

expression). In particular, the RNAi oligonucleotides herein are useful for treating a disease, disorder, or condition associated with mutant *PNPLA3* expression.

[009] In one aspect, the present disclosure refers to an RNAi oligonucleotide for reducing patatin-like phospholipase domain-containing protein 3 (PNPLA3) expression, the RNAi oligonucleotide comprising a sense strand and an antisense strand wherein: the antisense strand comprises a sequence as set forth in sequence: 5'- UUAACAUAUUAUUUAGUUAGGG-3' (SEQ ID NO: 1018), and the sense strand comprises a sequence as set forth in sequence: 5'- CUAACUAAAAUAAUGUUUAAGCAGCCGAAAGGCUGC-3' (SEQ ID NO: 1017). In another aspect, the present disclosure refers to an RNAi oligonucleotide for reducing patatin-like phospholipase domain-containing protein 3 (PNPLA3) expression, the RNAi oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein the sense strand comprises: 5'- [mCs][mU][mA][mA][mC][mU][mA][fA][fA][fA][fU][mA][mA][mU][mG][mU][mU][mU][mA][mA][mG][mC][mA][mG][mC][mC][mG][ademA-GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC]-3' (SEQ ID NO: 1250); and the antisense strand comprises:

[MePhosphonate-4O-mUs][fUs][fAs][fA][fA][mC][fA][mU][mU][fA][mU][mU][mU][fU][mA][mG][mU][mU][mA][mGs][mGs][mG] (SEQ ID NO: 1251); wherein: mCs represents 2'-OMe modified cytosine with a 3'-phosphorothioate linkage; mA represents 2'-OMe modified adenosine; mC represents 2'-OMe modified cytosine; mG represents 2'-OMe modified guanosine; mU represents 2'-OMe modified uridine; fA represents 2'-F modified adenosine; fU represents 2'-F modified uridine; mGs represents 2'-OMe modified guanosine with a 3'-phosphorothioate linkage; fAs represents 2'-F modified adenosine with a 3'-phosphorothioate linkage; fUs represents 2'-F modified uridine with a 3'-phosphorothioate linkage; ademA-GalNAc represents 2'-aminodiethoxymethanol-Adenine-GalNAc; and MePhosphonate-4O-mUs represents 5'-phosphonate-4'-oxy-2'-OMe uridine with a 3'-phosphorothioate linkage. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising (i) the RNAi oligonucleotide as described herein or a pharmaceutically acceptable salt thereof; and (ii) a pharmaceutically acceptable carrier, delivery agent, or excipient. In a further aspect, the present disclosure refers to an *in vitro* method for reducing PNPLA3 expression in a cell or a population of cells, wherein the method comprises: contacting the cell or the population of cells with the RNAi oligonucleotide as described herein or the pharmaceutical composition as described herein, wherein reducing PNPLA3 expression comprises reducing an amount or a

level of PNPLA3 mRNA, an amount or a level of PNPLA3 protein, or both. In another aspect, the present disclosure refers to the use of the RNAi oligonucleotide as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment of a disease, disorder or condition associated with PNPLA3 expression, wherein the disease, disorder or condition associated with PNPLA3 expression is cardiometabolic disease, alcoholic hepatitis (AH), alcoholic liver disease (ALD), cirrhosis, hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), primary sclerosing cholangitis (PSC), non-alcoholic fatty liver disease (NAFLD), or non-alcoholic steatohepatitis (NASH). In a further aspect, the present disclosure refers to a kit comprising: the RNAi oligonucleotide as described herein, or a pharmaceutically acceptable salt thereof; an optional pharmaceutically acceptable carrier; and a package insert comprising instructions for administration to a subject having a disease, disorder or condition associated with PNPLA3 expression, wherein the disease, disorder or condition associated with PNPLA3 expression is cardiometabolic disease, alcoholic hepatitis (AH), alcoholic liver disease (ALD), cirrhosis, hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), primary sclerosing cholangitis (PSC), non-alcoholic fatty liver disease (NAFLD), or non-alcoholic steatohepatitis (NASH). Accordingly, the disclosure describes RNAi oligonucleotides for reducing or inhibiting *PNPLA3* expression that includes a sense strand having a sequence as set forth in Table 1 (*e.g.*, SEQ ID NOs: 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, 591, 593, 595, 597, 599, 601, 603, 605, 607,

