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 18 to 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

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 18 to 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.

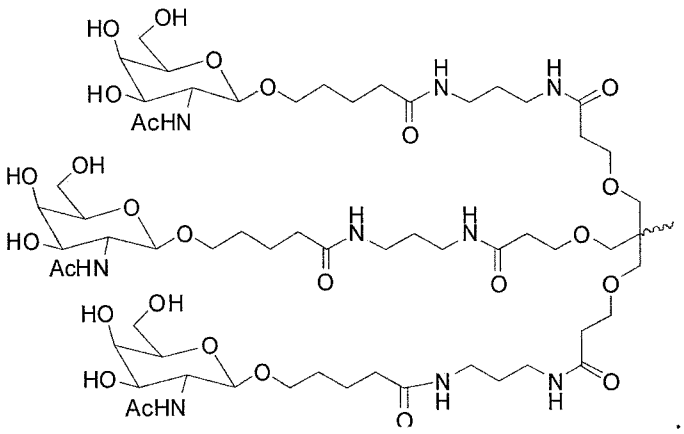
7. The double stranded RNAi agent of claim 1, wherein the double stranded region is 21-23 nucleotide pairs in length.

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.

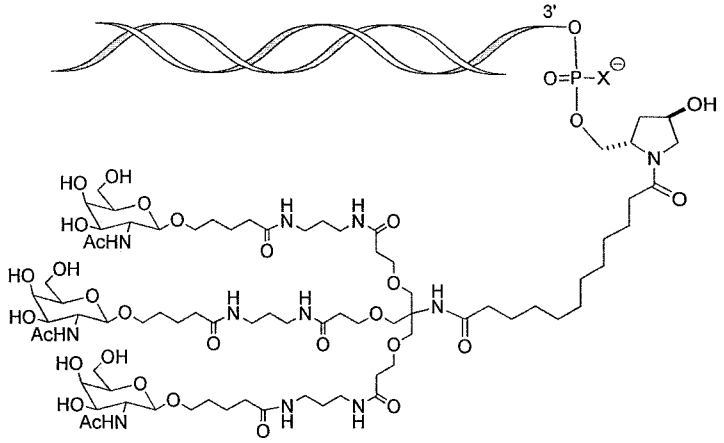
10. The double stranded RNAi agent of claim 1, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

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, and U, respectively; Af, Cf, Gf, and Uf are 2′-fluoro A, C, G, and U, respectively; s is a phosphorothioate linkage; and VP is a 5'-phosphate mimic.

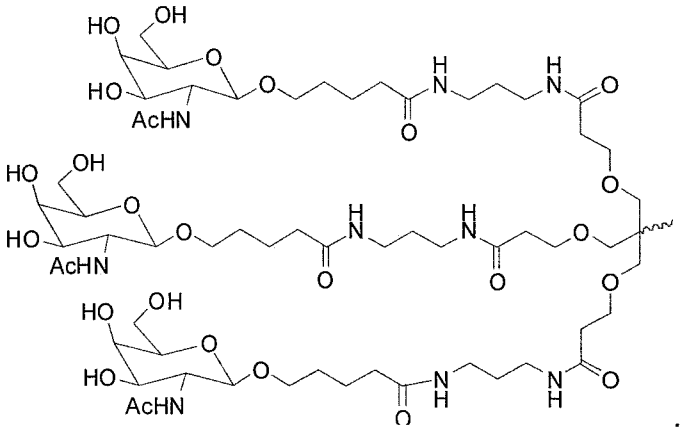
15. The double stranded RNAi agent of claim 1, wherein the sense and antisense strands comprise nucleotide sequences selected from the group consisting of
5'- usgsggauUfuCfAfUfguaaccaaga – 3' (SEQ ID NO: 10) and
5'- usCfsuugguuacaugAfaaucccasusc – 3' (SEQ ID NO: 6);
5'- usgsggauUfuCfAfUfguaaccaaga – 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, and U, respectively; Af, Cf, Gf, and Uf are 2'-fluoro A, C, G, and U, respectively; 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, and U, respectively; Af, Cf, Gf, and Uf are 2'-fluoro A, C, G, and U, respectively; and s is a phosphorothioate linkage.

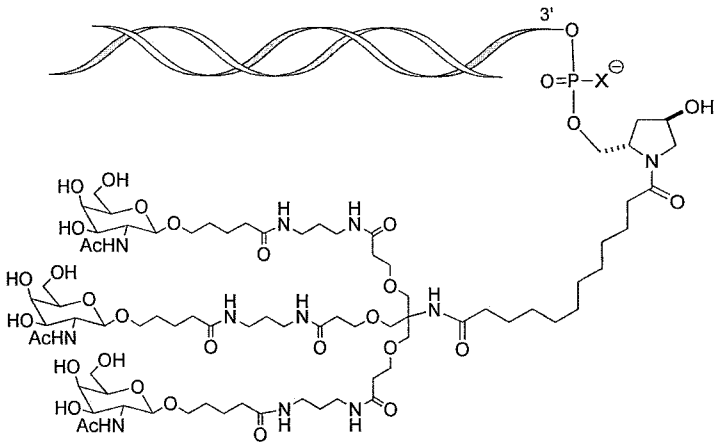
17. The double stranded RNAi agent of any one of claims 14 to 16, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

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 to be 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.