

activation and proliferation of hepatic stellate cells and excessive generation and deposition of extracellular matrix (Chen *et al.* (2015) *World J. Gastroenterol.*, 21(3):794-802).

Currently, treatments for NAFLD are directed towards weight loss and treatment of any secondary conditions, such as insulin resistance or dyslipidemia. To date, no pharmacologic
5 treatments for NAFLD have been approved. Therefore, there is a need for therapies for subjects suffering from NAFLD.

Summary

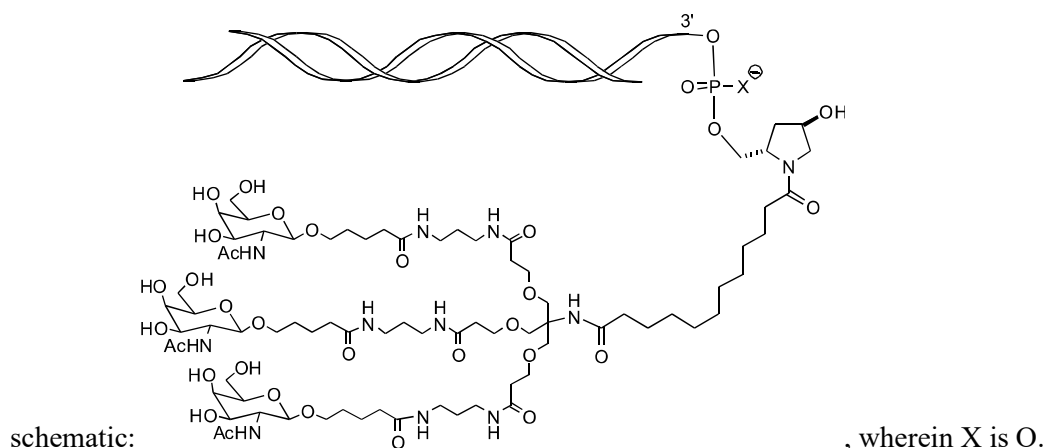
The present disclosure provides iRNA compositions which affect the RNA-induced silencing
10 complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3). The Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) may be within a cell, *e.g.*, a cell within a subject, such as a human subject.

In one aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA)
15 agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell or a salt thereof, wherein the dsRNA agent, or a salt thereof, comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence 5'-CAUUAGGAUAAUGUCUUAUGU-3' of SEQ ID NO:2480, and the antisense strand comprises the nucleotide sequence 5'-ACAUAAGACAUUAUCCUAAUGGG-3' of SEQ ID
20 NO:2564, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a nucleotide modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein both the sense strand and the antisense strand independently comprise at least one phosphorothioate or methylphosphonate internucleotide linkage, and wherein at least one strand is conjugated to a ligand.

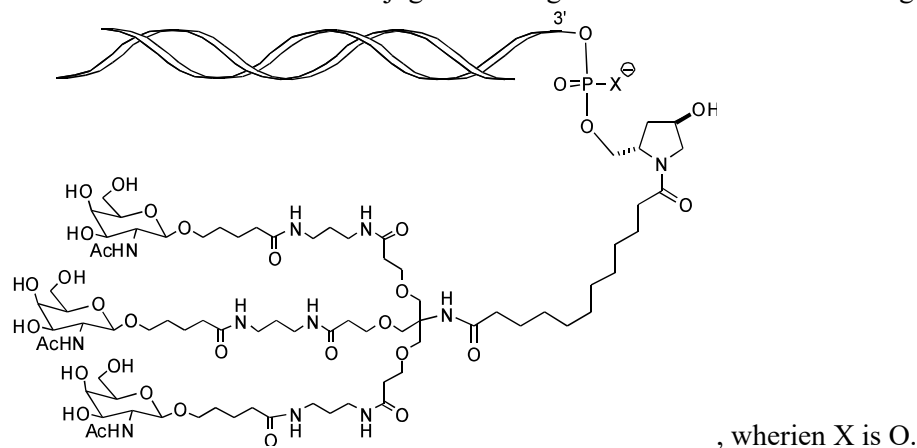
In another aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA)
25 agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, or a salt thereof, wherein the dsRNA agent, or a salt thereof, comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'-csasuuagGfaUfAfAfugucuuauugu-3' of SEQ ID NO:2648 and the antisense strand comprises the nucleotide sequence of 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3' of SEQ ID
30 NO:2732, 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; and s is a phosphorothioate linkage.

