In addition, elevated RBP4 in the circulation of type 2 diabetic patients was reported many years ago (Basualdo C, *et al.*, (1997). *J. Am. Coll. Nutr.* 16, 39–45; Abahusain *et al.*, (1999). *Eur. J. Clin. Nutr.* 53, 630–635) and transgenic overexpression of RBP4 or injection of human RBP4 in normal mice were shown to cause insulin resistance. In contrast, genetic deletion of RBP4 or lowering of circulating RBP4 levels had the opposite effect and protected mice from developing insulin resistance (Yang, Q., *et al*. (2005). *Nature* 436, 356–362). In addition, positive associations were documented for circulating RBP4 or RBP4 expression levels with established cardiovascular disease (CVD) risk factors, including metabolic syndrome, overall/central obesity, dyslipidemia, inflammatory markers, and hypertension (Qi Q, *et al., J Clin Endocrinol Metab*. 2007; 92:4827–4834; Ingelsson E, *et al., Atherosclerosis*. 2009; 206:239–244). As indicated above, TTR regulates RBP4 and, thus, decreasing TTR results in a decrease in RBP4.

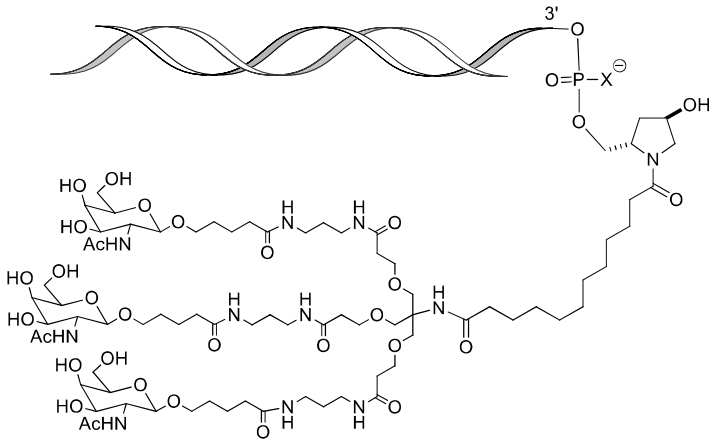
Any available current treatments for TTR-associated diseases such ocular diseases, *e.g.*, Stargardt's disease, diabetic retinopathy, age-related macular degeneration (AMD), *e.g*., dry AMD and wet AMD; a metabolic disorder, *e.g*., a disorder of glucose and lipid homeostasis, *e.g*., insulin resistance associated with type II diabetes, and a cardiovascular disease, are not fully effective and may result in serous side effects, such as conjunctival hemorrhage, increased eye pressure, infection, retinal detachment and eye inflammation. Similarly, current treatments for metabolic diseases, including dieting, exercise, and medications to help control blood pressure, cholesterol and blood sugar levels, are not always effecive.

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

SUMMARY OF THE INVENTION

In one aspect, provided herein is a double stranded ribonucleic acid (dsRNA) agent, or salt thereof, for inhibiting expression of transthyretin (TTR) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,
wherein the sense strand comprises the nucleotide sequence 5'-csasagagUfaUfUfCfcauuuuuacu-3' and the antisense strand comprises the nucleotide sequence 5'-asGfsuaaAfaauggaaUfaCfucuugsgsu-3', 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 wherein at least one strand is conjugated to an N-acetylgalactosamine (GalNAc) ligand.

In another aspect, provided herein is a double stranded ribonucleic acid (dsRNA) agent, or salt thereof, for inhibiting expression of transthyretin (TTR) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence 5'-csasagagUfaUfUfCfcauuuuuacu-3' and the antisense strand comprises the nucleotide sequence 5'-asGfsuaaAfaauggaaUfaCfucuugsgsu-3', 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 wherein the dsRNA agent is conjugated to a ligand as shown in the following schematic



, wherein X is O.

In another aspect, provided herein is a cell containing the dsRNA agent, or salt thereof, as described herein.

In another aspect, provided herein is a pharmaceutical composition for inhibiting expression of a gene encoding transthyretin (TTR) comprising the dsRNA agent, or salt thereof, as described herein, and a pharmaceutically acceptable carrier.

In another aspect, provided herein is an *in vitro* method of inhibiting expression of a transthyretin (TTR) gene in a cell, the method comprising contacting the cell with the dsRNA agent, or salt thereof, as described herein, or the pharmaceutical composition as described herein, thereby inhibiting expression of the TTR gene in the cell.

