

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 treatments for NAFLD have been approved. Therefore, there is a need for therapies for subjects suffering from NAFLD.

Summary of the Invention

The present ~~invention~~disclosure provides iRNA compositions which affect the RNA-induced silencing 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) 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 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of nucleotides 1214-1234 of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In another aspect, the present disclosure refers to 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 PNPLA3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 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 another aspect, the present disclosure refers to 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 a) the sense strand comprises at least 15 contiguous nucleotides differing by no more than three 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; 2121-2208; 2169-2208; 2176-2208; or 2239-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2; or b) the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 574-596; 677-699; 683-705; 699-721; 773-795; 795-817; 1185-1207; 1192-1214; 1202-1224; 1208-1230; 1209-1231; 1210-1232; 1211-1233; 1212-1234; 1213-1233; 1214-1234; 1214-1236; 1215-1237; 1216-1238; 1219-1237; 1219-1241; 1255-1275; 1256-1276; 1257-

1275; 1257-1277; 1258-1278; 1259-1279; 1260-1278; 1260-1280; 1261-1281; 1262-1282; 1263-1283; 1264-1282; 1264-1284; 1265-1285; 1267-1285; 1266-1286; 1266-1288; 1267-1285; 1267-1287; 1268-1290; 1269-1289; 1270-1290; 1271-1291; 1272-1292; 1273-1293; 1274-1294; 1275-1295; 1631-1653; 1738-1760; 1739-1761; 1740-1760; 1740-1762; 1741-1763; 1744-1766; 1746-1766; 1750-1772; 1751-1773; 1752-1774; 1753-1775; 1754-1776; 1755-1777; 1756-1778; 1757-1779; 1758-1780; 1759-1781; 1760-1782; 1761-1783; 1762-1782; 1762-1784; 1763-1785; 1764-1786; 1765-1787; 1766-1786; 1766-1788; 1767-1787; 1768-1788; 1767-1789; 1769-1789; 1770-1788; 1770-1790; 1771-1791; 1772-1792; 1815-1837; 1901-1923, 1920-1942, 1923-1945; 2112-2130; 2169-2191; 2171-2191; 2176-2198, 2177-2199, 2178-2200; 2179-2201, 2180-2202; 2181-2203; 2183-2205; 2184-2206; 2186-2208; 2239-2261; 2241-2263; 2242-2264; or 2243-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In another aspect, the present disclosure refers to a cell containing the dsRNA agent 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 as disclosed herein.

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 inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a 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.

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

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

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

In an aspect example, the invention 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 embodiment 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 ~~aspect~~example, the present ~~invention~~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
10 region of 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 ~~aspect~~example, the present ~~invention~~disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3
15 (PNPLA3) 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, *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,
20 2121-2166; 2121-2208; 2169-2208; 2176-2208; or 2239-2265 of the nucleotide sequence of SEQ ID NO:1, and

the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one ~~aspect~~example, the present ~~invention~~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 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 574-596; 677-699; 683-705; 699-721; 773-795; 795-817; 1185-1207; 1192-1214; 1202-1224; 1208-1230; 1209-1231; 1210-1232; 1211-1233; 1212-1234; 1213-1233; 1214-1234; 1214-1236; 1215-1237; 1216-1238; 1219-1237; 1219-1241; 1255-1275; 1256-1276; 1257-1275; 1257-1277; 1258-1278; 1259-1279; 1260-1278; 1260-1280; 1261-1281; 1262-1282; 1263-1283; 1264-1282; 1264-1284; 1265-1285; 1267-1285; 1266-1286; 1266-1288; 1267-1285; 1267-1287; 1268-1290; 1269-1289; 1270-1290; 1271-1291; 1272-1292; 1273-1293; 1274-1294; 1275-1295; 1631-1653; 1738-1760; 1739-1761; 1740-1760; 1740-1762; 1741-1763; 1744-1766; 1746-1766; 1750-1772; 1751-1773; 1752-1774; 1753-1775; 1754-1776; 1755-1777; 1756-1778; 1757-1779; 1758-1780; 1759-1781; 1760-1782; 1761-1783; 1762-1782; 1762-1784; 1763-1785; 1764-1786; 1765-1787; 1766-1786; 1766-1788; 1767-1787; 1768-1788; 1767-1789; 1769-1789; 1770-1788; 1770-1790; 1771-1791; 1772-1792; 1815-1837; 1901-1923, 1920-1942, 1923-1945; 2112-2130; 2169-2191; 2171-2191; 2176-2198, 2177-2199, 2178-2200; 2179-2201, 2180-2202; 2181-2203; 2183-2205; 2184-2206; 2186-2208; 2239-2261; 2241-2263; 2242-2264; or 2243-2265 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one ~~embodiment~~example, the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by nor more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-517197.2; AD-517258.2; AD-516748.2; AD-516851.2; AD-519351.2; AD-519754.2; AD-519828.2; AD-520018.2; AD-520035.2; AD-520062.2; AD-520064.2; AD-520065.2; AD-520067.2; AD-75289.2; AD-520069.2; AD-520099.2; AD-67575.7; AD-520101.2; AD-67605.7; AD-1193323.1; AD-1193344.1; AD-1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; and AD-1193481.1.