609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, and 775) or Table 3 (*e.g.*, SEQ ID NOs: 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1007, 1009, 1011, 1013, 1015, 1017, 1019, 1021, 1023, 1025, 1027, 1029, 1031, 1033, 1035, 1037, 1039, 1041, 1043, 1045, 1047, 1049, 1051, 1053,

CLAIMS

The invention claimed is:

1. An RNAi oligonucleotide for reducing patatin-like phospholipase domain-containing protein 3 (PNPLA3) expression, the RNAi oligonucleotide comprising a sense strand and an antisense strand,
wherein: the antisense strand comprises a sequence as set forth in sequence: 5'-UUAAACAUAUUUUAGUUAGGG-3' (SEQ ID NO: 1018), and
the sense strand comprises a sequence as set forth in sequence: 5'-CUAACUAAAAUAAUGUUUAAGCAGCCGAAAGGCUGC-3' (SEQ ID NO: 1017).
2. The RNAi oligonucleotide of claim 1, wherein the RNAi oligonucleotide comprises at least one modified nucleotide.
3. The RNAi oligonucleotide of claim 2, wherein the modified nucleotide comprises a 2'-modification.
4. The RNAi oligonucleotide of claim 3, wherein the 2'-modification is a modification selected from the group consisting of 2'-aminoethyl, 2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl, and 2'-deoxy-2'-fluoro- β -d-arabinonucleic acid.
5. The RNAi oligonucleotide of claim 3 or 4, wherein the 2'-modification is selected from the group consisting of: 2'-fluoro and 2'-O-methyl.
6. The RNAi oligonucleotide of any one of claims 1-5, wherein all nucleotides of the RNAi oligonucleotide are modified.
7. The RNAi oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises at least one modified internucleotide linkage.
8. The RNAi oligonucleotide of claim 7, wherein the at least one modified internucleotide linkage is a phosphorothioate linkage.

9. The RNAi oligonucleotide of any one of claims 1-8, wherein the 4'-carbon of the sugar of the 5'-nucleotide of the antisense strand comprises a phosphate analog.
10. The RNAi oligonucleotide of claim 9, wherein the phosphate analog is oxymethylphosphonate, vinylphosphonate or malonylphosphonate, or a 4'-phosphate analog comprising 5'-methoxyphosphonate-4'-oxy.
11. The RNAi oligonucleotide of any one of claims 1-10, wherein at least one nucleotide of the oligonucleotide is conjugated to one or more targeting ligands.
12. The RNAi oligonucleotide of claim 11, wherein each targeting ligand comprises a carbohydrate, amino sugar, cholesterol, polypeptide, or lipid.
13. The RNAi oligonucleotide of claim 12, wherein each targeting ligand comprises a N-acetylgalactosamine (GalNAc) moiety.
14. The RNAi oligonucleotide of claim 13, wherein the GalNAc moiety is a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety.
15. The RNAi oligonucleotide of any one of claims 1-14, wherein up to 4 nucleotides of a stem-loop of the RNAi oligonucleotide are each conjugated to a monovalent GalNAc moiety.
16. The RNAi oligonucleotide of any one of claims 1-15, wherein the sense strand comprises:
5'-
[mCs][mU][mA][mA][mC][mU][mA][fA][fA][fA][fU][mA][mA][mU][mG][mU][mU][mU]
[mA][mA][mG][mC][mA][mG][mC][mC][mG][ademA-GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC]-3' (SEQ ID NO: 1250), wherein:
mA represents 2'-OMe modified adenosine;
mC represents 2'-OMe modified cytosine;
mG represents 2'-OMe modified guanosine;
mU represents 2'-OMe modified uridine;

fA represents 2'-F modified adenosine;

fU represents 2'-F modified uridine;

mCs represents 2'-OMe modified cytosine with a 3'-phosphorothioate linkage; and

ademA-GalNAc represents: 2'-aminodiethoxymethanol-Adenine-GalNAc.