In another aspect, provided herein is use of the dsRNA agent, or salt thereof, as described herein, or the pharmaceutical composition as described herein, in the manufacture of a medicament for the treatment of a TTR-associated disease in a subject or a subject at risk for developing a TTR-associated disease.

In another aspect, provided herein is the dsRNA agent, or salt thereof, as described herein, or the pharmaceutical composition as described herein for use in therapy.

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding transthyretin (TTR). The TTR gene may be within a cell, *e.g.,* a cell within a subject, such as a human subject. The present

invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of an TTR gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of an TTR gene, *e.g.*, a subject suffering or prone to suffering from an TTR-associated disorder, *e.g.,* senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, hyperthyroxinemia, an ocular disease, *e.g.*, Stargardt's disease, diabetic retinopathy, age-related macular degeneration (AMD), *e.g*., dry AMD and wet AMD; a metabolic disorder, *e.g*., a disorder of glucose and lipid homeostasis, *e.g*., insulin resistance associated with type II diabetes, and a cardiovascular disease.

Accordingly, in one aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of transthyretin (TTR) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent, or salt thereof, for inhibiting expression of transthyretin (TTR) in a cell,

wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises the nucleotide sequence 5'-csasagagUfaUfUfCfcauuuuuacu-3' and the antisense strand comprises the nucleotide sequence 5'-asGfsuaaAfaauggaaUfaCfucuugsgsu-3',

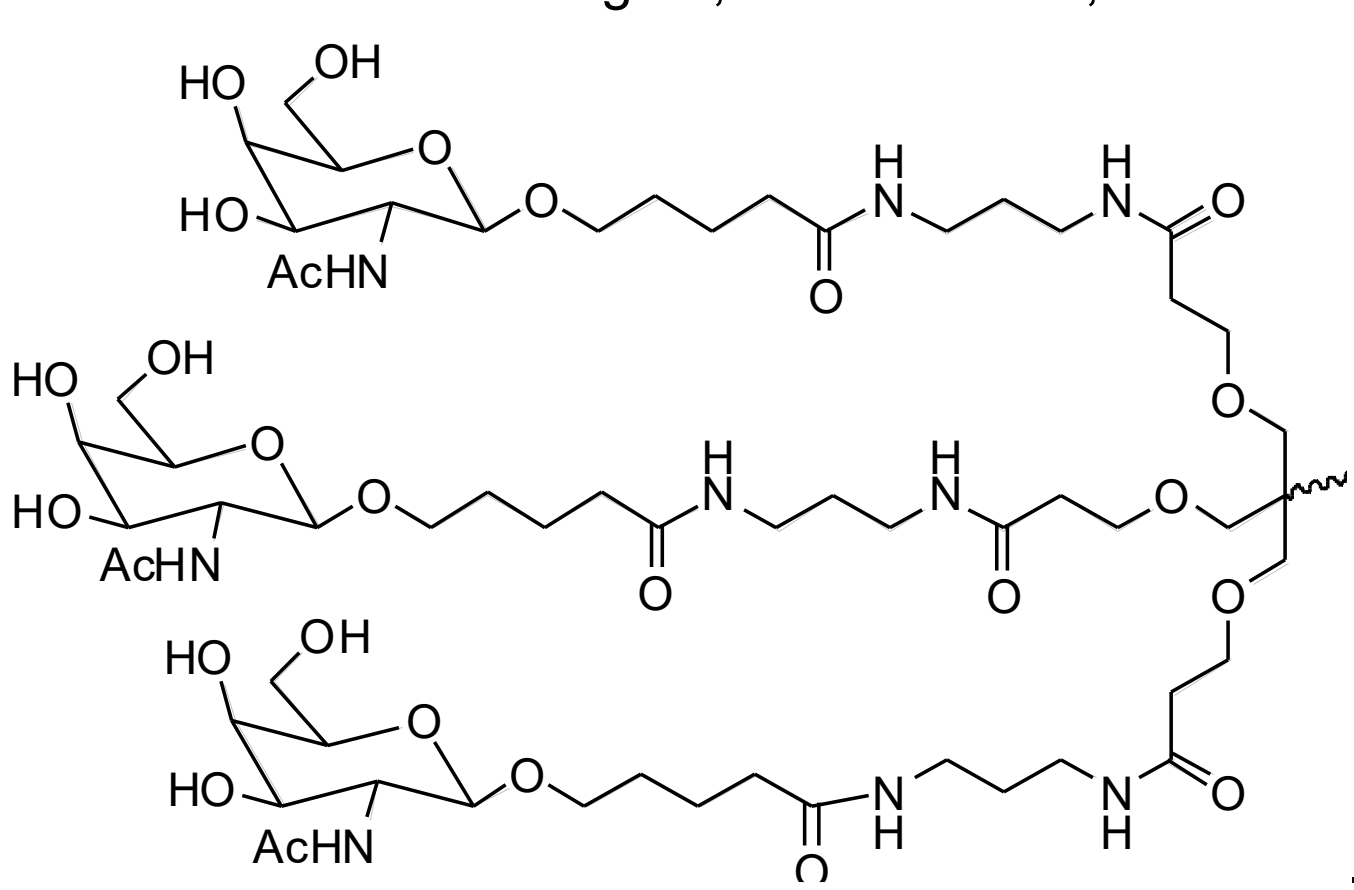
wherein a, c, g, and u are 2′-O-methyl (2′-OMe) A, C, G, and U, respectively; Af, Cf, Gf and Uf are 2′-fluoro A, C, G and U, respectively; s is a phosphorothioate linkage, and wherein at least one strand is conjugated to an N-acetylgalactosamine (GalNAc) ligand.

