## SUMMARY

**[0008a]** In an aspect of the present disclosure, there is provided an oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising an antisense strand comprising a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

**[0008b]** In another aspect of the present disclosure, there is provided an oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length, wherein the antisense strand has a region of complementarity to CYP27A1 that is complementary to at least 15 contiguous nucleotides of a sequence as set forth in SEQ ID NOs: 767-781.

**[0008c]** In a further aspect of the present disclosure, there is provided an oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity with CYP27A1,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: $S_1$-L-$S_2$, wherein $S_1$ is complementary to $S_2$, and wherein L forms a loop between $S_1$ and $S_2$ of 3 to 5 nucleotides in length,

and wherein the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length but are not covalently linked.

**[0008d]** In another aspect of the present disclosure, there is provided a composition comprising an oligonucleotide disclosed herein, and an excipient.

**[0008e]** In a further aspect of the present disclosure, there is provided a composition disclosed herein, for use in delivering an oligonucleotide to a subject.

**[0008f]** In another aspect of the present disclosure, there is provided a use of a composition disclosed herein, in the manufacture of a medicament for attenuating bile acid accumulation in liver of a subject.

**[0008g]** In a further aspect of the present disclosure, there is provided a use of a composition disclosed herein, in the manufacture of a medicament for decreasing the extent of liver fibrosis in a subject in need thereof.

**[0008h]** In another aspect of the present disclosure, there is provided a use of a composition disclosed herein, in the manufacture of a medicament for decreasing circulating bile acid concentrations in a subject in need thereof.

**[0008i**] In a further aspect of the present disclosure, there is provided a composition disclosed herein for use in attenuating bile acid accumulation in liver of a subject, decreasing the extent of liver fibrosis in a subject, and/or decreasing circulating bile acid concentrations in a subject.

**[0008j]** In another aspect of the present disclosure, there is provided an oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising a sense strand of 15 to 50 nucleotides in length and an antisense strand of 15 to 30 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand, wherein the sense strand comprises a sequence as set forth in any one of SEQ ID Nos: 577-578, 581-597, 759-762, 785, and 787 and wherein the antisense strand comprises a complementary sequence selected from SEQ ID Nos: 579-580, 598-614, 763-766, 786, and 788.

**[0008k]** In a further aspect of the present disclosure, there is provided an oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising a pair of sense and antisense strands selected from a row of the table set forth in Appendix A.

**[0009]** Examples of the disclosure relate to compositions and related methods for reducing expression of genes affecting liver metabolic functions, particularly genes affecting bile acid levels in a subject. In some examples, the disclosure relates to a recognition that CYP27A1 is a useful target for the treatment of hepatobiliary diseases, particularly such diseases that are associated with bile acid accumulation. In further examples it has been discovered that oligonucleotides for reducing expression or activity of CYP27A1 are useful for treating conditions in which the accumulation of bile acids in the liver contributes to cellular toxicity (*e.g.*, to toxicity hepatocytes and/or cholangiocytes) and/or promotes liver fibrosis. Accordingly, in some examples, the disclosure relates to the use of oligonucleotides, including RNAi oligonucleotides, antisense oligonucleotides, and other similar modalities, for reducing expression or activity of CYP27A1 for the treating of

hepatobiliary diseases, including, for example, cholestasis, cholangitis, nonalcoholic steatohepatitis (NASH) and/or alagille syndrome.

**[00010]** In further examples, potent RNAi oligonucleotides have been developed for selectively inhibiting CYP27A1 expression in a subject. In some examples, the RNAi oligonucleotides are useful for reducing CYP27A1 activity, and thereby decreasing or preventing the accumulation of bile acid in a subject. In some examples, key regions of CYP27A1 activity mRNA (referred to as hotspots) have been identified herein that are particularly amenable to targeting using such oligonucleotide-based approaches (See Example 1). In some examples, oligonucleotides developed herein to inhibit CYP27A1 expression are useful for reducing or preventing liver fibrosis associated with bile acid accumulation (see, e.g., Example 1, FIG. 7 and FIG. 8).

**[00011]** One example of the present disclosure provides oligonucleotides for reducing expression of CYP27A1. In some examples, the oligonucleotides comprise an antisense strand comprising a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788. In some examples, the oligonucleotides further comprise a sense strand that comprises a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787. In some examples, the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788. In some examples, the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787.