In another aspect, the present disclosure refers to a double stranded ribonucleic acid
(dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3
(PNPLA3) in a cell, or a salt thereof, wherein the dsRNA agent, or a salt thereof, comprises a sense
35 strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'-csasuuagGfaUfAfAfugucuuauugu-3' of SEQ ID NO:2648 and the antisense strand comprises the nucleotide sequence of 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3' of SEQ ID NO:2732, 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; 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



In another aspect, the present disclosure refers to a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, or a salt thereof, wherein the dsRNA agent, or a salt thereof, comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand consists of the nucleotide sequence of 5'-csasuagGfaUfAfAfugucuuaugu-3' of SEQ ID NO:2648 and the antisense strand consists of the nucleotide sequence of 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3' SEQ ID NO:2732, 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; 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, the present disclosure refers to a cell containing the dsRNA agent, or a salt thereof, as disclosed herein.

In another aspect, the present disclosure refers to a pharmaceutical composition for inhibiting expression of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) comprising the dsRNA agent, or a salt thereof, as disclosed herein.

In another aspect, the present disclosure refers to an *in vitro* method of inhibiting the expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell, comprising contacting the cell with the dsRNA agent, or a salt thereof, as disclosed herein, or the pharmaceutical composition as disclosed herein, thereby inhibiting expression of PNPLA3 in the cell.

In another aspect, the present disclosure refers to the use of the dsRNA agent as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

In another aspect, the present disclosure refers to use of the dsRNA agent as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for preventing at least one symptom in a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

In another aspect, the present disclosure refers to a kit comprising the dsRNA agent, or a salt thereof, as disclosed herein or the pharmaceutical composition as disclosed herein.

In another aspect, the present disclosure refers to a vial comprising the dsRNA agent, or a salt thereof, as disclosed herein or the pharmaceutical composition as disclosed herein.

In another aspect, the present disclosure refers to a syringe comprising the dsRNA agent, or a salt thereof, as disclosed herein or the pharmaceutical composition as disclosed herein.

In an example, the disclosure provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) 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, *e.g.*, 15, 16, 17, 18, 19, or 20, 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, *e.g.*, 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2. In one example, 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.

5 In another example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) 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 Patatin-Like Phospholipase Domain Containing 3 (PNPLA3), and wherein the region of
10 complementarity comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, or 20, 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 2-11, 21, 24, 27, 30, 32, 33, 36, 37, 49 or 50.

 In one example, the present disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell,
15 wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 677-721; 683-721; 773-817; 1185-1295; 1185-1241; 1202-1295; 1202-1241; 1255-1295; 1738-1792; 1901-1945; 1920-1945; 2108-2208; 2108-2166; 2108-2136, 2121-2166;
20 2121-2208; 2169-2208; 2176-2208; or 2239-2265 of the nucleotide sequence of SEQ ID NO:1, and

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell or a salt thereof,

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

wherein the sense strand comprises the nucleotide sequence 5'-CAUUAGGAUAAUGUCUUAUGU-3' of SEQ ID NO:2480, and the antisense strand comprises the nucleotide sequence 5'-ACAUAAAGACAUUAUCCUAAUGGG-3' of SEQ ID NO:2564,

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

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

15 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 2-6 2'-fluoro modified nucleotides.

20 3. The dsRNA agent, or a salt thereof, of claim 2, wherein the sense strand comprises 4 2'-fluoro modified nucleotides.

4. The dsRNA agent, or a salt thereof, of any one of claims 1-3, wherein the antisense strand comprises 2-8 2'-fluoro modified nucleotides.

25 5. The dsRNA agent, or a salt thereof, of claim 4, wherein the antisense strand comprises 6 2'-fluoro modified nucleotides.