In one ~~embodiment~~example, the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by nor more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-519345.1, AD-519346.1, AD-519347.1, AD-67554.7, AD-519752.3, AD-1010731.1, AD-1010732.1, AD-519343.1, AD-519344.1, AD-519349.1, AD-519350.1, AD-519753.2, AD-519932.1, AD-519935.2, AD-520018.6, AD-517837.2, AD-805635.2, AD-519329.2, AD-520063.2, AD-519757.2, AD-805631.2, AD-516917.2, AD-516828.2, AD-518983.2, AD-805636.2, AD-519754.7, AD-520062.2, AD-67575.9, AD-518923.3, AD-520053.4, AD-519667.2, AD-519773.2, AD-519354.2, AD-520060.4, AD-520061.4, AD-1010733.2, AD-1010735.2; AD-1193323.1; AD-1193344.1; AD-

1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; and AD-1193481.1.

In one ~~aspect~~example, the present ~~invention~~disclosure provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in a cell, wherein the 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 three nucleotides from any one of the nucleotide sequence of nucleotides 1200-1250, 1205-1250, 11210-1250, 1200-1245, 1200-1240, 1200-1235, 1200-1237, 1205-1237, 1210-1232, 1212-1237, 1212-1234, 1250-1300, 1255-1300, 1260-1300, 1250-1295, 1250-1390, 1250-1285, 1250-1282, 1255-1282, 1260-1282, 1262-1300, 1262-1295, 1262-1390, and 1262-1282 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one ~~embodiment~~example, the antisense 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 antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-519345, AD-1193350, AD-1193365, AD-1193437 and AD-519347.

In one ~~embodiment~~example, the antisense 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 antisense strand nucleotide sequence of duplex AD-519351.

In one ~~embodiment~~example, the antisense 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 antisense strand nucleotide sequence of duplex AD-1193350.

In one ~~embodiment~~example, the antisense 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 antisense strand nucleotide sequence of duplex AD-1193365.

In one ~~embodiment~~example, the antisense 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 antisense strand nucleotide sequence of duplex AD-1193437.

In one ~~embodiment~~example, the antisense 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 antisense strand nucleotide sequence of duplex AD-519347.

In one ~~embodiment~~example, the dsRNA agent comprises at least one modified nucleotide; substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

In one ~~embodiment~~example, all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one ~~embodiment~~example, at least one of the modified nucleotides is 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, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

In one ~~embodiment~~example, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

In one ~~embodiment~~example, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), *e.g.*, Ggn, Cgn, Tgn, or Agn, and, a vinyl-phosphonate nucleotide; and combinations thereof.

In another ~~embodiment~~example, at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

In one ~~embodiment~~example, the thermally destabilizing nucleotide modification is selected from the group consisting of 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), and a glycerol nucleic acid (GNA).

In some ~~embodiment~~examples, the modified nucleotide comprises a short sequence of 3'-terminal deoxy-thymine nucleotides (dT).

In some ~~embodiment~~examples, the modifications on the nucleotides are 2'-O-methyl, GNA and 2'-fluoro modifications.

