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(54) Title: MODULATION OF THE NOTCH SIGNALING PATHWAY FOR TREATMENT OF RESPIRATORY DISORDERS

(57) Abstract: Provided herein are methods, compounds, and compositions for modulating expression of at least one member of the Notch signaling pathway in a cell or individual. Such methods, compounds, and compositions are useful to treat, prevent, delay, or ameliorate a respiratory disorder associated with excessive mucus production in an individual.

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MODULATION OF THE NOTCH SIGNALING PATHWAY FOR TREATMENT OF RESPIRATORY DISORDERS

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0319WOSEQ_ST25.txt, created on August 7, 2018 which is 524 KB in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Background

The Notch signaling pathway is a highly conserved pathway that is involved in a large variety of developmental processes, diseases, and other biological functions and processes. (*See, e.g.,* Bray, S. *Nat. Rev. Mol. Cell Biol.* 17, 723 (2016).) Ligands of the Notch signaling pathway activate Notch receptors, which ultimately leads to activation of target gene transcription.

Antisense technology is an effective means for modulating the expression of one or more specific gene products and can therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications. Chemically modified nucleosides may be incorporated into antisense compounds to enhance one or more properties, such as nuclease resistance, pharmacokinetics or affinity for a target nucleic acid.

Summary

Provided herein are compositions, compounds and methods for modulating expression of the Notch signaling pathway. In certain embodiments, one or more members of the Notch signaling pathway is modulated. In certain embodiments, the Notch signaling pathway is modulated by a compound comprising or consisting of a modified oligonucleotide complementary to a transcript encoding a member of the Notch signaling pathway. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, Jagged1 (hereinafter referred to as JAG1 signaling pathway), Jagged2 (hereinafter referred to as JAG2), Delta-like1 (hereinafter referred to as DLL1), Delta-like3 (hereinafter referred to as DLL3), Delta-like4 (hereinafter referred to as DLL4), or Hes family bHLH transcription factor 1 (hereinafter referred to as Hes-1) transcript. In certain such embodiments, the compound decreases expression or activity of one or more members of the Notch signaling pathway. In certain embodiments, the modified oligonucleotide of the compound can be single-stranded or part of a duplex.

Certain embodiments are directed to compounds useful for inhibiting the Notch signaling pathway, which can be useful for treating, ameliorating, or slowing progression of a respiratory disorder associated with excessive mucus production. Certain embodiments relate to the novel findings of antisense inhibition of

the Notch signaling pathway resulting in improvement of symptoms or endpoints associated with such respiratory disorders and/or lung function. Certain embodiments are directed to compounds useful in improving trans-differentiation from club cells or goblet cells to ciliated cells, decreased mucus in the lungs, and increased lung function.

Provided herein are embodiments including but not limited to:

1. A method of treating, preventing, delaying the onset, slowing the progression, or ameliorating a respiratory disorder associated with excessive mucus production in an individual having, or at risk of having, a respiratory disorder associated with excessive mucus production comprising administering a compound comprising a Notch signaling pathway inhibitor to the individual, thereby treating, preventing, delaying the onset, slowing the progression, or ameliorating the respiratory disorder associated with excessive mucus production in the individual.

2. The method of embodiment 1, wherein the respiratory disorder associated with excessive mucus production is asthma, chronic obstructive pulmonary disorder (COPD), idiopathic pulmonary fibrosis (IPF), or cystic fibrosis (CF).

3. The method of embodiment 2, wherein the respiratory disorder associated with excessive mucus production is asthma.

4. The method of embodiment 2, wherein the respiratory disorder associated with excessive mucus production is COPD.

5. The method of embodiment 2, wherein the respiratory disorder associated with excessive mucus production is IPF.

6. The method of embodiment 2, wherein the respiratory disorder associated with excessive mucus production is CF.

7. The method of any of embodiments 1-6, wherein the compound increases trans-differentiation from club cells or goblet cells to ciliated cells, decreases mucus in the lungs, and/or increases lung function.

8. The method of embodiment 7, wherein the compound decreases mucus in the lungs.

9. The method of embodiment 7, wherein the compound increases lung function.

10. A method of inhibiting expression or activity of the Notch signaling pathway in a cell comprising contacting the cell with a compound comprising a Notch signaling pathway inhibitor, thereby inhibiting expression or activity of at least one member of the Notch signaling pathway in the cell.

11. The method of embodiment 10, wherein the cell is a lung cell.

12. The method of embodiment11, wherein the cell is in an individual.

13. The method of embodiment 12, wherein the individual has, or is at risk of having asthma, COPD, IPF, or CF.

14. The method of any of embodiments 1-9 or 12-13, wherein the individual is human.

15. The method of any of embodiments 1-14, comprising administering to the individual or contacting the cell with no more than one compound comprising a Notch signaling pathway inhibitor.

