacities, carpal tunnel syndrome, and hyperthyroxinemia, and wherein the double stranded 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), and 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 aspect, the present disclosure refers to the use of a pharmaceutical composition comprising a double stranded RNAi agent in the manufacture of a medicament for improving at least one indicia of neurological impairment or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease, wherein the pharmaceutical composition is to be administered at a fixed dose of about 25 mg, wherein the TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), leptomeningeal/Central Nervous System (CNS) amyloidosis, and carpal tunnel syndrome, and wherein the double stranded 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.

Accordingly, in one ~~aspect~~example, the present ~~invention~~disclosure provides methods of treating a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg to about 50 mg (*e.g.,* about 25, 30, 35, 40, 45, or about 50 mg) of a double stranded RNAi agent, wherein the double stranded 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 human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In another ~~aspect~~example, the present ~~invention~~disclosure provides method sof improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg to about 50 mg of a double stranded RNAi agent, wherein the double stranded 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 improving the at least one indicia of neurological impairment or quality of life in the human subject.

In one ~~embodiment~~example, the indicia is a neurological impairment indicia, *e.g.,* a Neuropathy Impairment (NIS) score or a modified NIS (mNIS+7) score. In another ~~embodiment~~example, the indicia is a quality of life indicia, *e.g.,* a quality of life indicia selected from the group consisting of a SF-36® health surveyscore, a Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) score, a NIS-W score, a Rasch-built Overall Disability Scale (R-ODS) score, a composite autonomic symptom score (COMPASS-31), a median body mass index (mBMI) score, a 6-minute walk test (6MWT) score, and a 10-meter walk test score.

In another ~~aspect~~example, the present ~~invention~~disclosure provides methods of reducing, slowing, or arresting a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg to about 50 mg (*e.g.,* about 25, 30, 35, 40, 45, or about 50 mg) of a

double stranded RNAi agent, wherein the double stranded 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 human subject.

In another ~~aspect~~example, the present ~~invention~~disclosure provides methods of increasing a 6-minute walk test (6MWT) in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg to about 50 mg (*e.g.,* about 25, 30, 35, 40, 45, or about 50 mg) of a double stranded RNAi agent, wherein the double stranded 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, therebyincreasing a 6-minute walk test (6MWT) in the human subject.

In one ~~embodiment~~example, the human subject is a human subject suffering from a TTR-associated disease. In another ~~embodiment~~example, the human subject is a human subject at risk for developing a TTR-associated disease. In another ~~embodiment~~example, the human subject carries a TTR gene mutation that is associated with the development of a TTR-associated disease. In one ~~embodiment~~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 another ~~embodiment~~example, the human subject has a transthyretin-mediated amyloidosis (ATTR amyloidosis) and the method reduces an amyloid TTR deposit in the human subject. The ATTR may be hereditary ATTR (h-ATTR) or non-heriditary ATTR (wt ATTR).

The double stranded RNAi agent may be administered to the human 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 one ~~embodiment~~example, the double stranded RNAi agent is administered to the human subject *via* subcutaneous, intramuscular or intravenous administration. In another ~~embodiment~~example, the double stranded RNAi agent is administered to the human subject *via* subcutaneous administration. In one ~~embodiment~~example, the subcutaneous administration is self administration. In one ~~embodiment~~example, the self-administration is *via* a pre-filled syringe or auto-injector syringe.

The methods of the ~~invention~~disclosure may further include assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the human subject.

The double stranded RNAi agent may be administered to the human subject every 3 months, every four months, every five months, or every six months. In one ~~embodiment~~example, the fixed dose of the double stranded RNAi agent is administered to the human subject once about every 3 months. In another ~~embodiment~~example, the fixed dose of the double stranded RNAi agent is administered to the human subject once about every six months.

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

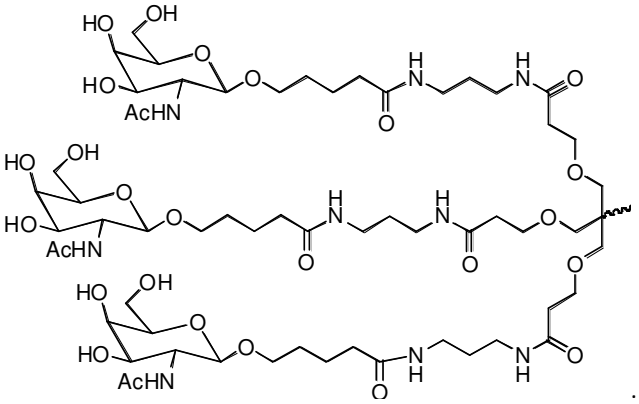
In one ~~embodiment~~example, the double stranded RNAi agent is administered to the human subject at a fixed dose of about 25 mg. In another ~~embodiment~~example, the double stranded RNAi agent is administered to the human subject at a fixed dose of about 50 mg.