In some ~~embodiment~~examples, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage. In some ~~embodiment~~examples, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages. In one ~~embodiment~~example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another ~~embodiment~~example, the strand is the sense strand. In a related ~~embodiment~~example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another ~~embodiment~~example, the strand is the sense strand. In another ~~embodiment~~example, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another ~~embodiment~~example, the strand is the sense strand.

The double stranded region may be 19-30 nucleotide pairs in length; 19-25 nucleotide pairs in length; 19-23 nucleotide pairs in length; 23-27 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

In one ~~embodiment~~example, each strand is independently no more than 30 nucleotides in length.

In one ~~embodiment~~example, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

The region of complementarity may be at least 17 nucleotides in length; between 19 and 23 nucleotides in length; or 19 nucleotides in length.

In one ~~embodiment~~example, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another ~~embodiment~~example, at least one strand comprises a 3' overhang of at least 2 nucleotides.

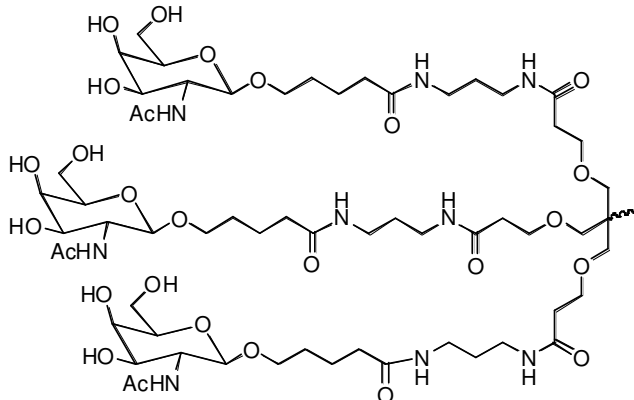
In one ~~embodiment~~example, the dsRNA agent further comprises a ligand.

In one ~~embodiment~~example, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

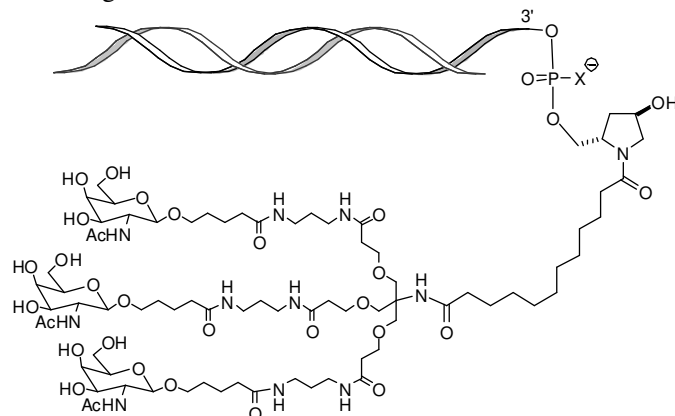
In one ~~embodiment~~example, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

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

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



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



and, wherein X is O or S.

In one ~~embodiment~~example, the X is O.

In one ~~embodiment~~example, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

In one ~~embodiment~~example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

5 In another ~~embodiment~~example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

In one ~~embodiment~~example, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In one ~~embodiment~~example, the strand is the antisense strand.

10 In one ~~embodiment~~example, the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

The present ~~invention~~disclosure also provides cells containing any of the dsRNA agents of the ~~invention~~disclosure and pharmaceutical compositions comprising any of the dsRNA agents of the ~~invention~~disclosure.

15 The pharmaceutical composition of the ~~invention~~disclosure may include dsRNA agent in an unbuffered solution, *e.g.*, saline or water, or the pharmaceutical composition of the ~~invention~~disclosure may include the dsRNA agent is in a buffer solution, *e.g.*, a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

20 In one ~~aspect~~example, the present ~~invention~~disclosure provides a method of inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell. The method includes contacting the cell with any of the dsRNAs of the ~~invention~~disclosure or any of the pharmaceutical compositions of the ~~invention~~disclosure, thereby inhibiting expression of the PNPLA3 gene in the cell.