16. The method of any of embodiments 1-15, wherein the compound inhibits the expression of at least one Notch signaling pathway member transcript.

17. The method of any of embodiments 1-16, wherein the compound inhibits the expression of at least two Notch signaling pathway members.

18. The method of any of embodiments 1-17, wherein the Notch signaling pathway inhibitor is a modified oligonucleotide complementary to a Notch signaling pathway member transcript.

19. The method of any of embodiments 1-17, wherein the compound comprises a modified oligonucleotide complementary to a member of the Notch signaling pathway.

20. The method of embodiment 18 or 19, wherein the modified oligonucleotide is single-stranded.

21. The method of embodiment 18 or 19, wherein the modified oligonucleotide is part of a double-stranded duplex.

22. The method of any of embodiments 18-21, wherein the modified oligonucleotide is 12 to 30 linked nucleosides in length.

23. The method of any of embodiments 18-22, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

24. The method of embodiment 23, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.

25. The method of any of embodiments 18-24, wherein the modified oligonucleotide comprises at least one modified sugar moiety.

26. The method of embodiment 25, wherein the at least one modified sugar moiety is a bicyclic sugar or 2'-O-methyoxyethyl modified sugar moiety.

27. The method of embodiment 26, wherein the at least one modified sugar is a cEt, LNA, or ENA.

28. The method of any of embodiments 18-27, wherein the modified oligonucleotide comprises at least one 5-methylcytosine modified nucleobase.

29. The method of any of embodiments 24-28, wherein each modified internucleoside linkage is a phosphorothioate linkage.

30. The method of any of embodiments 18-29, wherein each cytosine nucleobase is a 5-methylcytosine.

31. The method of any one of embodiments 18-30, wherein the modified oligonucleotide comprises:
a gap segment consisting of 7-11 linked 2'-deoxynucleosides;
a 5' wing segment consisting of 1-7 linked nucleosides;
a 3' wing segment consisting of 1-7 linked nucleosides;
wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein the nucleoside of each wing segment that is immediately adjacent to the gap segment each comprises a modified sugar.

32. The method of any of embodiments 18-31, wherein the modified oligonucleotide is at least 90% complementary to a Notch signaling pathway member nucleic acid.

33. The method of any of embodiments 18-31, wherein the modified oligonucleotide is 100% complementary to a Notch signaling pathway member nucleic acid.

34. The method of embodiments 32 or 33, wherein the Notch signaling pathway member nucleic acid is a Notch signaling pathway member transcript.

35. The method of embodiment 34, wherein the Notch signaling pathway member transcript is a Notch signaling pathway member pre-mRNA.

36. The method of embodiment 34, wherein the Notch signaling pathway member transcript is a Notch signaling pathway member mRNA.

37. The method of any of embodiments 32-36, wherein the Notch signaling pathway member is a Notch receptor, ligand of a Notch receptor, or intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

38. The method of embodiment 37, wherein the Notch signaling pathway member is a Notch receptor or a ligand of a Notch receptor.

39. The method of embodiment 38, wherein the Notch signaling pathway member is a Notch receptor.

40. The method of embodiment 39, wherein the Notch receptor is Notch1, Notch2, Notch 3, or Notch4.

41. The method of embodiment 40, wherein the Notch receptor is Notch1, Notch2, or Notch3.

42. The method of embodiment 41, wherein the Notch receptor is Notch1.

43. The method of embodiment 41, wherein the Notch receptor is Notch2.

44. The method of embodiment 41, wherein the Notch receptor is Notch3.

45. The method of embodiment 38, wherein the Notch signaling pathway member is a ligand of a Notch receptor.

46. The method of embodiment 45, wherein the ligand is DLL1, DLL3, DLL4, JAG1, or JAG2.

47. The method of embodiment 46, wherein the ligand is DLL4, JAG1, or JAG2.

48. The method of embodiment 47, wherein the ligand is DLL4.

49. The method of embodiment 47, wherein the ligand is JAG1.

50. The method of embodiment 47, wherein the ligand is JAG2.

51. The method of embodiment 37, wherin the Notch signaling pathway member is an intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

52. The method of embodiment 51, wherein the intracellular protein that transmits the Notch signal to or within in the nucleus of a cell is Hes-1.

53. The method of any of embodiments 16-52, wherein the at least one Notch signaling pathway member that is inhibited is the target transcript.

54. The method of any of embodiments 17-53, wherein the expression or activity of at least one Notch signaling pathway member that is not the target transcript is inhibited.

55. The method of any of embodiments 1-9 or 12-54, wherein the compound is administered parenterally.

56. The method of embodiment 55, wherein the compound is administered parenterally by subcutaneous administration.

57. The method of any of embodiments 1-9 or 12-54, wherein the compound is administered via inhalation.

58. The method of any of the preceding embodiments, comprising co-administering the compound and at least one additional therapy, wherein the additional therapy is not a Notch signaling pathway inhibitor.