17. The RNAi oligonucleotide of any one of claims 1-15, wherein the antisense strand comprises:

5'-[MePhosphonate-4O-

mUs][fUs][fAs][fA][fA][mC][fA][mU][mU][fA][mU][mU][mU][fU][mA]

[mG][mU][mU][mA][mGs][mGs][mG]-3' (SEQ ID NO: 1251), wherein:

mA represents 2'-OMe modified adenosine;

mC represents 2'-OMe modified cytosine;

mG represents 2'-OMe modified guanosine;

mU represents 2'-OMe modified uridine;

fA represents 2'-F modified adenosine;

fU represents 2'-F modified uridine;

mGs represents 2'-OMe modified guanosine with a 3'-phosphorothioate linkage;

fAs represents 2'-F modified adenosine with a 3'-phosphorothioate linkage;

fUs represents 2'-F modified uridine with a 3'-phosphorothioate linkage; and

MePhosphonate-4O-mUs represents 5'-phosphonate-4'-oxy-2'-OMe uridine with a 3'-phosphorothioate linkage.

18. An RNAi oligonucleotide for reducing patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) expression, the RNAi oligonucleotide comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand form a duplex region, wherein

the sense strand comprises: 5'-

[mCs][mU][mA][mA][mC][mU][mA][fA][fA][fA][fU][mA][mA][mU][mG][mU][mU][mU]

[mA][mA][mG][mC][mA][mG][mC][mC][mG][ademA-GalNAc][ademA-GalNAc][ademA-GalNAc][mG][mG][mC][mU][mG][mC]-3' (SEQ ID NO: 1250); and

the antisense strand comprises: [MePhosphonate-4O-

mUs][fUs][fAs][fA][fA][mC][fA][mU][mU][fA][mU][mU][mU][fU][mA][mG][mU][mU][

mA][mGs][mGs][mG] (SEQ ID NO: 1251);

wherein:

mCs represents 2'-OMe modified cytosine with a 3'-phosphorothioate linkage;
 mA represents 2'-OMe modified adenosine;
 mC represents 2'-OMe modified cytosine;
 mG represents 2'-OMe modified guanosine;
 mU represents 2'-OMe modified uridine;
 fA represents 2'-F modified adenosine;
 fU represents 2'-F modified uridine;
 mGs represents 2'-OMe modified guanosine with a 3'-phosphorothioate linkage;
 fAs represents 2'-F modified adenosine with a 3'-phosphorothioate linkage;
 fUs represents 2'-F modified uridine with a 3'-phosphorothioate linkage;
 ademA-GalNAc represents 2'-aminodiethoxymethanol-Adenine-GalNAc; and
 MePhosphonate-4O-mUs represents 5'-phosphonate-4'-oxy-2'-OMe uridine with a 3'-phosphorothioate linkage .

19. A pharmaceutical composition comprising:

- (i) the RNAi oligonucleotide of any one of claims 1-18 or a pharmaceutically acceptable salt thereof; and
- (ii) a pharmaceutically acceptable carrier, delivery agent, or excipient.

20. An *in vitro* method for reducing PNPLA3 expression in a cell or a population of cells, wherein the method comprises:

contacting the cell or the population of cells with the RNAi oligonucleotide of any one of claims 1-18 or the pharmaceutical composition of claim 19,
 wherein reducing PNPLA3 expression comprises reducing an amount or a level of PNPLA3 mRNA, an amount or a level of PNPLA3 protein, or both.

21. Use of the RNAi oligonucleotide of any one of claims 1-18 or the pharmaceutical composition of claim 19 in the manufacture of a medicament for the treatment of a disease, disorder or condition associated with *PNPLA3* expression,
 wherein the disease, disorder or condition associated with *PNPLA3* expression is cardiometabolic disease, alcoholic hepatitis (AH), alcoholic liver disease (ALD), cirrhosis, hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), primary sclerosing cholangitis (PSC), non-alcoholic fatty liver disease (NAFLD), or non-alcoholic steatohepatitis (NASH).

22. The use of claim 21, wherein the medicament is to be administered in combination with a second composition or therapeutic agent.

23. The RNAi oligonucleotide of any one of claims 1 to 18, or the pharmaceutical composition of claim 19, for use as a medicament.

24. A kit comprising:

the RNAi oligonucleotide of any one of claims 1-18, or a pharmaceutically acceptable salt thereof;

an optional pharmaceutically acceptable carrier; and

a package insert comprising instructions for administration to a subject having a disease, disorder or condition associated with *PNPLA3* expression,

wherein the disease, disorder or condition associated with *PNPLA3* expression is cardiometabolic disease, alcoholic hepatitis (AH), alcoholic liver disease (ALD), cirrhosis, hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), primary sclerosing cholangitis (PSC), non-alcoholic fatty liver disease (NAFLD), or non-alcoholic steatohepatitis (NASH).