25 In one ~~embodiment~~example, the cell is within a subject, *e.g.*, a human subject, *e.g.*, a subject having a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder, such as a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder 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, or nonalcoholic fatty liver disease (NAFLD).

30 In one ~~embodiment~~example, contacting the cell with the dsRNA agent inhibits the expression of PNPLA3 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one ~~embodiment~~example, inhibiting expression of PNPLA3 decreases PNPLA3 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

35 In one ~~aspect~~example, the present ~~invention~~disclosure provides a method of treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression. The method includes administering to the subject a therapeutically effective amount of any of the dsRNAs of the ~~invention~~disclosure or any of the pharmaceutical compositions of the ~~invention~~disclosure, thereby treating the subject having the disorder that would benefit from reduction in PNPLA3 expression.

40 In another ~~aspect~~example, the present ~~invention~~disclosure provides a method of 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. The method includes administering to the subject a prophylactically effective amount of any of the dsRNAs of the ~~invention~~disclosure or
45 any of the

pharmaceutical compositions of the ~~invention~~disclosure, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in PNPLA3 expression.

In one ~~embodiment~~example, the disorder is a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder, *e.g.*, a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3)-associated disorder is 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, or nonalcoholic fatty liver disease (NAFLD).

In one ~~embodiment~~example, the PNPLA3-associated disorder is NAFLD.

In one ~~embodiment~~example, the subject is human.

In one ~~embodiment~~example, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

In one ~~embodiment~~example, the dsRNA agent is administered to the subject subcutaneously.

In one ~~embodiment~~example, the methods of the ~~invention~~disclosure include further determining the level of PNPLA3 in a sample(s) from the subject.

In one ~~embodiment~~example, the level of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) in the subject sample(s) is a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) protein level in a blood or serum sample(s).

In certain ~~embodiment~~examples, the methods of the ~~invention~~disclosure further comprise administering to the subject an additional therapeutic agent. In a further ~~embodiment~~example, the additional therapeutic agent is selected from the group consisting of an HMG-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 gene-based therapy, a 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, or fish oil.

The present ~~invention~~disclosure also provides kits comprising any of the dsRNAs of the ~~invention~~disclosure or any of the pharmaceutical compositions of the ~~invention~~disclosure, and optionally, instructions for use.

Brief Description of the Drawings

Figure 1 is a graph showing human PNPLA3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single 3 mg/kg or 10 mg/kg dose of the indicated dsRNA duplexes, on day 7 or day14 post-dose. Human PNPLA3 mRNA levels are shown relative to control levels detected with PBS treatment.

Figure 2 is a graph showing human PNPLA3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single dose of the indicated dsRNA duplexes, on day 7 post-dose as described in Example 3. Human PNPLA3 mRNA levels are shown relative to control levels detected with PBS treatment.

We claim:

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

5 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 3 nucleotides from the nucleotide sequence of nucleotides 1214-1234 of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide
10 sequence of SEQ ID NO:2.

~~12.~~ 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
15 comprises a region of complementarity to an mRNA encoding PNPLA3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 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.

20 ~~23.~~ 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

25 a) the sense strand comprises at least 15 contiguous nucleotides differing by no more than three 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; 2121-2208; 2169-2208; 2176-2208; or 2239-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the
30 corresponding nucleotide sequence of SEQ ID NO:2; ~~or~~

~~3. 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 b) the sense strand~~

35 ~~comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 574-596; 677-699; 683-705; 699-721; 773-795; 795-817; 1185-1207; 1192-1214; 1202-1224; 1208-1230; 1209-1231; 1210-1232; 1211-1233; 1212-1234; 1213-1233; 1214-1234; 1214-1236; 1215-1237; 1216-1238; 1219-1237; 1219-1241; 1255-~~