59. The method of embodiment 58, wherein the compound and the additional therapy are administered concomitantly.

60. The method of embodiment 58, wherein the compound and the additional therapy are administered consecutively.

61. Use of a compound comprising a modified oligonucleotide complementary to a Notch signaling pathway member transcript for the manufacture or preparation of a medicament for treating a respiratory disorder associated with excessive mucus production.

62. Use of a compound comprising a modified oligonucleotide complementary to a Notch signaling pathway member transcript for the treatment of a respiratory disorder associated with excessive mucus production.

63. The use of embodiment 61 or 62, wherein the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF.

64. The use of any one of embodiments 61-63, wherein the compound is capable of increasing trans-differentiation from club cells or goblet cells to ciliated cells, decreasing mucus in the lungs, and/or increasing lung function.

65. The use of any one of embodiments 61-64, wherein the modified oligonucleotide is at least 90% complementary to the Notch signaling pathway member transcript.

66. The use of embodiment 65, wherein the modified oligonucleotide is at least 100% complementary to the Notch signaling pathway member transcript.

67. The use of any one of embodiments 61-66, wherein the Notch signaling pathway member transcript is a Notch receptor transcript, a transcript of a ligand of a Notch receptor, or a transcript of an intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

68. The use of embodiment 67, wherein the Notch signaling pathway member transcript is a Notch receptor transcript or a transcript of a ligand of a Notch receptor.

69. The use of embodiment 68, wherein the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, or Notch4 trasncript.

70. The use of embodiment 69, wherein the Notch signaling pathway member transcript is a Notch1, Notch2, or Notch3 transcript.

71. The use of embodiment 70, wherein the Notch signaling pathway member transcript is a Notch1 transcript.

72. The use of embodiment 70, wherein the Notch signaling pathway member transcript is a Notch2 transcript.

73. The use of embodiment 70, wherein the Notch signaling pathway member transcript is a Notch3 transcript.

74. The use of embodiment 68, wherein the Notch signaling pathway member transcript is a DLL1, DLL3, DLL4, JAG1, or JAG2 transcript.

75. The use of embodiment 74, wherein the Notch signaling pathway member transcript is a DLL4, JAG1, or JAG2 transcript.

76. The use of embodiment 75, wherein the Notch signaling pathway member transcript is a DLL4 transcript.

77. The use of embodiment 75, wherein the Notch signaling pathway member transcript is a JAG1 transcript.

78. The use of embodiment 75, wherein the Notch signaling pathway member transcript is a JAG2 transcript.

79. The use of embodiment 67, wherin the Notch signaling pathway member transcript is a transcript of an intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

80. The use of embodiment 79, wherein the transcript of an intracellular protein that transmits the Notch signal to or within in the nucleus of a cell is a Hes-1 transcript.

81. The use of any one of embodiments 61-80, wherein the modified oligonucleotide is single-stranded.

82. The use of any one of embodiments 61-80, wherein the modified oligonucleotide is part of a double-stranded duplex.

83. The use of any one of embodiments 61-82, wherein the modified oligonucleotide is 12 to 30 linked nucleosides in length.

84. The use of any one of embodiments 61-83, wherein the modified oligonucleotide comprises at least one phosphorothioate internucleoside linkage, at least one bicyclic sugar moiety or 2'-O-methyoxyethyl modified sugar moiety, and at least one 5-methylcytosine modified nucleobase.

85. The use of embodiment 84, wherein at least one modified sugar is a cEt, LNA, or ENA.

86. The use of any of embodiments 61-85, wherein each modified internucleoside linkage of the modified oligonucleotide is a phosphorothioate linkage.

87. The use of any one of embodiments 61-86, wherein each cytosine nucleobase of the modified oligonucleotide is a 5-methylcytosine.

88. The use of any one of embodiments 61-87, wherein the modified oligonucleotide comprises:

a gap segment consisting of 7-11 linked 2'-deoxynucleosides;

a 5' wing segment consisting of 1-7 linked nucleosides;

a 3' wing segment consisting of 1-7 linked nucleosides;

wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein the nucleoside of each wing segment that is immediately adjacent to the gap segment comprises a modified sugar moiety.

Detailed Description

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the embodiments, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, treatises, and GenBank and NCBI reference sequence records are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

It is understood that the sequence set forth in each SEQ ID NO in the examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase.

As used herein, "2'-deoxynucleoside" means a nucleoside comprising 2'-H(H) deoxyribosyl sugar moiety, as found in naturally occurring deoxyribonucleic acids (DNA). In certain embodiments, a 2'-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (uracil).