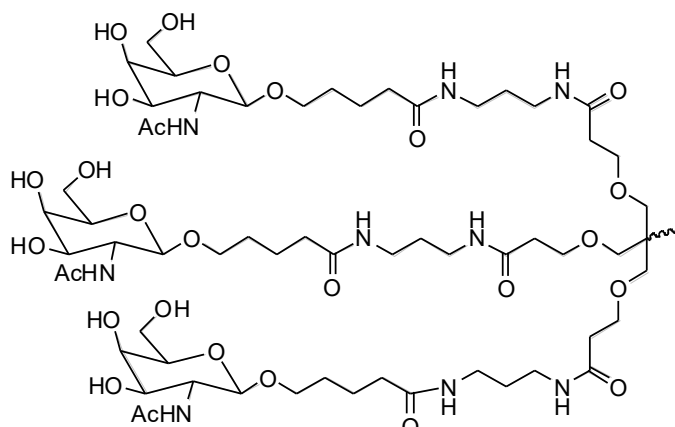
6. The dsRNA agent, or a salt thereof, of any one of claims 1-5, wherein the sense strand comprises 4 2'-fluoro modified nucleotides at nucleotides 7 and 9-11, counting from the 5'-end, and the antisense strand comprises 6 2'-fluoro modified nucleotides at nucleotides 2, 6, 8, 9, 14 and 16, counting from the 5'-end.

7. The dsRNA agent, or a salt thereof, of claim 1, wherein the double stranded region is 19-30 nucleotide pairs in length.

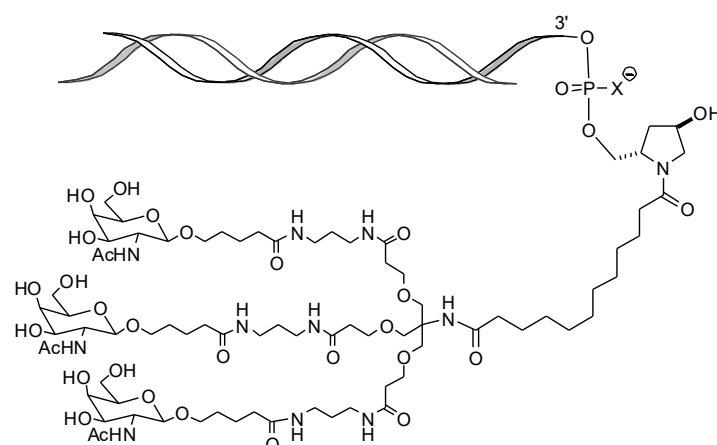
8. The dsRNA agent, or a salt thereof, of claim 7, wherein the double stranded region is 19-25 nucleotide pairs in length.

9. The dsRNA agent, or a salt thereof, of claim 7, wherein the double stranded region is 19-23 nucleotide pairs in length.
- 5 10. The dsRNA agent, or a salt thereof, of claim 7, wherein the double stranded region is 23-27 nucleotide pairs in length.
11. The dsRNA agent, or a salt thereof, of claim 7, wherein the double stranded region is 21-23 nucleotide pairs in length.
- 10 12. The dsRNA agent, or a salt thereof, of any one of claims 1 and 7-11, wherein each strand is independently no more than 30 nucleotides in length.
13. The dsRNA agent, or salt thereof, of any one of claims 1 and 7-12, wherein the sense strand is 15 21 nucleotides in length and the antisense strand is 23 nucleotides in length.
14. The dsRNA agent, or a salt thereof, of any one of claims 1 and 7-13, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.
- 20 15. The dsRNA agent, or a salt thereof, of any one of claims 1 and 7-13, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.
- 25 16. The dsRNA agent, or a salt thereof, of any one of claims 1 and 7-15, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent, or a salt thereof.
17. The dsRNA agent, or a salt thereof, of any one of claims 1 and 7-16, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.
- 30 18. The dsRNA agent, or a salt thereof, of any one of claims 1 and 7-17, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

19. The dsRNA agent, or a salt thereof, of claim 17 or 18, wherein the ligand is



20. The dsRNA agent, or a salt thereof, of claim 19, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

21. The dsRNA agent, or a salt thereof, of claim 20, wherein X is O.

22. The dsRNA agent, or a salt thereof, of any one of claims 1 and 7-21, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