1275; 1256-1276; 1257-1275; 1257-1277; 1258-1278; 1259-1279; 1260-1278; 1260-1280; 1261-1281; 1262-1282; 1263-1283; 1264-1282; 1264-1284; 1265-1285; 1267-1285; 1266-1286; 1266-1288; 1267-1285; 1267-1287; 1268-1290; 1269-1289; 1270-1290; 1271-1291; 1272-1292; 1273-1293; 1274-1294; 1275-1295; 1631-1653; 1738-1760; 1739-1761; 1740-1760; 1740-1762; 1741-1763; 1744-1766; 1746-1766; 1750-1772; 1751-1773; 1752-1774; 1753-1775; 1754-1776; 1755-1777; 1756-1778; 1757-1779; 1758-1780; 1759-1781; 1760-1782; 1761-1783; 1762-1782; 1762-1784; 1763-1785; 1764-1786; 1765-1787; 1766-1786; 1766-1788; 1767-1787; 1768-1788; 1767-1789; 1769-1789; 1770-1788; 1770-1790; 1771-1791; 1772-1792; 1815-1837; 1901-1923, 1920-1942, 1923-1945; 2112-2130; 2169-2191; 2171-2191; 2176-2198, 2177-2199, 2178-2200; 2179-2201, 2180-2202; 2181-2203; 2183-2205; 2184-2206; 2186-2208; 2239-2261; 2241-2263; 2242-2264; or 2243-2265 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

4. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-517197.2; AD-517258.2; AD-516748.2; AD-516851.2; AD-519351.2; AD-519754.2; AD-519828.2; AD-520018.2; AD-520035.2; AD-520062.2; AD-520064.2; AD-520065.2; AD-520067.2; AD-75289.2; AD-520069.2; AD-520099.2; AD-67575.7; AD-520101.2; AD-1193323.1; AD-1193344.1; AD-1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; AD-1193481.1 and AD-67605.7.

5. The dsRNA agent of any one of claims 1-3, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by nor more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-519345.1, AD-519346.1, AD-519347.1, AD-67554.7, AD-519752.3, AD-1010731.1, AD-1010732.1, AD-519343.1, AD-519344.1, AD-519349.1, AD-519350.1, AD-519753.2, AD-519932.1, AD-519935.2, AD-520018.6, AD-517837.2, AD-805635.2, AD-519329.2, AD-520063.2, AD-519757.2, AD-805631.2, AD-516917.2, AD-516828.2, AD-518983.2, AD-805636.2, AD-519754.7, AD-520062.2, AD-67575.9, AD-518923.3, AD-520053.4, AD-519667.2, AD-519773.2, AD-519354.2, AD-520060.4, AD-520061.4, AD-1010733.2, and AD-1010735.2.

6. ~~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 sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 1200-1250, 1205-1250, 11210-1250, 1200-1245, 1200-1240, 1200-1235, 1200-1237, 1205-1237, 1210-1232, 1212-1237, 1212-1234, 1250-1300, 1255-1300, 1260-1300, 1250-1295, 1250-1390, 1250-1285, 1250-1282, 1255-1282, 1260-1282, 1262-1300,~~

1262-1295, 1262-1390, and 1262-1282 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

7. ~~The dsRNA agent of any one of claims 1-6, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by not more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-519351, AD-519345, AD-1193350, AD-1193365, AD-1193437 and AD-519347.~~

86. The dsRNA agent of any one of claims 1-75, wherein the dsRNA agent comprises at least one modified nucleotide.

97. The dsRNA agent of any one of claims 1-86, wherein substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

108. The dsRNA agent of any one of claims 1-97, wherein all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

119. The dsRNA agent of any one of claims 86-108, wherein at least one of the modified nucleotides is 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'-hydroxyl-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, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

1210. The dsRNA agent of any one of claims 86-108, wherein the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

~~13~~11. The dsRNA of any one of claims ~~86-108~~, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), and, a vinyl-phosphonate nucleotide; and combinations thereof.

~~14~~12. The dsRNA of any one of claims ~~86-108~~, wherein at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

~~15~~13. The dsRNA of claim ~~14~~12, wherein the thermally destabilizing nucleotide modification is selected from the group consisting of 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), and a glycerol nucleic acid (GNA).

~~16~~14. The dsRNA agent of any one of claims 1-~~15~~13, wherein the double stranded region is 19-30 nucleotide pairs in length.

~~17~~15. The dsRNA agent of claim ~~16~~14, wherein the double stranded region is 19-25 nucleotide pairs in length.