As used herein, "2'-substituted nucleoside" or "2'-modified nucleoside" means a nucleoside comprising a 2'-substituted or 2'-modified ribosyl sugar moiety. As used herein, "2'-substituted" or "2-modified" in reference to a sugar moiety means a ribosyl sugar moiety comprising at least one 2'-substituent group other than H or OH.

As used herein, "antisense activity" means any detectable and/or measurable change attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

As used herein, "antisense compound" means a compound comprising an antisense oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group.

As used herein, "antisense oligonucleotide" means an oligonucleotide having a nucleobase sequence that is complementary to a target nucleic acid.

As used herein, "ameliorate" refers to an improvement or lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. In certain embodiments, amelioration includes a decrease in severity and/or a delay or slowing in the progression of one or more symtpoms or indicators of a condition or disease. The severity or progression of symptoms or indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

As used herein, "animal" refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

As used herein, "bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar moiety. As used herein, "bicyclic sugar" or "bicyclic sugar moiety" means a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In certain embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety.

As used herein, "cEt" or "constrained ethyl" means a β-D ribosyl bicyclic sugar moiety wherein the second ring of the bicyclic sugar is formed via a bridge connecting the 4'-carbon and the 2'-carbon of the β-D ribosyl sugar moiety, wherein the bridge has the formula 4'-$CH(CH_3)$-O-2', and wherein the methyl group of the bridge is in the *S* configuration.

As used herein, "cleavable moiety" means a bond or group of atoms that is cleaved under physiological conditions, for example, inside a cell, an animal, and/or a human.

As used herein, "complementary" in reference to an oligonucleotide or region thereof means that at least 70% of the nucleobases of such oligonucleotide or region thereof and the nucleobases of another nucleic acid or one or more regions thereof are capable of hydrogen bonding with one another when the nucleobase sequences of the oligonucleotide and the other nucleic acid are aligned in opposing directions. Complementary nucleobases means nucleobases that are capable of forming hydrogen bonds with one another. Complementary nucleobase pairs include adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (^mC) and guanine (G). Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside. Rather, some mismatches are tolerated. In contrast, "fully complementary" or "100% complementary" in reference to an oligonucleotides means that such oligonucleotide is complementary to another nucleic acid at each nucleoside of the oligonucleotide.

As used herein, “conjugate group” means a group of atoms that is directly or indirectly attached to an oligonucleotide. Conjugate groups include a conjugate moiety and a conjugate linker that attaches the conjugate moiety to the oligonucleotide.

As used herein, “conjugate linker” means a group of atoms comprising at least one bond that connects a conjugate moiety to an oligonucleotide.

As used herein, “conjugate moiety” means a group of atoms that is attached to an oligonucleotide via a conjugate linker.

As used herein, "contiguous" in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, “contiguous nucleobases” means nucleobases that are immediately adjacent to each other in a sequence.

As used herein, “double-stranded antisense compound” means an antisense compound comprising two oligomeric compounds that are complementary to each other and form a duplex, and wherein one of the two said oligomeric compounds comprises an antisense oligonucleotide.

As used herein, “expression” means the formation of the structures into which a gene’s coded information is converted in a cell, including the products of transcription and translation.

As used herein, “gapmer” means an oligonucleotide comprising an internal region having a plurality of nucleosides that support RNase H cleavage positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from at least one of the nucleoside or nucleosides comprising each of the external regions. The internal region may be referred to as the “gap” and the external regions may be referred to as the “wings.”

As used herein, "hybridization" means the pairing or annealing of complementary oligonucleotides and/or nucleic acids. While not limited to a particular mechanism, the most common mechanism of hybridization involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases.

As used herein, "inhibiting the expression or activity" refers to a reduction or blockade of the expression or activity relative to the expression or activity in an untreated or control sample or relative to the expression or activity prior to the onset of inhibition. Such inhibition does not necessarily indicate a total elimination of expression or activity.

As used herein, the term “internucleoside linkage” means a group or bond that forms a covalent linkage between adjacent nucleosides in an oligonucleotide. As used herein “modified internucleoside linkage” means any internucleoside linkage other than a naturally occurring, phosphate internucleoside linkage. Non-phosphate linkages are referred to herein as modified internucleoside linkages. “Phosphorothioate linkage” means a modified phosphate linkage in which one of the non-bridging oxygen atoms is replaced with a sulfur atom. A phosphorothioate internucleoside linkage is a modified internucleoside linkage.

As used herein, "linker-nucleoside" means a nucleoside that links, either directly or indirectly, an oligonucleotide to a conjugate moiety. Linker-nucleosides are located within the conjugate linker of an oligomeric compound. Linker-nucleosides are not considered part of the oligonucleotide portion of an oligomeric compound even if they are contiguous with the oligonucleotide.

As used herein, "linked nucleosides" are nucleosides that are connected in a continuous sequence (*i.e.* no additional nucleosides are present between those that are linked). Linked nucleosides are linked together by internucleoside linkages.