**[00012]** One example of the present disclosure provides oligonucleotides for reducing

expression of CYP27A1, in which the oligonucleotides comprise an antisense strand of 15 to 30 nucleotides in length. In some examples, the antisense strand has a region of complementarity to a target sequence of CYP27A1 as set forth in any one of SEQ ID NOs: 767-781. In some examples, the region of complementarity is at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, or at least 22 contiguous nucleotides in length. In some examples, the region of complementarity is fully complementary to the target sequence of CYP27A1. In some examples, the region of complementarity to CYP27A1 is at least 19 contiguous nucleotides in length. In some examples, the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787. In some examples, the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787. In some examples, the antisense strand comprises a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788. In some examples, the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

**[00013]** In some examples, the antisense strand is 19 to 27 nucleotides in length. In some examples, the antisense strand is 21 to 27 nucleotides in length. In some examples, the oligonucleotide further comprises a sense strand of 15 to 40 nucleotides in length, in which the sense strand forms a duplex region with the antisense strand. In some examples, the sense strand is 19 to 40 nucleotides in length. In some examples, the antisense strand is 27 nucleotides in length and the sense strand is 25 nucleotides in length. In some examples, the duplex region is at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 nucleotides in length. In some examples, the antisense strand and sense strand form a duplex region of 25 nucleotides in length.

**[00014]** In some examples, an oligonucleotide comprises an antisense strand and a sense strand that are each in a range of 21 to 23 nucleotides in length. In some examples, an oligonucleotide comprises a duplex structure in a range of 19 to 21 nucleotides in length. In some examples, an oligonucleotide further comprises a 3′-overhang sequence on the antisense strand of two nucleotides in length. In some examples, an oligonucleotide comprises a 3′-overhang sequence of one or more nucleotides in length, in which the 3′-overhang sequence is present on the antisense strand, the sense strand, or the antisense strand and sense strand. In some examples, an oligonucleotide comprises a 3′-overhang sequence of two nucleotides in length, in which the 3′-overhang sequence is present on the antisense strand, and in which the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length, such that the sense strand and antisense strand form a duplex of 21

nucleotides in length.

**[00015]** In some examples, the sense strand comprises at its 3′-end a stem-loop set forth as: $S_1$-L-$S_2$, in which $S_1$ is complementary to $S_2$, and in which L forms a loop between $S_1$ and $S_2$ of 3 to 5 nucleotides in length.

**[00016]** Another example of the present disclosure provides an oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising an antisense strand and a sense strand, in which the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity to CYP27A1, in which the sense strand comprises at its 3′-end a stem-loop set forth as: S1-L-S2, in which S1 is complementary to S2, and in which L forms a loop between S1 and S2 of 3 to 5 nucleotides in length, and in which the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length but are not covalently linked. In some examples, the region of complementarity to CYP27A1 mRNA is fully complementary to at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21 contiguous nucleotides of CYP27A1 mRNA. In some examples, L is a tetraloop. In some examples, L is 4 nucleotides in length. In some examples, L comprises a sequence set forth as GAAA.

**[00017]** In some examples, an oligonucleotide comprises at least one modified nucleotide. In some examples, the modified nucleotide comprises a 2′-modification. In some examples, the 2′-modification is a modification selected from: 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, and 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid. In some examples, all of the nucleotides of an oligonucleotide are modified.

**[00018]** In some examples, an oligonucleotide comprises at least one modified internucleotide linkage. In some examples, the at least one modified internucleotide linkage is a phosphorothioate linkage. In some examples, the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog. In some examples, the phosphate analog is oxymethyl phosphonate, viny lphosphonate, or malonyl phosphonate.

**[00019]** In some examples, at least one nucleotide of an oligonucleotide is conjugated to one or more targeting ligands. In some examples, each targeting ligand comprises a carbohydrate, amino sugar, cholesterol, polypeptide, or lipid. In some examples, each targeting ligand comprises a N-acetylgalactosamine (GalNAc) moiety. In some examples, the GalNac moiety is a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety. In some examples, up to 4 nucleotides of L of a stem-loop are each conjugated to a monovalent GalNAc moiety. In other examples, a bi-valent, tri-valent or tetravalent GalNac moiety is conjugated to a

single nucleotide, e.g., of the nucleotides of L of a stem loop. In some examples, the targeting ligand comprises an aptamer.

**[00020]** Another example of the present disclosure provides a composition comprising an oligonucleotide of the present disclosure and an excipient. Another example of the present disclosure provides a method comprising administering a composition of the present disclosure to a subject. In some examples, such methods are useful for attenuating bile acid accumulation in liver of a subject. In some examples, such methods are useful for decreasing the extent of liver fibrosis in a subject in need thereof. In some examples, such methods are useful for decreasing circulating bile acid concentrations in a subject in need thereof. In some examples, such methods are useful for treating hepatobiliary disease. In some examples, the subject suffers from PNALD.