2. The dsRNA agent, or salt thereof, of claim 1, wherein the sense strand consists of the nucleotide sequence 5'-csasagagUfaUfUfCfcauuuuuacu-3' and the antisense strand consists of the nucleotide sequence 5'-asGfsuaaAfaauggaaUfaCfucuugsgsu-3'.

3. The dsRNA agent, or salt thereof, of claim 11, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

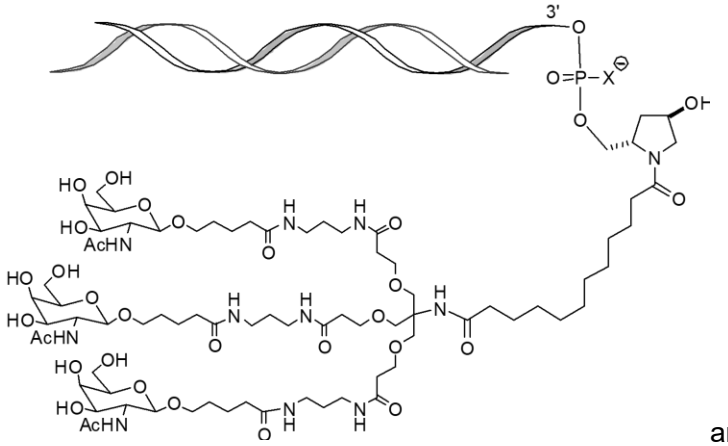
4. The dsRNA agent, or salt thereof, of claim 1, wherein the ligand is one or more GalNAc ligands attached through a monovalent, bivalent, or trivalent branched linker.

5. The dsRNA agent, or salt thereof, of claim 4, wherein the ligand is



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6. The dsRNA agent, or salt thereof, of claim 5, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

7. The dsRNA agent, or salt thereof, of claim 6, wherein X is O.

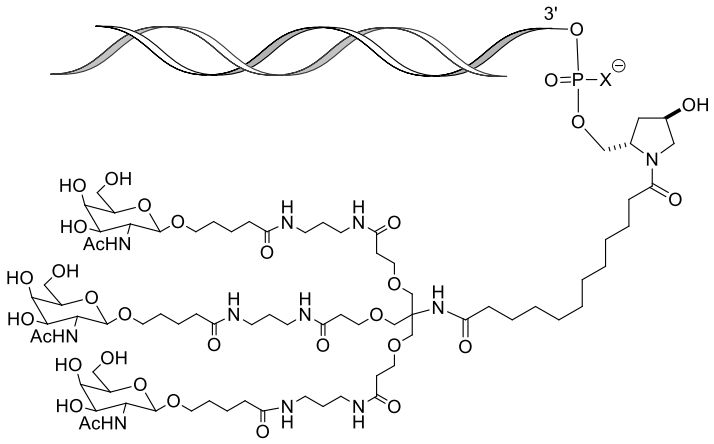
8. A double stranded ribonucleic acid (dsRNA) agent, or salt thereof, for inhibiting expression of transthyretin (TTR) in a cell,

wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises the nucleotide sequence 5'-csasagagUfaUfUfCfcauuuuuacu-3' and the antisense strand comprises the nucleotide sequence 5'-asGfsuaaAfaauggaaUfaCfucuugsgsu-3',

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

wherein the dsRNA agent is conjugated to a ligand as shown in the following schematic



, wherein X is O.