As used herein, "lung cell" means any cell found within the lungs or the airways leading to and inside of the lungs. As described herein, lung cells include but are not limited to cells of the trachea, bronchi, bronchioles, and alveoli.

As used herein, "mismatch" means a nucleobase of a first oligonucleotide that is not complementary with the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligomeric compound are aligned.

As used herein, "modulating" refers to changing a feature in a cell, tissue, organ or organism. For example, modulating the Notch signaling pathway can mean increasing or decreasing the level of at least one member of the Notch signaling pathway in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, a compound can be a modulator of the Notch signaling pathway that decreases the amount of at least one Notch signaling pathway member transcript in a cell, tissue, organ or organism.

As used herein, "MOE" means methoxyethyl. "2'-MOE" means a 2'-$OCH_2CH_2OCH_3$ group in place of the 2'-OH group of a ribosyl sugar moiety.

As used herein, "motif" means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

As used herein, "naturally occurring" means found in nature.

As used herein, "non-bicyclic modified sugar" or "non-bicyclic modified sugar moiety" means a modified sugar moiety that comprises a modification, such as a substitutent, that does not form a bridge between two atoms of the sugar to form a second ring.

As used herein, "Notch signaling pathway" or "Notch signaling pathway members" means the Notch receptors, ligands of the Notch receptors, and intracellular proteins that transmit the Notch signal to or within the nucleus of a cell, as well as the nucleic acids encoding said Notch signaling pathway members. Notch signaling pathway members include the DNA sequences encoding Notch signaling pathway members and the RNA transcripts transcribed from said DNA sequences.

As used herein, "Notch signaling pathway inhibitor" refers to any agent that binds to a member of the Notch signaling pathway and is capable of inhibiting expression and/or activity of at least one member of the Notch signaling pathway.

As used herein, "nucleobase" means a naturally occurring nucleobase or a modified nucleobase. As used herein a “naturally occurring nucleobase” is adenine (A), thymine (T), cytosine (C), uracil (U), and guanine (G). As used herein, a modified nucleobase is a group of atoms capable of pairing with at least one naturally occurring nucleobase. A universal base is a nucleobase that can pair with any one of the five unmodified nucleobases. As used herein, “nucleobase sequence” means the order of contiguous nucleobases in a nucleic acid or oligonucleotide independent of any sugar or internucleoside linkage modification.

As used herein, “nucleoside” means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified. As used herein, “modified nucleoside” means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety.

As used herein, "oligomeric compound" means a compound consisting of an oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group.

As used herein, "oligonucleotide" means a strand of linked nucleosides connected via internucleoside linkages, wherein each nucleoside and internucleoside linkage may be modified or unmodified. Unless otherwise indicated, oligonucleotides consist of 8-80 linked nucleosides. As used herein, “modified oligonucleotide” means an oligonucleotide, wherein at least one nucleoside or internucleoside linkage is modified. As used herein, “unmodified oligonucleotide” means an oligonucleotide that does not comprise any nucleoside modifications or internucleoside modifications.

As used herein, “pharmaceutically acceptable carrier or diluent” means any substance suitable for use in administering a compound or composition to an animal. Certain such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject. In certain embodiments, a pharmaceutically acceptable carrier or diluent is sterile water; sterile saline; or sterile buffer solution.

As used herein “pharmaceutically acceptable carrier or diluent” means any substance suitable for use in administering to an individual. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution, such as PBS or water-for-injection.

As used herein “pharmaceutical composition” means a mixture of substances suitable for administering to a subject. For example, a pharmaceutical composition may comprise an antisense compound and a sterile aqueous solution.

As used herein, “phosphorus moiety” means a group of atoms comprising a phosphorus atom. In certain embodiments, a phosphorus moiety comprises a mono-, di-, or tri-phosphate, or phosphorothioate.

As used herein “prodrug” means a therapeutic agent in a form outside the body that is converted to a differentform within the body or cells thereof. Typically conversion of a prodrug within the body is facilitated by the action of an enzymes (e.g., endogenous or viral enzyme) or chemicals present in cells or tissues and/or by physiologic conditions.

As used herein, “RNAi compound” means an antisense compound that acts, at least in part, through RISC or Ago2 to modulate a target nucleic acid and/or protein encoded by a target nucleic acid. RNAi compounds include, but are not limited to double-stranded siRNA, single-stranded RNA (ssRNA), and microRNA, including microRNA mimics. In certain embodiments, an RNAi compound modulates the amount, activity, and/or splicing of a target nucleic acid. The term RNAi compound excludes antisense oligonucleotides that act through RNase H.