~~18~~16. The dsRNA agent of claim ~~16~~14, wherein the double stranded region is 19-23 nucleotide pairs in length.

~~19~~17. The dsRNA agent of claim ~~16~~14, wherein the double stranded region is 23-27 nucleotide pairs in length.

~~20~~18. The dsRNA agent of claim ~~16~~14, wherein the double stranded region is 21-23 nucleotide pairs in length.

~~21~~19. The dsRNA agent of any one of claims 1-~~20~~18, wherein each strand is independently no more than 30 nucleotides in length.

~~22~~20. The dsRNA agent of any one of claims 1-~~21~~19, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

~~23~~21. The dsRNA agent of any one of claims 1-~~22~~20, wherein the region of complementarity is at least 17 nucleotides in length.

2422. The dsRNA agent of any one of claims 1-2321, wherein the region of complementarity is between 19 and 23 nucleotides in length.

2523. The dsRNA agent of any one of claims 1-2422, wherein the region of complementarity is 19 nucleotides in length.

2624. The dsRNA agent of any one of claims 1-2523, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

2725. The dsRNA agent of any one of claims 1-2523, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

2826. The dsRNA agent of any one of claims 1-2725, further comprising a ligand.

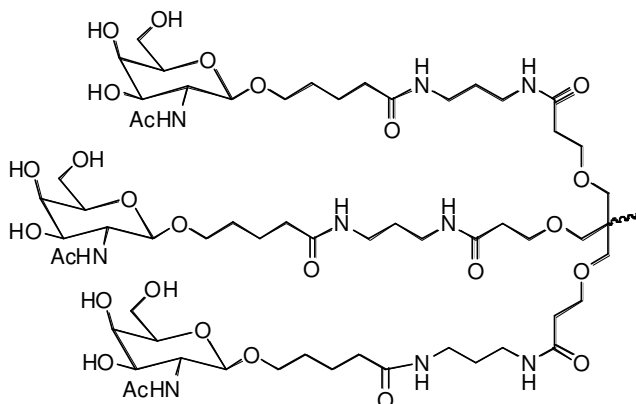
2927. The dsRNA agent of claim 2826, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