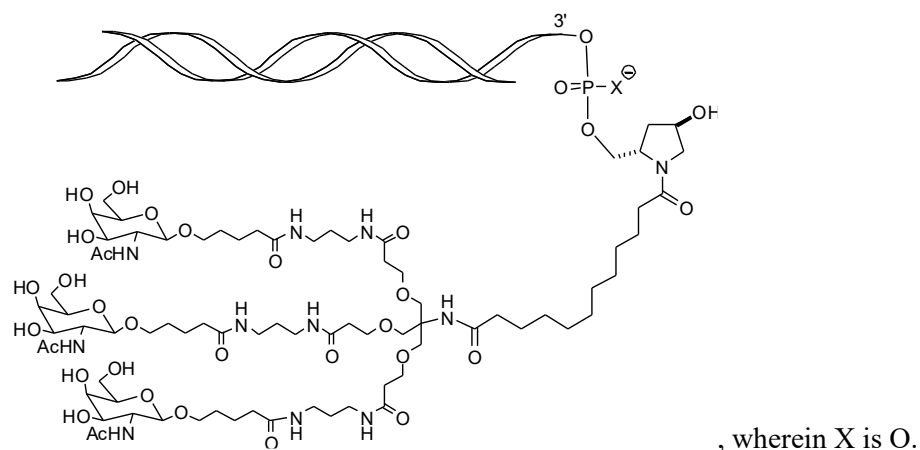
23. The dsRNA agent, or a salt thereof, of claim 22, wherein the strand is the antisense strand.

24. The dsRNA agent, or a salt thereof, of claim 22, wherein the strand is the sense strand.

25. The dsRNA agent, or a salt thereof, of claim 22, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

26. The dsRNA agent, or a salt thereof, of claim 25, wherein the strand is the antisense strand.

27. The dsRNA agent, or a salt thereof, of claim 25, wherein the strand is the sense strand.
28. The dsRNA agent, or a salt thereof, of claim 22, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.
- 5 29. The dsRNA agent, or a salt thereof, of claim 28, wherein the strand is the antisense strand.
30. The dsRNA agent, or a salt thereof, of any one of claims 1, and 7-29, wherein the sense strand comprises the nucleotide sequence 5'-CAUUAGGAUAAUGUCUUAUGU-3' of SEQ ID
10 NO:2480 and the antisense strand comprises the nucleotide sequence 5'-
ACAUAAGACAUAUCCUAAUGGG-3' of SEQ ID NO:2564.
31. The dsRNA agent, or a salt thereof, of claim 30, wherein the sense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'- csasuuagGfaUfAfAfugucuuaugu-3' of SEQ ID NO:2648 and the antisense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'- asCfsauaAfgAfCfauuaUfcCfuaaugsgsg -3' of SEQ ID NO:2732, 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; and s is a phosphorothioate linkage.
- 15 32. The dsRNA agent, or a salt thereof, of claim 30, wherein the sense strand differs by no more than 2 modified nucleotides from the nucleotide sequence of 5'-csasuuagGfaUfAfAfugucuuaugu-3' of SEQ ID NO:2648 and the antisense strand differs by no more than 2 modified nucleotides from the nucleotide sequence of 5'- asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3' of SEQ ID NO:2732.
- 20 33. The dsRNA agent, or a salt thereof, of claim 30, wherein the sense strand differs by no more than 1 modified nucleotide from the nucleotide sequence of 5'-csasuuagGfaUfAfAfugucuuaugu-3' (SEQ ID NO:2648) and the antisense strand differs by no more than 1 modified nucleotide from the nucleotide sequence of 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3' (SEQ ID NO:2732).
- 25 34. The dsRNA agent, or a salt thereof, of claim 30, wherein the sense strand comprises the nucleotide sequence of 5'- csasuuagGfaUfAfAfugucuuaugu-3' of SEQ ID NO:2648 and the antisense strand comprises the nucleotide sequence of 5'- asCfsauaAfgAfCfauuaUfcCfuaaugsgsg -3' of SEQ ID NO:2732.
- 30 35. The dsRNA agent, or a salt thereof, of any one of claims 30-34, wherein the sense strand of the dsRNA agent, or a salt thereof, is conjugated to the ligand as shown in the following schematic
- 35