**[00021]** Another example of the present disclosure provides an oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising a sense strand of 15 to 40 nucleotides in length and an antisense strand of 15 to 30 nucleotides in length, in which the sense strand forms a duplex region with the antisense strand, in which the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787 and the antisense strand comprises a complementary sequence selected from SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

**[00022]** In some examples, the oligonucleotide comprises a pair of sense and antisense strands selected from a row of the table set forth in Appendix A.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[00023]** The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain embodiments, and together with the written description, serve to provide non-limiting examples of certain aspects of the compositions and methods disclosed herein.

**[00024]** FIG. 1 is a flowchart depicting the experimental design used to select compounds for testing in cell and animal models and to develop oligonucleotides for reducing expression of CYP27A1. SAR: Structure-Activity Relationship.

**[00025]** FIG. 2 is a schematic showing a non-limiting example of a double-stranded oligonucleotide with a nicked tetraloop structure that has been conjugated to four GalNAc moieties (yellow diamonds).

**[00026]** FIG. 3 is a graph showing the percent of CYP27A1 mRNA remaining after a

## CLAIMS

What is claimed is:

1. An oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising an antisense strand comprising a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

2. The oligonucleotide of claim 1, further comprising a sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787.

3. The oligonucleotide of claim 1 or 2, wherein the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

4. The oligonucleotide of any one of claims 1 to 3, wherein the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787.

5. An oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising an antisense strand of 15 to 30 nucleotides in length, wherein the antisense strand has a region of complementarity to CYP27A1 that is complementary to at least 15 contiguous nucleotides of a sequence as set forth in SEQ ID NOs: 767-781.

6. The oligonucleotide of claim 1, wherein the antisense strand is 19 to 27 nucleotides in length.

7. The oligonucleotide of claim 1, wherein the antisense strand is 21 to 27 nucleotides in length.

8. The oligonucleotide of any one of claims 2 to 4, wherein the sense strand is 15 to 50 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand.

9. The oligonucleotide of claim 5, wherein the sense strand is 19 to 50 nucleotides in length.

10. The oligonucleotide of claim 5 or 6, wherein the duplex region is at least 19 nucleotides in length.

11. The oligonucleotide of any one of claims 1 to 7, wherein the region of complementarity with CYP27A1 is complementary to at least 19 contiguous nucleotides of a sequence as set forth in SEQ ID NOs: 767-781.

12. The oligonucleotide of any one of claims 5 to 9, wherein the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787.

13. The oligonucleotide of any one of claims 10, wherein the antisense strand comprises a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

14. The oligonucleotide of any one of claims 5 to 9, wherein the sense strand consists of a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787.

15. The oligonucleotide of any one of claims 10, wherein the antisense strand consists of a sequence as set forth in any one of SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

16. The oligonucleotide of any one of claims 8 to 15, wherein the sense strand comprises at its 3′-end a stem-loop set forth as: $S_1$-L-$S_2$, wherein $S_1$ is complementary to $S_2$, and wherein L forms a loop between $S_1$ and $S_2$ of 3 to 5 nucleotides in length.

17. An oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising an antisense strand and a sense strand,

wherein the antisense strand is 21 to 27 nucleotides in length and has a region of complementarity with CYP27A1,

wherein the sense strand comprises at its 3′-end a stem-loop set forth as: $S_1$-L-$S_2$, wherein $S_1$ is complementary to $S_2$, and wherein L forms a loop between $S_1$ and $S_2$ of 3 to 5 nucleotides in length,

and wherein the antisense strand and the sense strand form a duplex structure of at least 19 nucleotides in length but are not covalently linked.

18. The oligonucleotide of claim 17, wherein the region of complementarity is complementary to at least 19 contiguous nucleotides of CYP27A1 mRNA.

19. The oligonucleotide of any one of claims 16 to 18, wherein L is a tetraloop.

20. The oligonucleotide of any one of claims 16 to 19, wherein L is 4 nucleotides in length.

21. The oligonucleotide of any one of claims 16 to 20, wherein L comprises a sequence set forth as GAAA.

22. The oligonucleotide of any one of claims 8 to 15, wherein the antisense strand is 27 nucleotides in length and the sense strand is 25 nucleotides in length.

23. The oligonucleotide of claim 22, wherein the antisense strand and sense strand form a duplex region of 25 nucleotides in length.