9. The dsRNA agent, or salt thereof, of claim 8, wherein the sense strand consists of the nucleotide sequence 5'-csasagagUfaUfUfCfcauuuuuacu-3' and the antisense strand consists of the nucleotide sequence 5'-asGfsuaaAfaauggaaUfaCfucuugsgsu-3'.

10. A cell containing the dsRNA agent, or salt thereof, of any one of claims 1-9.

11. A pharmaceutical composition for inhibiting expression of a gene encoding transthyretin (TTR) comprising the dsRNA agent, or salt thereof, of any one of claims 1-9, and a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of claim 11, wherein dsRNA agent, or salt thereof, is in an unbuffered solution.

13. The pharmaceutical composition of claim 12, wherein the unbuffered solution is saline or water.

14. The pharmaceutical composition of claim 11, wherein the dsRNA agent, or salt thereof, is in a buffer solution.

15. The pharmaceutical composition of claim 14, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

16. The pharmaceutical composition of claim 14, wherein the buffer solution is phosphate buffered saline (PBS).

17. The pharmaceutical composition of claim 11, wherein dsRNA agent is in a salt form.

18. The pharmaceutical composition of claim 11, wherein dsRNA agent is in a sodium salt form.

19. The pharmaceutical composition of claim 11, wherein dsRNA agent is in a free acid form.

20. An *in vitro* method of inhibiting expression of a transthyretin (TTR) gene in a cell, the method comprising contacting the cell with the dsRNA agent, or salt thereof, of any one of claims 1-9, or the pharmaceutical composition of any one of claims 10-19, thereby inhibiting expression of the TTR gene in the cell.

21. Use of the dsRNA agent, or salt thereof, of any one of claims 1-9, or the pharmaceutical composition of any one of claims 10-19, in the manufacture of a medicament for the treatment of a TTR-associated disease in a subject or a subject at risk for developing a TTR-associated disease.

22. The use of claim 21, wherein the subject is a human.

23. The use of claim 21 or 22, wherein said subject is a subject suffering from a TTR-associated disease.

24. The use of claim 21 or 22, wherein said subject is a subject at risk for developing a TTR-associated disease.

25. The use of any one of claims 21-24, wherein said subject carries a TTR gene mutation that is associated with the development of a TTR-associated disease.

26. The use of any one of claims 21-25, 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, and hyperthyroxinemia.

27. The use of any one of claims 21-25, wherein said TTR-associated disease is an ocular disease.

28. The use of claim 27, wherein said ocular disease is Stargardt’s disease, diabetic retinopathy, or age-related macular degeneration (AMD).

29. The use of any one of claims 21-25, wherein said TTR-associated disease is a metabolic disorder.

30. The use of claim 29, wherein the metabolic disorder is a disorder of glucose and lipid homeostasis.

31. The use of claim 30, wherein the disorder of glucose and lipid homeostasis is insulin resistance associated with type II diabetes.

32. The use of any one of claims 21-25, wherein said TTR-associated disease is a cardiovascular disease.

33. The use of any one of claims 21-32, wherein said subject has a transthyretin-mediated amyloidosis (ATTR amyloidosis) and said use reduces an amyloid TTR deposit in said subject.

34. The use of claim 33, wherein the ATTR is hereditary ATTR (h-ATTR).

35. The use of claim 33, wherein the ATTR is non-hereditary ATTR (wt ATTR).

36. The use of any one of claims 21-35, wherein the dsRNA agent, or salt thereof, or the pharmaceutical composition is to be administered to the subject and improves at least one indicia of neurological impairment, quality of life, ongoing nerve damage, or cardiovascular impairment in the subject.

37. The use of any one of claims 21-36, wherein the dsRNA agent, or salt thereof, or the pharmaceutical composition is to be administered to the human subject by subcutaneous administration or intravenous administration.

38. The use of claim 37, wherein the subcutaneous administration is self-administration.

39. The use of claim 38, wherein the self-administration is via a pre-filled syringe or auto-injector syringe.

40. The use of any one of claims 21-39, further comprising assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the human subject.

41. The dsRNA agent, or salt thereof, of any one of claims 1-9, or the pharmaceutical composition of any one of claims 10-19 for use in therapy.