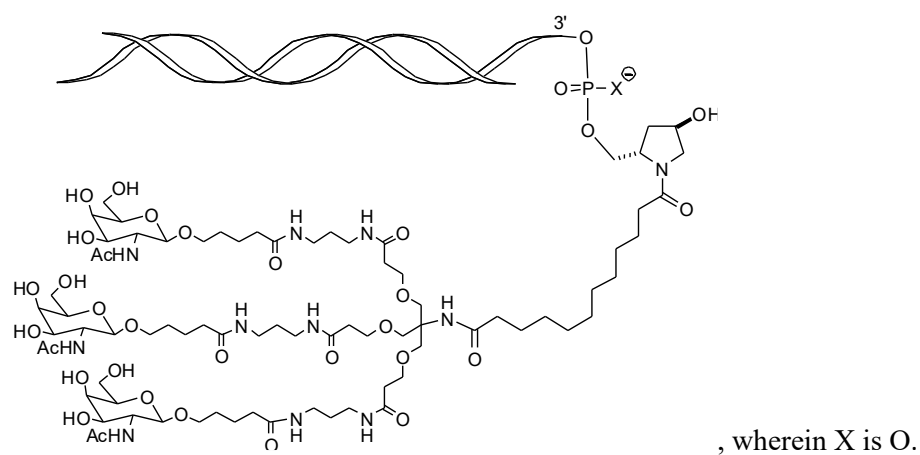
36. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, or a salt thereof,

5 wherein the dsRNA agent, or a salt thereof, comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'-csasuuagGfaUfAfAfugucuuaugu-3' of SEQ ID NO:2648 and the antisense strand comprises the nucleotide sequence of 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg -3' of SEQ ID NO:2732,

10 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; and s is a phosphorothioate linkage.

37. The dsRNA agent, or a salt thereof, of claim 36, further comprising a ligand.

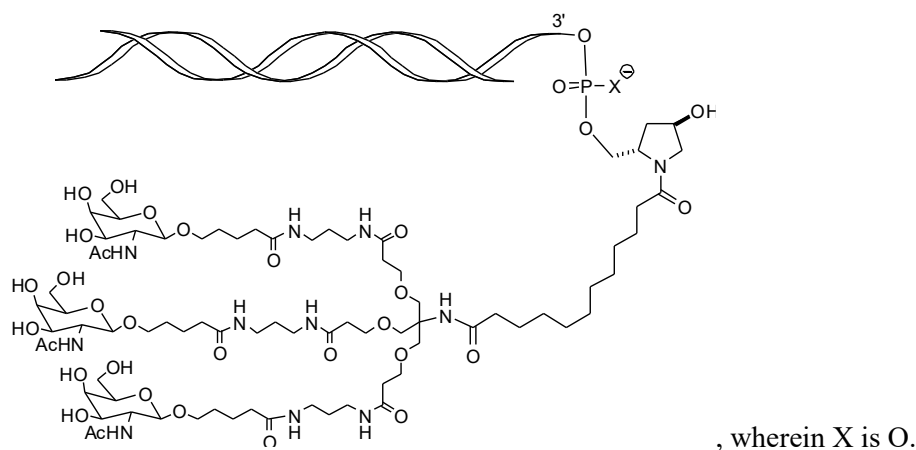
38. The dsRNA agent, or a salt thereof, of claim 36 or 37, wherein the 3'-end of the sense strand
15 is conjugated to a ligand as shown in the following schematic:



39. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, or a salt thereof,

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

wherein the sense strand comprises the nucleotide sequence of 5'-
 csasuuagGfaUfAfAfugucuuauugu-3' of SEQ ID NO:2648 and the antisense strand comprises the
 nucleotide sequence of 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3' of SEQ ID NO:2732,
 wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and
 5 Uf are 2'-fluoro A, G, C and U respectively; 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:



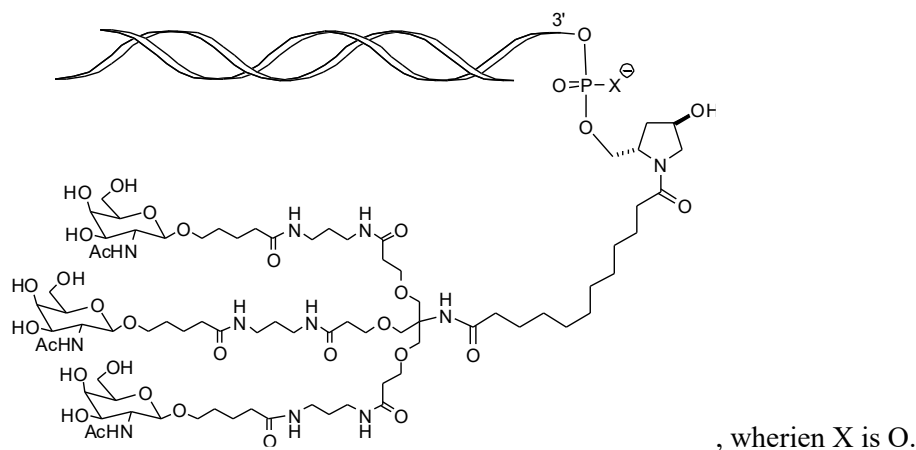
40. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Patatin-Like
 Phospholipase Domain Containing 3 (PNPLA3) in a cell, or a salt thereof,