3028. The dsRNA agent of claim 2826 or 2927, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

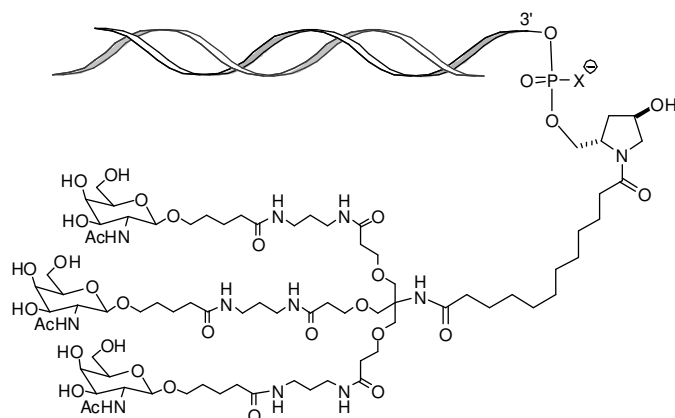
20

3129. The dsRNA agent of any one of claims 2826-3028, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

3230. The dsRNA agent of claim 3028 or 3129, wherein the ligand is



3331. The dsRNA agent of claim 3230, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

3432. The dsRNA agent of claim 3331, wherein the X is O.

5

3533. The dsRNA agent of any one of claims 1-3432, wherein the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

3634. The dsRNA agent of claim 3533, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

10

3735. The dsRNA agent of claim 3634, wherein the strand is the antisense strand.

3836. The dsRNA agent of claim 3634, wherein the strand is the sense strand.

15

3937. The dsRNA agent of claim 3533, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

4038. The dsRNA agent of claim 3937, wherein the strand is the antisense strand.

20

4139. The dsRNA agent of claim 3937, wherein the strand is the sense strand.

4240. The dsRNA agent of claim 3533, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.

25

4341. The dsRNA agent of claim 4240, wherein the strand is the antisense strand.

4442. The dsRNA agent of any one of claims 1-4341, wherein the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

30

4543. A cell containing the dsRNA agent of any one of claims 1-4442.
4644. A pharmaceutical composition for inhibiting expression of a gene encoding Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) comprising the dsRNA agent of any one of claims 1-4442.
4745. The pharmaceutical composition of claim 4644, wherein dsRNA agent is in an unbuffered solution.
4846. The pharmaceutical composition of claim 4745, wherein the unbuffered solution is saline or water.
4947. The pharmaceutical composition of claim 4644, wherein said dsRNA agent is in a buffer solution.
5048. The pharmaceutical composition of claim 4947, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.
5149. The pharmaceutical composition of claim 5048, wherein the buffer solution is phosphate buffered saline (PBS).
- ~~5250. Use of A method of inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell, the method comprising contacting the cell with the dsRNA agent of any one of claims 1-4442, or the pharmaceutical composition of any one of claims 4846-5149 in the manufacture of a medicament for inhibiting expression of a Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene in a cell, thereby inhibiting expression of the PNPLA3 gene in the cell.~~
- ~~5351. The method-use of claim 5250, wherein the cell is within a subject.~~
- ~~5452. The method-use of claim 5351, wherein the subject is a human.~~
- ~~5553. The method-use of claim 5250, wherein the subject has a PNPLA3-associated disorder.~~
5654. The ~~method-use~~ of claim 5553, wherein the PNPLA3-associated disorder is 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).

5755. The ~~method-use~~ of any one of claims 5250-5654, wherein contacting the cell with the dsRNA agent inhibits the expression of PNPLA3 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

5 5856. The ~~method-use~~ of any one of claims 5250-5755, wherein inhibiting expression of PNPLA3 decreases PNPLA3 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

10 5957. ~~Use of A method of treating a subject having a disorder that would benefit from reduction in Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) expression, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-4442, or the pharmaceutical composition of any one of claims 4644-5149 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, thereby treating the subject having the~~
15 ~~disorder that would benefit from reduction in PNPLA3 expression.~~

6058. ~~Use of A method of 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, comprising administering to the subject a prophylactically effective amount of the~~
20 ~~dsRNA agent of any one of claims 1-4442, or the pharmaceutical composition of any one of claims 4644-5149 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, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in PNPLA3 expression.~~

25 6159. The ~~method-use~~ of claim 5957 or 6058, wherein the disorder is a PNPLA3-associated disorder.

6260. The ~~method-use~~ of claim 6159, wherein the PNPLA3-associated disorder is selected from the
30 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).

6361. The ~~method-use~~ of claim 6159, wherein the PNPLA3-associated disorder is NAFLD.

35 6462. The ~~method-use~~ of claim 6159, wherein the subject is human.

6563. The ~~method-use~~ of claim 5957 or 6058, wherein the administration of the agent to the subject causes a decrease in PNPLA3 protein accumulation.

6664. The ~~method-use~~ of any one of claims 5957-6563, wherein the dsRNA agent is to be administered to the subject at a dose of ~~about~~ 0.01 mg/kg to ~~about~~ 50 mg/kg.

6765. The ~~method-use~~ of any one of claims 5957-6664, wherein the dsRNA agent is to be administered to the subject subcutaneously.

6866. The ~~method-use~~ of any one of claims 5957-6765, further comprising determining the level of PNPLA3 in a sample(s) from the subject.

6967. The ~~method-use~~ of claim 6866, wherein the level of PNPLA3 in the subject sample(s) is a PNPLA3 protein level in a blood or serum sample(s).

7068. The ~~method-use~~ of any one of claims 5957-6967, further comprising ~~administering to the subject that~~ an additional therapeutic agent for treatment of a PNPLA3-associated disorder is to be administered to the subject.

7169. A kit comprising the dsRNA agent of any one of claims 1-44-42 or the pharmaceutical composition of any one of claims 4644-5149.

7270. A vial comprising the dsRNA agent of any one of claims 1-44-42 or the pharmaceutical composition of any one of claims 4644-5149.

7371. A syringe comprising the dsRNA agent of any one of claims 1-44-42 or the pharmaceutical composition of any one of claims 4644-5149.