24. The oligonucleotide of claim 19, further comprising a 3′-overhang sequence on the antisense strand of two nucleotides in length.

25. The oligonucleotide of any one of claims 8 to 15, wherein the oligonucleotide comprises an antisense strand and a sense strand that are each in a range of 21 to 23 nucleotides in length.

26. The oligonucleotide of claim 25, wherein the oligonucleotide comprises a duplex structure in a range of 19 to 21 nucleotides in length.

27. The oligonucleotide of claim 25 or 26, wherein the oligonucleotide comprises a 3′-overhang sequence of one or more nucleotides in length, wherein the 3′-overhang sequence is present on the antisense strand, the sense strand, or the antisense strand and sense strand.

28. The oligonucleotide of claim 25 or 26, wherein the oligonucleotide comprises a 3′-overhang sequence of two nucleotides in length, wherein the 3′-overhang sequence is present on the antisense strand, and wherein the sense strand is 21 nucleotides in length and the

antisense strand is 23 nucleotides in length, such that the sense strand and antisense strand form a duplex of 21 nucleotides in length.

29. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises at least one modified nucleotide.

30. The oligonucleotide of claim 29, wherein the modified nucleotide comprises a 2′-modification.

31. The oligonucleotide of claim 30, wherein the 2′-modification is a modification selected from: 2′-aminoethyl, 2′-fluoro, 2′-O-methyl, 2′-O-methoxyethyl, and 2′-deoxy-2′-fluoro-β-d-arabinonucleic acid.

32. The oligonucleotide of any one of claims 29 to 31, wherein all of the nucleotides of the oligonucleotide are modified.

33. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises at least one modified internucleotide linkage.

34. The oligonucleotide of claim 33, wherein the at least one modified internucleotide linkage is a phosphorothioate linkage.

35. The oligonucleotide of any one of the preceding claims, wherein the 4′-carbon of the sugar of the 5′-nucleotide of the antisense strand comprises a phosphate analog.

36. The oligonucleotide of claim 35, wherein the phosphate analog is oxymethyl phosphonate, vinyl phosphonate, or malonyl phosphonate.

37. The oligonucleotide of any one of the preceding claims, wherein at least one nucleotide of the oligonucleotide is conjugated to one or more targeting ligands.

38. The oligonucleotide of claim 37, wherein each targeting ligand comprises a carbohydrate, amino sugar, cholesterol, polypeptide or lipid.

39. The oligonucleotide of claim 38, wherein each targeting ligand comprises a N-acetylgalactosamine (GalNAc) moiety.

40. The oligonucleotide of claim 39, wherein the GalNac moiety is a monovalent GalNAc moiety, a bivalent GalNAc moiety, a trivalent GalNAc moiety, or a tetravalent GalNAc moiety.

41. The oligonucleotide of any one of claims 16 to 19, wherein up to 4 nucleotides of L of the stem-loop are each conjugated to a monovalent GalNAc moiety.

42. The oligonucleotide of claim 37, wherein the targeting ligand comprises an aptamer.

43. A composition comprising an oligonucleotide of any one of the preceding claims and an excipient.

44. A composition of claim 43, for use in delivering an oligonucleotide to a subject.

45. Use of a composition of claim 43 in the manufacture of a medicament for attenuating bile acid accumulation in liver of a subject.

46. Use of a composition of claim 43 in the manufacture of a medicament for decreasing the extent of liver fibrosis in a subject in need thereof.

47. Use of a composition of claim 43 in the manufacture of a medicament for decreasing circulating bile acid concentrations in a subject in need thereof.

48. A composition of claim 43 for use in attenuating bile acid accumulation in liver of a subject, decreasing the extent of liver fibrosis in a subject, and/or decreasing circulating bile acid concentrations in a subject.

49. The use or composition for use of any one of claims 44 to 48, wherein the subject suffers from hepatobiliary disease.

50. An oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising a sense strand of 15 to 50 nucleotides in length and an antisense strand of 15 to 30 nucleotides in length, wherein the sense strand forms a duplex region with the antisense strand, wherein the sense strand comprises a sequence as set forth in any one of SEQ ID NOs: 577-578, 581-597, 759-762, 785, and 787 and wherein the antisense strand comprises a complementary sequence selected from SEQ ID NOs: 579-580, 598-614, 763-766, 786, and 788.

51. An oligonucleotide for reducing expression of CYP27A1, the oligonucleotide comprising a pair of sense and antisense strands selected from a row of the table set forth in Appendix A.

52. The use or composition for use of any one of claims 44 to 48, wherein the subject suffers from PNALD.