

The binding of chylomicrons and VLDL particles to the lipolysis-stimulated receptor (LSR) is also significantly inhibited by APOC3.

An increase in APOC3 levels induces the development of hypertriglyceridemia, or high (hyper-) blood levels (-emia) of triglycerides. Elevated levels of triglycerides are associated with a variety of diseases, including cardiovascular disease, atherosclerosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension and skin lesions (xanthomas). Very high triglyceride levels also increase the risk of acute pancreatitis. Therefore, regulating APOC3 metabolism is an important therapeutic approach to managing hypertriglyceridemia and the associated diseases.

Accordingly, there is a need in the art for regulators of APOC3 expression for treating apolipoprotein C3-associated disorders, such as hypertriglyceridemia.

Summary of the Invention

The present invention provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding apolipoprotein C3 (APOC3). The apolipoprotein C3 (APOC3) may be within a cell, *e.g.*, a cell within a subject, such as a human subject.

In one aspect, provided herein is a double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 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 at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of CUUAAAAGGGACAGUAUUCUA of SEQ ID NO:13, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of UAGAAUACUGUCCCUUUUAAGCC of SEQ ID NO:14, wherein all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification, a 2'-fluoro modification, and a deoxy-modification, wherein the sense strand comprises 4 2'-fluoro modified nucleotides at nucleotides 7 and 9-11, counting from the 5'-end, and the antisense strand comprises 2 2'-fluoro modified nucleotides at nucleotides 14 and 16, counting from the 5'-end, and 3 2'-deoxy-modified nucleotides at nucleotides 2, 5, and 7, counting from the 5'-end, wherein both the sense strand and the antisense strand independently further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage, and wherein at least one strand is conjugated to a ligand.

In another aspect, provided herein is a double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand differs by no more than 3 modified nucleotides from the nucleotide sequence 5'-

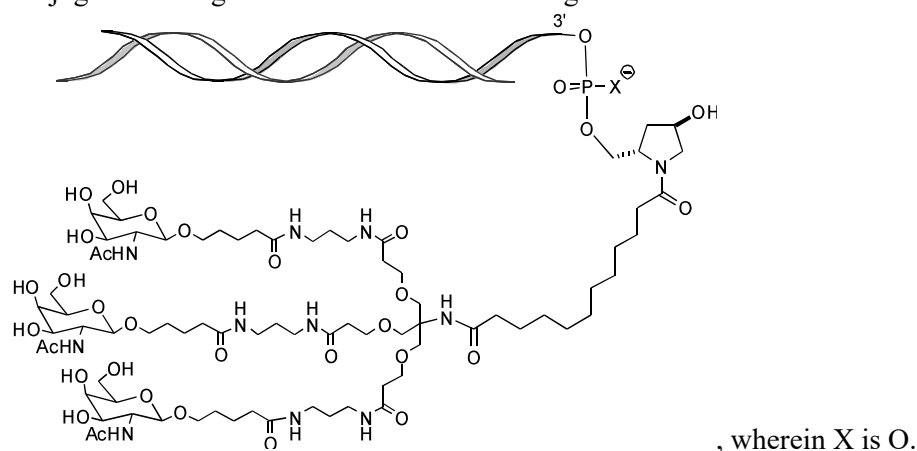
csusuaaaAfgGfGfAfcagauucua-3' of SEQ ID NO:15, wherein the antisense strand differs by no more than 3 modified nucleotides from the nucleotide sequence 5'-

usdAsgadAudAcuguccCfuUfuuaagscsc-3' of SEQ ID NO:16, wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively;

dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

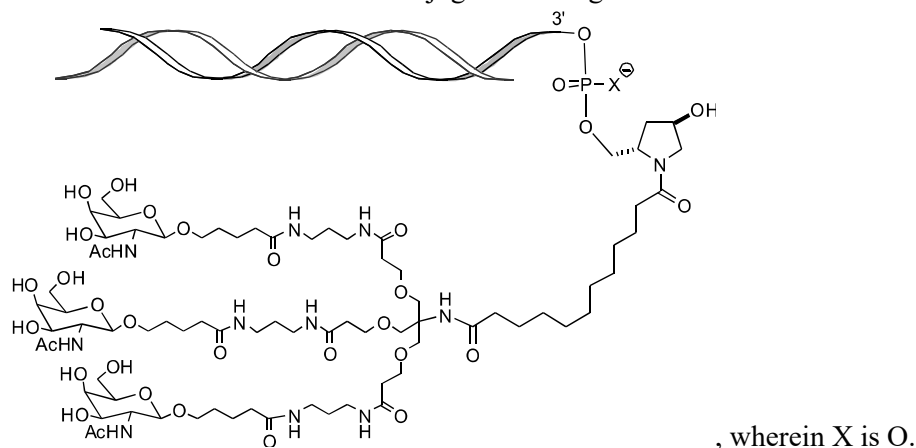
In another aspect, provided herein is a double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence 5'- csusuaaaAfgGfGfAfcagauucua-3' of SEQ ID NO:15, and the antisense strand comprises the nucleotide sequence 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' of SEQ ID NO:16, wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

In another aspect, provided herein is a double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 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'- csusuaaaAfgGfGfAfcagauucua-3' of SEQ ID NO:15, and the antisense strand comprises the nucleotide sequence 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' of SEQ ID NO:16, wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage; and wherein the 3'-end of the sense strand is conjugated to a ligand as shown in the following schematic:



In another aspect, provided herein is a double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent

comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand consists of the nucleotide sequence 5'-csusuaaaAfgGfGfAfcagauucua-3' of SEQ ID NO:15, and the antisense strand consists of the nucleotide sequence 5'-
 5 usdAsgadAudAcuguccCfuUfuuaagscsc-3' of SEQ ID NO:16, wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage; and wherein the 3'-end of the sense strand is conjugated to a ligand as shown in the following schematic:



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

In another aspect, provided herein is a pharmaceutical composition for inhibiting expression of a gene encoding apolipoprotein C3 (APOC3) comprising the dsRNA agent, or a salt thereof, as described herein.

In another aspect, provided herein is an *in vitro* method of inhibiting expression of an
 15 apolipoprotein C3 (APOC3) gene in a cell, the method comprising contacting the cell with the dsRNA agent, or a salt thereof, as described herein, or the pharmaceutical composition as described herein, thereby inhibiting expression of the APOC3 gene in the cell.

In another aspect, provided herein is use of the dsRNA agent, or a salt thereof, as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for
 20 treating a subject having an apolipoprotein C3-associated disorder.

In another aspect, provided herein is a kit comprising the dsRNA agent, or a salt thereof, as described herein or the pharmaceutical composition as described herein.

In another aspect, provided herein is a vial comprising the dsRNA agent, or a salt thereof, as described herein or the pharmaceutical composition as described herein.

In yet another aspect, provided herein is a syringe comprising the dsRNA agent, or a salt thereof, as described herein or the pharmaceutical composition as described herein.

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

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for
 30 inhibiting expression of apolipoprotein C3 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 at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2. In one embodiment, the dsRNA agent comprises at least one thermally destabilizing nucleotide modification, *e.g.*, an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), or a glycerol nucleic acid (GNA), *e.g.*, the antisense strand comprises at least one thermally destabilizing nucleotide modification.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding apolipoprotein C3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-5, 14, and 15.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 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 at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of CUUAAAAGGGACAGUAUUCUA of SEQ ID NO:13, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of

UAGAAUACUGUCCCUUUUAAGCC of SEQ ID NO:14,

wherein all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification, a 2'-fluoro modification, and a deoxy-modification,

wherein the sense strand comprises 4 2'-fluoro modified nucleotides at nucleotides 7 and 9-11, counting from the 5'-end, and the antisense strand comprises 2 2'-fluoro modified nucleotides at nucleotides 14 and 16, counting from the 5'-end, and 3 2'-deoxy-modified nucleotides at nucleotides 2, 5, and 7, counting from the 5'-end,

wherein both the sense strand and the antisense strand independently further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage, and

wherein at least one strand is conjugated to a ligand.

2. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at the 5'-terminus.

3. The dsRNA agent, or a salt thereof, of claim 1, wherein the antisense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at both the 5'- and the 3'-terminus.

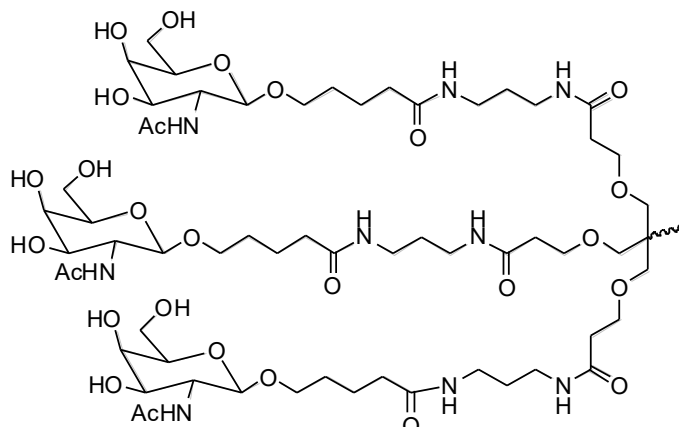
4. The dsRNA agent, or a salt thereof, of claim 1, wherein the sense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at the 5'-terminus and the antisense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at both the 5'- and the 3'-terminus.

5. The dsRNA agent, or a salt thereof, of any one of claims 1-4, wherein the ligand is conjugated to the 3'-end of the sense strand.

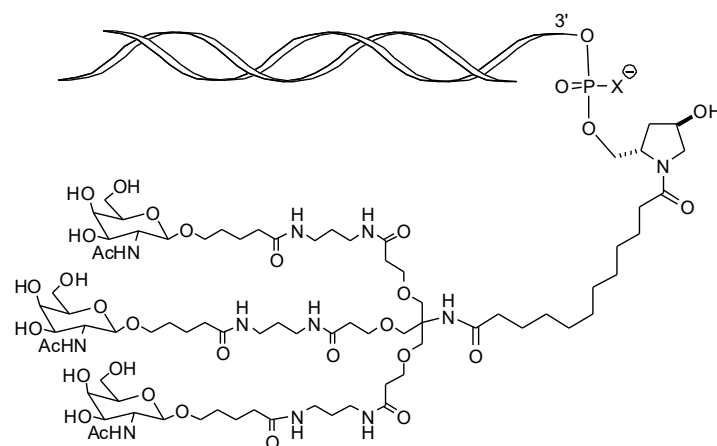
6. The dsRNA agent, or a salt thereof, of claim 1 or 5, wherein the ligand is an N-acetylgalactosamine (GalNAc) ligand.

7. The dsRNA agent, or a salt thereof, of claim 6, wherein the ligand is a GalNAc ligand attached through a monovalent, bivalent, or trivalent branched linker.

5 8. The dsRNA agent, or a salt thereof, of claim 7, wherein the ligand is



9. The dsRNA agent, or a salt thereof, of claim 8, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



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and, wherein X is O.

10. A double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an
15 antisense strand forming a double stranded region,

wherein the sense strand differs by no more than 3 modified nucleotides from the nucleotide sequence 5'- csusuaaaAfgGfGfAfcagauucua-3' of SEQ ID NO:15,