In one ~~embdoiment~~example, the methods of the ~~invention~~disclosure may further include providing to the subject an additional therapeutic treatment, *e.g.,* an orthotopic liver tanslplant, implantation of a pacemaker, a heart transplant, and/or administering to the human subject an additional therapeutic agent useful for treating the TTR-associated disease, *e.g.,* a TTR tetramer stabilizer, *e.g.,* tafamidis, and/or a nonsteroidal anti-inflammatory drug (NSAID), *e.g.,* diflunisal.

In one ~~embodiment~~example, the sense strand of the double stranded RNAi agent is conjugated to at least one ligand.

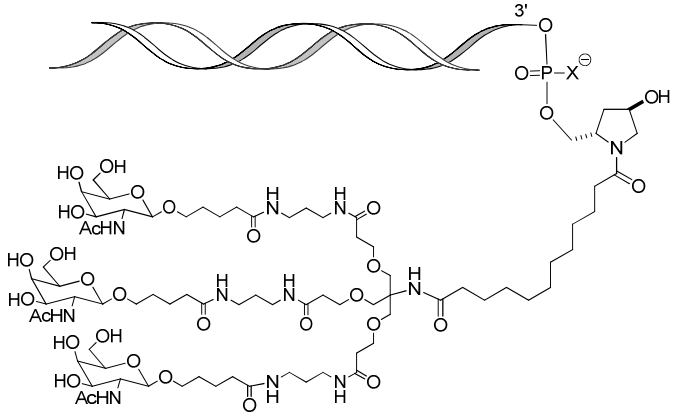
In one ~~embodiment~~example, the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one ~~embodiment~~example, the ligand is



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

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



wherein X is O or S.

In one ~~embodiment~~example, the sense strand of the RNAi agent comprises the nucleotide sequence 5’- usgsggauUfuCfAfUfguaaccaagaL96 – 3’ (SEQ ID NO:15)and the antisense strand of the RNAi agent 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

In one ~~aspect~~example, the present ~~invention~~disclosure provides methods of treating a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg of a double stranded RNAi agent about once every three months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5’- usgsggauUfuCfAfUfguaaccaagaL96 – 3’ (SEQ ID NO: 17) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby treating the human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In another ~~aspect~~example, the present ~~invention~~disclosure provides methods of treating a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg of a double stranded RNAi agent about once every six months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaagaL96 – 3' (SEQ ID NO: 15) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby treating the human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In one ~~aspect~~example, the present ~~invention~~disclosure provides methods of treating a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 50 mg of a double stranded RNAi agent about once every three months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaagaL96 – 3' (SEQ ID NO: 15) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby treating the human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In another ~~aspect~~example, the present ~~invention~~disclosure provides methods of treating a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 50 mg of a double stranded RNAi agent about once every six months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'-

usgsggauUfuCfAfUfguaaccaagaL96 – 3’ (SEQ ID NO: 15) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby treating the human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In one ~~aspect~~example, the present ~~invention~~disclosure provides methods of improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg of a double stranded RNAi agent about once every three months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5’- usgsggauUfuCfAfUfguaaccaagaL96 – 3’ (SEQ ID NO: 15) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In another ~~aspect~~example, the present ~~invention~~disclosure provides methods of improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 25 mg of a double stranded RNAi agent about once every six months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5’- usgsggauUfuCfAfUfguaaccaagaL96 – 3’ (SEQ ID NO: 15) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In one ~~aspect~~example, the present ~~invention~~disclosure provides methods of improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 50 mg of a double stranded RNAi agent about once every three months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaagaL96 – 3' (SEQ ID NO: 15) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In another ~~aspect~~example, the present ~~invention~~disclosure provides methods of improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease. The methods include administering to the human subject a fixed dose of about 50 mg of a double stranded RNAi agent about once every six months, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaagaL96 – 3' (SEQ ID NO: 15) 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol, thereby improving at least one indicia of neurological impairement or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease.

In one ~~embodiment~~example, the indicia is a neurological impairment indicia, *e.g.,* a Neuropathy Impairment (NIS) score or a modified NIS (mNIS+7) score. In another ~~embodiment~~example, the indicia is a quality of life indicia, *e.g.,* a quality of life indicia selected from the group consisting of a SF-36® health surveyscore, a Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) score, a NIS-W score, a Rasch-built Overall Disability Scale (R-ODS) score, a composite autonomic symptom score (COMPASS-31), a median body mass index (mBMI) score, a 6-minute walk test (6MWT) score, and a 10-meter walk test score.