As used herein, the term “single-stranded” in reference to an antisense compound, oligomeric compound, or oligonucleotide means that the compound or oligonucleotide is not paired with a second compound or oligonucleotide to form a duplex. “Self-complementary” in reference to an oligonucleotide means an oligonucleotide that at least partially hybridizes to itself. A compound consisting of one oligomeric compound, wherein the oligonucleotide of the oligomeric compound is self-complementary, is a single-stranded compound.

As used herein, “sugar moiety” means an unmodified sugar moiety or a modified sugar moiety. As used herein, “unmodified sugar moiety” means a 2’-OH(H) ribosyl moiety, as found in RNA (an “unmodified RNA sugar moiety”), or a 2’-H(H) deoxyribosyl moiety, as found in DNA (an “unmodified DNA sugar moiety”).. As used herein, “modified sugar moiety” or “modified sugar” means a modified furanosyl sugar moiety or a sugar surrogate. As used herein, modified furanosyl sugar moiety means a furanosyl sugar comprising a non-hydrogen substituent in place of at least one hydrogen of an unmodified sugar moiety. In certain embodiments, a modified furanosyl sugar moiety is a 2’-substituted sugar moiety. Such modified furanosyl sugar moieties include bicyclic sugars and non-bicyclic sugars. As used herein, "sugar surrogate" means a modified sugar moiety having other than a furanosyl moiety that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group in an oligonucleotide. Modified nucleosides comprising sugar surrogates can be incorporated into one or more positions within an oligonucleotide and such oligonucleotides are capable of hybridizing to complementary oligomeric compounds or nucleic acids.

As used herein, “target nucleic acid,” “target RNA,” “target transcript” and “nucleic acid target” mean a nucleic acid that an antisense compound is designed to affect.

As used herein, “target region” means a portion of a target nucleic acid to which an antisense compound is complementary.

As used herein, "terminal group" means a chemical group or group of atoms that is covalently linked to a terminus of an oligonucleotide.

As used herein, “therapeutically effective amount” means an amount of a compound, pharmaceutical agent, or composition that provides a therapeutic benefit to an individual.

Certain Embodiments

Certain embodiments provide methods, compounds, and compositions for treating a respiratory disorder associated with excessive mucus production, or a symptom thereof, in an individual by administering the compound or composition to the individual, wherein the compound or composition comprises a Notch signaling pathway modulator. Modulation of one member of the Notch signaling pathway can lead to a decrease of the level or expression of one or more Notch signaling pathway members in order to treat, prevent, ameliorate or delay a respiratory disorder associated with excessive mucus production, or a symptom thereof. In certain embodiments, the Notch signaling pathway modulator is a compound comprising or consisting of a modified oligonucleotide complementary to a transcript encoding a member of the Notch signaling pathway. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the compound decreases expression or activity of one or more members of the Notch signaling pathway. In certain embodiments, the individual is human. In certain embodiments, no more than one compound comprising a Notch signaling pathway modulator is administered. In certain such embodiments, one compound comprising a Notch signaling pathway modulator is administered, and a second agent that does not comprise a Notch signaling pathway modulator is administered.

Certain embodiments disclosed herein provide compounds or compositions comprising a Notch signaling pathway modulator. Such compounds or compositions are useful to treat, prevent, ameliorate or delay a respiratory disorder associated with excessive mucus production, or a symptom thereof. In certain embodiments, the compound or composition comprises no more than one Notch signaling pathway inhibitor. In certain embodiments, the compound comprises a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the modified oligonucleotide is single-stranded. In certain embodiments, the modified oligonucleotide is part of a duplex. In certain such embodiments, the compound or composition comprises an antisense compound. In any of the foregoing embodiments, the compound or composition comprises an oligomeric compound. In certain embodiments, the compound comprises 2'-deoxyribonucleotides. In certain embodiments, the composition is double-stranded and comprises two oligomeric compounds that comprise ribonucleotides.

In any of the foregoing embodiments, the compound can comprise a modified oligonucleotide consisting of 8 to 80, 10 to 30, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked nucleosides.

In certain embodiments, at least one internucleoside linkage of said modified oligonucleotide is a modified internucleoside linkage. In certain embodiments, at least one internucleoside linkage is a

phosphorothioate internucleoside linkage. In certain embodiments, the internucleoside linkages are phosphorothioate linkages and phosphate linkages.

In certain embodiments, any of the foregoing oligonucleotides comprises at least one modified sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl ("2'-MOE") group. In certain embodiments, at least one modified sugar is a bicyclic sugar, such as a 4'-$CH(CH_3)$-O-2' ("cEt") group, a 4'-CH_2-O-2' ("LNA") group, or a 4'-$(CH_2)_2$-O-2'("ENA") group.