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

wherein the sense strand consists of the nucleotide sequence of 5'-
 csasuuagGfaUfAfAfugucuuauugu-3' of SEQ ID NO:2648 and the antisense strand consists of the
 nucleotide sequence of 5'-asCfsauaAfgAfCfauuaUfcCfuaaugsgsg-3' SEQ ID NO:2732,

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; 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:



41. A cell containing the dsRNA agent, or a salt thereof, of any one of claims 1 and 7-40.

42. A pharmaceutical composition for inhibiting expression of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) comprising the dsRNA agent, or a salt thereof, of any one of claims 1 and 7-40.

43. The pharmaceutical composition of claim 42, wherein dsRNA agent, or a salt thereof, is in an unbuffered solution.

44. The pharmaceutical composition of claim 43, wherein the unbuffered solution is saline or water.

45. The pharmaceutical composition of claim 42, wherein said dsRNA agent, or a salt thereof, is in a buffer solution.

46. The pharmaceutical composition of claim 45, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

47. The pharmaceutical composition of claim 46, wherein the buffer solution is phosphate buffered saline (PBS).

48. An *in vitro* method of inhibiting the expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell, comprising contacting the cell with the dsRNA agent, or a salt thereof, of any one of claims 1 and 7-40, or the pharmaceutical composition of any one of claims 44-47, thereby inhibiting expression of PNPLA3 in the cell.

49. Use of the dsRNA agent of any one of claims 1 and 7-40, or the pharmaceutical composition of any one of claims 42-47 in the manufacture of a medicament for treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

50. Use of the dsRNA agent of any one of claims 1 and 7-40, or the pharmaceutical composition of any one of claims 42-47 in the manufacture of a medicament for preventing at least one symptom in a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression selected from the group consisting of fatty liver

(steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, and nonalcoholic fatty liver disease (NAFLD).

- 5 51. The use of claim 49 or 50, wherein the disorder that would benefit from reduction in PNPLA3 is NAFLD.
52. The use of any one of claims 49-51, wherein the subject is human.
- 10 53. The use of any one of claims 49-52, wherein the dsRNA agent, or a salt thereof, is to be administered to the subject subcutaneously.
54. The use of any one of claims 49-53, further comprising that an additional therapeutic agent for treatment of the PNPLA3-associated disorder selected from the group consisting of an HMG-
- 15 CoA reductase inhibitor, a fibrate, a bile acid sequestrant, niacin, an antiplatelet agent, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, an acylCoA cholesterol acetyltransferase (ACAT) inhibitor, a cholesterol absorption inhibitor, a cholesterol ester transfer protein (CETP) inhibitor, a microsomal triglyceride transfer protein (MTTP) inhibitor, a cholesterol modulator, a bile acid modulator, a peroxisome proliferation activated receptor (PPAR) agonist, a
- 20 composite vascular protectant, a glycoprotein IIb/IIIa inhibitor, aspirin or an aspirin- like compound, an IBAT inhibitor, a squalene synthase inhibitor, a monocyte chemoattractant protein (MCP)-I inhibitor, and fish oil is to be administered to the subject.
55. A kit comprising the dsRNA agent, or a salt thereof, of any one of claims 1 and 7-40 or the
- 25 pharmaceutical composition of any one of claims 42-47.
56. A vial comprising the dsRNA agent, or a salt thereof, of any one of claims 1 and 7-40 or the pharmaceutical composition of any one of claims 42-47.
- 30 57. A syringe comprising the dsRNA agent, or a salt thereof, of any one of claims 1 and 7-40 or the pharmaceutical composition of any one of claims 42-47.