The present ~~invention~~disclosure also provides kits for performing any of the methods of the ~~invention~~disclosure. The kits may include the double stranded RNAi agent; and a label comprising instructions for use.

The present ~~invention~~disclosure is further illustrated by the following detailed description and drawings.

Brief Description of the Drawings

Figure 1 is a graph depicting TTR protein suppression in healthy human volunteers subcutaneously administered a single 5 mg, 25 mg, 50 mg, 100 mg, 200 mg, or 300 mg dose of AD-65492. The graph shows the mean [+/- SEM] TTR relative to baseline over time by cohort.

Detailed Description of the Invention

The present invention provides methods of inhibiting expression of TTR and methods of treating or preventing a Transthyretin- (TTR-) associated disease in a human subject using RNAi agents, *e.g.*, double stranded RNAi agents, targeting the TTR gene. The present invention 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 (*e.g.,* no more than 7 2′-fluoro modifications, no more than 6 2′-fluoro modifications, no more than 5 2′-fluoro modifications, no more than 4 2′-fluoro modifications, 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) on the sense strand, no more than 6 2'-fluoro modifications (*e.g.,* 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) 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 selectively 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.

We claim:

1. Use of a pharmaceutical composition comprising a double stranded RNAi agent in the manufacture of a medicament for ~~A method of~~ treating a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease,

wherein the pharmaceutical composition is to be administered at ~~the method comprising administering to the human subject~~ a fixed dose of about 25 mg ~~to about 50 mg of a double stranded RNAi agent~~,

wherein 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, amyloidotic vitreous opacities, carpal tunnel syndrome, and hyperthyroxinemia, and

wherein the double stranded 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), and

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 human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease~~.

2. Use of a pharmaceutical composition comprising a double stranded RNAi agent in the manufacture of a medicament for ~~A method of~~ improving at least one indicia of neurological impair~~e~~ment or quality of life in a human subject suffering from a TTR-associated disease or at risk for developing a TTR-associated disease,

wherein the pharmaceutical composition is to be administered at ~~the method comprising administering to the human subject~~ a fixed dose of about 25 mg ~~to about 50 mg of a double stranded RNAi agent~~,

wherein the TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), leptomeningeal/Central Nervous System (CNS) amyloidosis, and carpal tunnel syndrome, and

wherein the double stranded 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 improving the at least one indicia of neurological impairment or quality of life in the human subject~~.

3. The ~~method~~ use of claim 2, wherein the indicia is a neurological impairment indicia.

4. The ~~method~~ use of claim 3, wherein the neurological impairment indicia is a Neuropathy Impairment (NIS) score or a modified NIS (mNIS+7) score.

5. The ~~method~~ use of claim 2, wherein the indicia is a quality of life indicia.

6. The ~~method~~ use of claim 5, wherein the quality of life indicia is selected from the group consisting of a SF-36® health survey score, a Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) score, a NIS-W score, a Rasch-built Overall Disability Scale (R-ODS) score, a composite autonomic symptom score (COMPASS-31), a median body mass index (mBMI) score, a 6-minute walk test (6MWT) score, and a 10-meter walk test score.

~~7. The method of any one of claims1-6, wherein the human subject is a human subject suffering from a TTR-associated disease.~~

~~8. The method of any one of claims 1-6, wherein the human subject is a human subject at risk for developing a TTR-associated disease.~~

~~9~~7. The ~~method~~ use of any one of claims 1-6, wherein the human subject carries a TTR gene mutation that is associated with the development of a TTR-associated disease.

~~10. The method of any one of claims 1-6, wherein 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.~~

~~11~~8. The ~~method~~ use of any one of claims 1-6, wherein the human subject has a transthyretin-mediated amyloidosis (ATTR amyloidosis) and the ~~method~~ use reduces an amyloid TTR deposit in the human subject.

~~12~~9. The ~~method~~ use of claim ~~11~~8, wherein the ATTR is hereditary ATTR (h-ATTR).

~~13~~10. The ~~method~~ use of claim ~~11~~8, wherein the ATTR is non-her~~i~~editary ATTR (wt ATTR).

~~14~~11. The ~~method~~ use of any one of claims 1-~~13~~10, wherein the double stranded RNAi agent is to be administered to the human subject by an administration means selected from the group consisting of subcutaneous, intravenous, intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof.

~~15~~12. The ~~method~~ use of any one of claims 1-~~13~~10, wherein the double stranded RNAi agent is to be administered to the human subject *via* subcutaneous, intramuscular or intravenous administration.

~~16~~13. The ~~method~~ use of any one of claims 1-~~13~~12, wherein the double stranded RNAi agent is to be administered to the human subject *via* subcutaneous administration.