In certain embodiments, at least one nucleoside of said modified oligonucleotide comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, a compound or composition comprises a modified oligonucleotide comprising: a) a gap segment consisting of linked 2'-deoxynucleosides; b) a 5' wing segment consisting of linked nucleosides; and c) a 3' wing segment consisting of linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment. In certain embodiments, each nucleoside of each wing segment comprises a modified sugar moiety. In certain embodiments, the nucleosides immediately adjacent to the gap each comprise a modified sugar moiety, and at least one wing comprises an unmodified sugar moiety. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises a modified oligonucleotide 12 to 80 linked nucleosides in length and having a nucleobase sequence complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain embodiments, the compound is single-stranded. In certain embodiments, the compound is part of a double-stranded duplex. In certain embodiments, the modified oligonucleotide is 12 to 30 linked nucleosides in length.

In certain embodiments, the compounds or compositions disclosed herein comprise a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the compound or composition is co-administered with a second agent. In certain embodiments, the compound or composition and the second agent are administered concomitantly. In certain embodiments, the second agent is not an inhibitor of a Notch signaling pathway member.

In certain embodiments, compounds and compositions described herein targeting the Notch signaling pathway can be used in methods of inhibiting expression of the Notch signaling pathway in a cell. In certain embodiments, compounds and compositions described herein targeting the Notch signaling pathway can be used in methods of treating, preventing, delaying or ameliorating a respiratory disease or disorder associated with excessive mucus production, including, but not limited to, asthma, chronic obstructive pulmonary disorder (COPD), idiopathic pulmonary fibrosis (IPF), and cystic fibrosis (CF).

Certain Indications

Certain embodiments provided herein relate to methods of inhibiting the expression or activity of at least one member of Notch signaling pathway, which can be useful for treating, preventing, or ameliorating a disease or disorder associated with the Notch signaling pathway in an individual, by administration of one compound or composition that targets a member of the Notch signaling pathway. In certain embodiments, such a compound or composition comprises a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the compound comprises or consists of an antisense compound or an oligomeric compound targeted to the Notch signaling pathway.

In certain embodiments, a method of inhibiting expression or activity of at least one member of the Notch signaling pathway in a cell comprises contacting the cell with a compound or composition comprising a modified oligonucleotide complementary to a Notch signaling pathway member transcript, thereby inhibiting expression or activity of the Notch signaling pathway in the cell. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the cell is a lung cell. In certain embodiments, the cell is in the lung. In certain embodiments, the cell is in the lung of an individual who has, or is at risk of having a respiratory disease, disorder, condition, symptom, or physiological marker associated with excessive mucus production. In certain embodiments, the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF. In certain embodiments, the Notch signaling pathway inhibitor is an antisense compound or an oligomeric compound targeted to the Notch signaling pathway. In certain embodiments, the Notch signaling pathway inhibitor is an oligonucleotide complementary to a member of the Notch signaling pathway. In certain embodiments, the compound or composition comprises a modified oligonucleotide 8 to 80 linked nucleosides in length. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length. In certain embodiments, the compound comprising a modified oligonucleotide can be single-stranded. In certain embodiments, the compound comprising a modified oligonucleotide can be part of a double-stranded duplex.

In certain embodiments, a method of treating, preventing, delaying the onset, slowing the progression, or ameliorating one or more diseases, disorders, conditions, symptoms or physiological markers associated with the Notch signaling pathway comprises administering to the individual a compound or composition comprising a Notch signaling pathway inhibitor, wherein the Notch sinaling pathway inhibitor comprises a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In

certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, a method of treating, preventing, delaying the onset, slowing the progression, or ameliorating a respiratory disease, disorder, condition, symptom, or physiological marker associated with excessive mucus production in an individual comprises administering to the individual a compound or composition comprising one Notch signaling pathway inhibitor, thereby treating, preventing, delaying the onset, slowing the progression, or ameliorating the disease. In certain embodiments, the individual is identified as having, or at risk of having, the disease, disorder, condition, symptom or physiological marker. In certain embodiments, the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF. In certain embodiments, the Notch signaling pathway inhibitor is administered to the individual via inhalation. In certain embodiments, the individual is human. In certain embodiments, the Notch signaling pathway inhibitor is an antisense compound or an oligomeric compound comprising a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the modified oligonucleotide is 8 to 80 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 10 to 30 linked nucleosides in length. In certain embodiments, the modified oligonucleotide can be single-stranded. In certain embodiments, the modified oligonucleotide can be part of a double-stranded duplex.