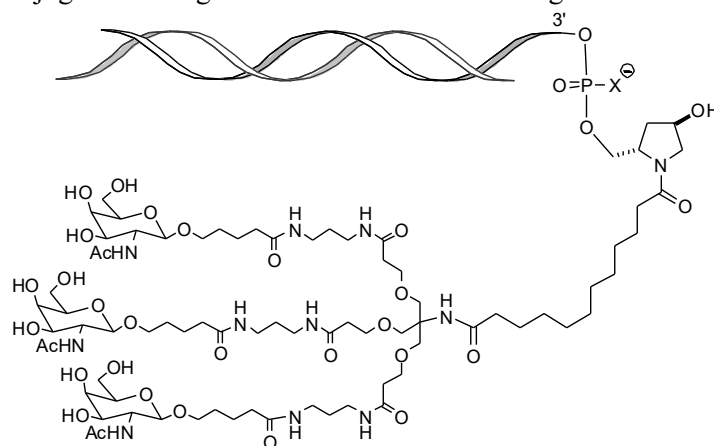
wherein the antisense strand differs by no more than 3 modified nucleotides from the nucleotide sequence 5'- usdAsgadAudAcuguccCfuUfuuaagscsc -3' of SEQ ID NO:16,

wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

11. The dsRNA agent, or a salt thereof, of claim 10, wherein the sense strand differs by no more than 2 modified nucleotides from the nucleotide sequence 5'-csusuaaaAfgGfGfAfcagauuucua-3' of SEQ ID NO: 15 and wherein the antisense strand differs by no more than 2 modified nucleotides from the nucleotide sequence 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' of SEQ ID NO:16.

12. The dsRNA agent, or a salt thereof, of claim 10, wherein the sense strand differs by no more than 1 modified nucleotide from the nucleotide sequence 5'-csusuaaaAfgGfGfAfcagauuucua-3' of SEQ ID NO: 15 and wherein the antisense strand differs by no more than 1 modified nucleotide from the nucleotide sequence 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' of SEQ ID NO:16.

13. The dsRNA agent, or a salt thereof, of any one of claims 10-12, wherein the dsRNA agent is conjugated to a ligand as shown in the following schematic



, and wherein X is O.

14. A double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 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'-csusuaaaAfgGfGfAfcagauuucua-3' of SEQ ID NO:15, and the antisense strand comprises the nucleotide sequence 5'- usdAsgadAudAcuguccCfuUfuuaagscsc -3' of SEQ ID NO:16,

wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

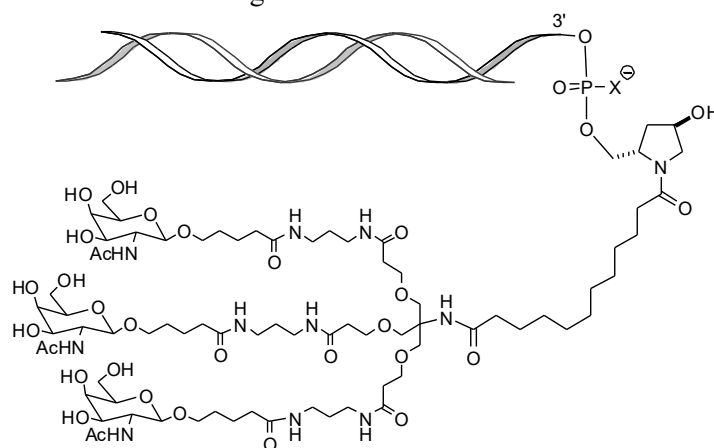
15. A double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises the nucleotide sequence 5'-

5 csusuaaaAfgGfGfAfcagauucua-3' of SEQ ID NO:15, and the antisense strand comprises the nucleotide sequence 5'- usdAsgadAudAcuguccCfuUfuaagscsc -3' of SEQ ID NO:16,

wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage; and wherein the 3'-end of the sense strand is conjugated to a ligand as

10 shown in the following schematic:



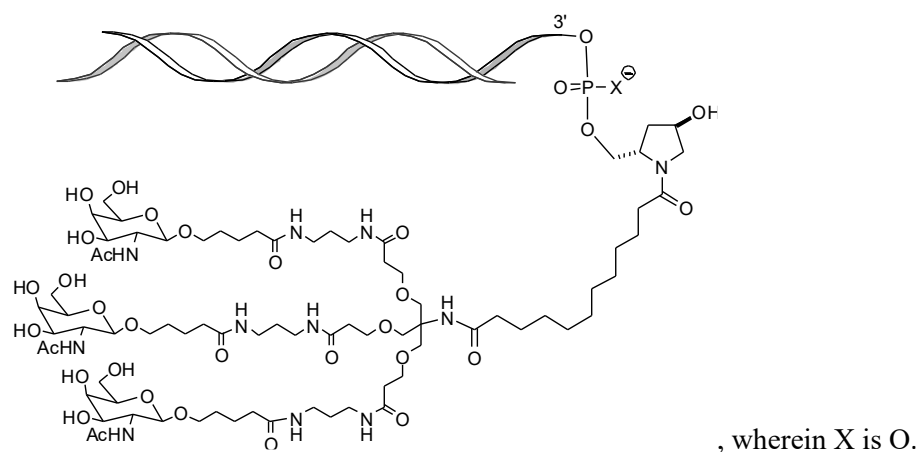
, wherein X is O.

16. A double stranded ribonucleic acid (dsRNA) agent, or a salt thereof, for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand consists of the nucleotide sequence 5'-

15 csusuaaaAfgGfGfAfcagauucua-3' of SEQ ID NO:15, and the antisense strand consists of the nucleotide sequence 5'- usdAsgadAudAcuguccCfuUfuaagscsc -3' of SEQ ID NO:16,

20 wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage; and wherein the 3'-end of the sense strand is conjugated to a ligand as shown in the following schematic:



17. A cell containing the dsRNA agent, or a salt thereof, of any one of claims 1-16.
- 5 18. A pharmaceutical composition for inhibiting expression of a gene encoding apolipoprotein C3 (APOC3) comprising the dsRNA agent, or a salt thereof, of any one of claims 1-16.
19. The pharmaceutical composition of claim 18, wherein dsRNA agent, or a salt thereof, is in an unbuffered solution.
- 10 20. The pharmaceutical composition of claim 19, wherein the unbuffered solution is saline or water.
21. The pharmaceutical composition of claim 18, wherein said dsRNA agent, or a salt thereof, is
- 15 in a buffer solution.
22. The pharmaceutical composition of claim 21, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.
- 20 23. The pharmaceutical composition of claim 22, wherein the buffer solution is phosphate buffered saline (PBS).
24. An *in vitro* method of inhibiting expression of an apolipoprotein C3 (APOC3) gene in a cell, the method comprising contacting the cell with the dsRNA agent, or a salt thereof, of any one of
- 25 claims 1-16, or the pharmaceutical composition of any one of claims 18-23, thereby inhibiting expression of the APOC3 gene in the cell.
25. The method of claim 24, wherein contacting the cell with the dsRNA agent, or a salt thereof, inhibits the expression of apolipoprotein C3 by at least 50%.

26. Use of the dsRNA agent, or a salt thereof, of any one of claims 1-16, or the pharmaceutical composition of any one of claims 18-23 in the manufacture of a medicament for treating a subject having an apolipoprotein C3-associated disorder.

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27. The use of claim 26, wherein the apolipoprotein C3-associated disorder is selected from the group consisting of hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension, atherosclerosis and pancreatitis.

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28. The use of claim 26, wherein the apolipoprotein C3-associated disorder is hypertriglyceridemia.

29. The use of any one of claims 26-28, wherein the subject is human.

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30. The use of any one of claims 26-29, wherein the agent that is to be administered to the subject causes a decrease in hypertriglyceridemia and/or a decrease in APOC3 protein accumulation.

31. The use of any one of claims 26-30, wherein the dsRNA agent, or a salt thereof, is to be administered to the subject subcutaneously.

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32. A kit comprising the dsRNA agent, or a salt thereof, of any one of claims 1-16 or the pharmaceutical composition of any one of claims 18-23.

33. A vial comprising the dsRNA agent, or a salt thereof, of any one of claims 1-16 or the pharmaceutical composition of any one of claims 18-23.

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34. A syringe comprising the dsRNA agent, or a salt thereof, of any one of claims 1-16 or the pharmaceutical composition of any one of claims 18-23.

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35. The dsRNA agent, or a salt thereof, of any one of claims 1-16, or the pharmaceutical composition of any one of claims 18-23 for use in therapy.