~~17~~14. The ~~method~~ use of claim ~~16~~13, wherein the subcutaneous administration is self administration.

~~18~~15. The ~~method~~ use of claim ~~17~~14, wherein the self-administration is *via* a pre-filled syringe or auto-injector syringe.

~~19~~16. The ~~method~~ use of any one of claims 1-~~18~~15, wherein ~~further comprising assessing~~ the level of TTR mRNA expression or TTR protein expression in a sample derived from the human subject is assessed.

~~20~~17. The ~~method~~ use of any one of claims 1-~~19~~16, wherein the double stranded RNAi agent is to be administered to the human subject every three months, every four months, every five months, every six months, every nine months, or every twelve months.

~~21~~18. The ~~method~~ use of any one of claims 1-~~19~~16, wherein the fixed dose of the double stranded RNAi agent is to be administered to the human subject once about every three months.

~~22. The method of any one of claims 1-19, wherein the fixed dose of the double stranded RNAi agent is administered to the human subject once about every six months.~~

~~23~~19. The ~~method~~ use of any one of claims 1-~~22~~18, wherein the double stranded RNAi agent is to be chronically administered to the human subject.

~~24. The method of any one of claims 1-23, wherein the double stranded RNAi agent is administered to the human subject at a fixed dose of about 25 mg.~~

~~25. The method of any one of claims 1-23, wherein the double stranded RNAi agent is administered to the human subject at a fixed dose of about 50 mg.~~

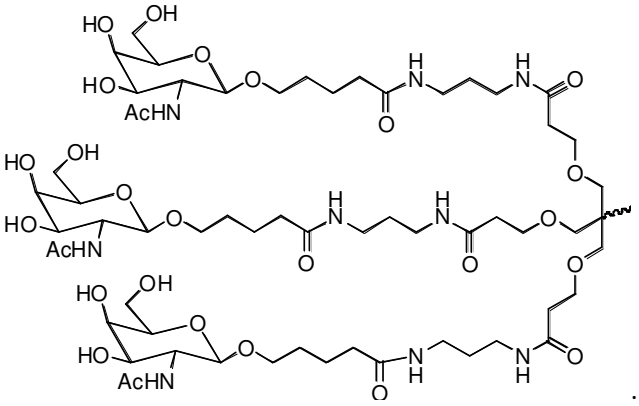
~~26~~20. The ~~method~~ use of any one of claims 1-~~25~~19, ~~further comprising administering to the human subject~~wherein the use further comprises that an additional therapeutic agent is to be administered.

~~27~~21. The ~~method~~ use of claim ~~26~~20, wherein the additional therapeutic agent is a TTR tetramer stabilizer and/or a non-steroidal anti-inflammatory agent.

~~28~~22. The ~~method~~ use of any one of claims 1-~~27~~21, wherein the sense strand of the double stranded RNAi agent is conjugated to at least one ligand.

~~29~~23. The ~~method~~ use of claim ~~28~~22, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

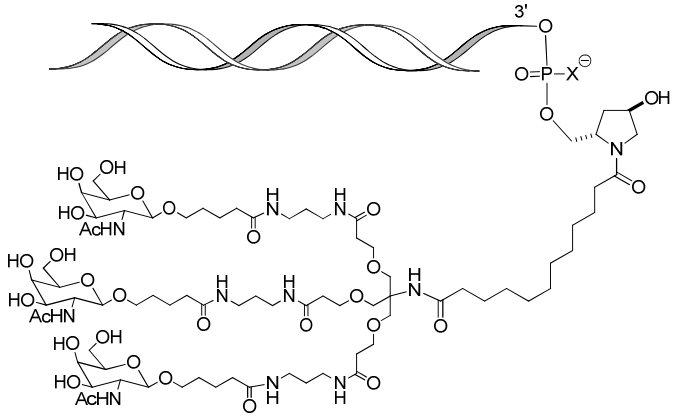
~~30~~24. The ~~method~~ use of claim ~~28~~22, wherein the ligand is



.

~~31~~25. The ~~method~~ use of claim ~~28~~22, wherein the ligand is attached to the 3′ end of the sense strand.

~~32~~26. The ~~method~~ use of claim ~~31~~25, wherein the double stranded RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

~~33~~27. The ~~method~~ use of any one of claims 1-~~32~~26, wherein the sense strand of the double stranded RNAi agent comprises the nucleotide sequence 5'- usgsggauUfuCfAfUfguaaccaagaL96 – 3' (SEQ ID NO: 15) and the antisense strand of the RNAi agent 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; s is a phosphorothioate linkage; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

~~34. A kit for performing the methods of any one of claims 1-33, comprising the double stranded RNAi agent; and a label comprising instructions for use.~~