In certain embodiments, a method of reducing, improving, or regulating trans-differentiation from club cells or goblet cells to ciliated cells, decreased mucus in the lungs, and increased lung function, or a combination thereof, in an individual comprises administering to the individual a compound or composition comprising one Notch signaling pathway inhibitor. In certain embodiments, administering the compound or composition reduces, improves, or regulates increased lung function in the individual. In certain embodiments, the individual is identified as having, or at risk of having a respiratory disease, disorder, condition, symptom, or physiological marker associated with excessive mucus production. In certain embodiments, the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF. In certain embodiments, the Notch signaling pathway inhibitor is administered to the individual via inhalation. In certain embodiments, the individual is human. In certain embodiments, the Notch signaling pathway inhibitor is an antisense compound or an oligomeric compound targeted to the Notch signaling pathway. In certain embodiments, the Notch signaling pathway inhibitor comprises or consists of a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the modified oligonucleotide is 8 to 80 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 10 to 30 linked nucleosides in length. In certain

embodiments, the compound comprising or consisting of the modified oligonucleotide can be single-stranded. In certain embodiments, the compound can be part of a duplex that is double-stranded.
In certain embodiments, lung function is increased by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45% or at least 50%.

Certain embodiments are drawn to compounds and compositions described herein for use in therapy. Certain embodiments are drawn to a compound or composition comprising a Notch signaling pathway inhibitor for use in treating, preventing, delaying the onset, slowing the progression, or ameliorating one or more diseases, disorders, conditions, symptoms or physiological markers associated with the Notch signaling pathway. Certain embodiments are drawn to a compound or composition for use in treating, preventing, delaying the onset, slowing the progression, or ameliorating a respiratory disorder associated with excessive mucus production, or a symptom or physiological marker thereof. In certain embodiments, the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF. In certain embodiments, the Notch signaling pathway inhibitor is an antisense compound or an oligomeric compound targeted to the Notch signaling pathway. In certain embodiments, the Notch signaling pathway inhibitor is a compound comprising or consisting of a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the compound or composition comprises a modified oligonucleotide 8 to 80 linked nucleosides in length. In certain embodiments, the compound or composition comprises a modified oligonucleotide 10 to 30 linked nucleosides in length. In certain embodiments, the compound comprising a modified oligonucleotide can be single-stranded. In certain embodiments, the compound comprising a modified oligonucleotide can be part of a double-stranded duplex.

Certain embodiments are drawn to a compound or composition comprising a Notch signaling pathway inhibitor for use in reducing, improving, or regulating trans-differentiation from club cells or goblet cells to ciliated cells, decreased mucus in the lungs, and increased lung function, or a combination thereof, in an individual. In certain embodiments, the compound or composition is provided for use in improving and/or increasing lung function in the individual. In certain embodiments, the individual is identified as having, or at risk of having a respiratory disease, disorder, condition, symptom, or physiological marker associated with excessive mucus production. In certain embodiments, the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF. In certain embodiments, the individual is human. In certain embodiments, the Notch signaling pathway inhibitor is an antisense compound or an oligomeric compound targeted to the Notch signaling pathway. In certain embodiments, the Notch signaling pathway inhibitor comprises or consists of a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch

signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the modified oligonucleotide is 8 to 80 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 10 to 30 linked nucleosides in length. In certain embodiments, the compound comprising or consisting of the modified oligonucleotide can be single-stranded. In certain embodiments, the compound comprising or consisting of the modified oligonucleotide can be part of a double-stranded duplex.

Certain embodiments are drawn to use of compounds or compositions described herein for the manufacture or preparation of a medicament for therapy. Certain embodiments are drawn to the use of one compound or composition as described herein in the manufacture or preparation of a medicament for treating, preventing, delaying the onset, slowing the progression, or ameliorating one or more diseases, disorders, conditions, symptoms or physiological markers associated with the Notch signaling pathway. In certain embodiments, a compound or composition as described herein is used in the manufacture or preparation of a medicament for treating, ameliorating, delaying or preventing a respiratory disorder associated with excessive mucus production, or a symptom or physiological marker thereof. In certain embodiments, the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF. In certain embodiments, the compound or composition comprises an antisense compound or an oligomeric compound targeted to the Notch signaling pathway. In certain embodiments, the compound or composition comprises or consists of a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the modified oligonucleotide is 8 to 80 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 10 to 30 linked nucleosides in length. In certain embodiments, the compound or composition comprising or consisting of the modified oligonucleotide can be single-stranded. In certain embodiments, the compound or composition comprising or consisting of the modified oligonucleotide can be part of a duplex that is double-stranded.

Certain embodiments are drawn to the use of a compound or composition for the manufacture or preparation of a medicament for reducing, improving, or regulating trans-differentiation from club cells or goblet cells to ciliated cells, decreased mucus in the lungs, and increased lung function, or a combination thereof, in an individual having or at risk of having a respiratory disorder associated with excessive mucus production. Certain embodiments are drawn to use of one compound or composition in the manufacture or preparation of a medicament for reducing, improving, or regulating increased lung function in the individual. In certain embodiments, the compound or composition comprises one antisense compound or an oligomeric compound targeted to the Notch signaling pathway. In certain embodiments, the compound or composition comprises or consists of a modified oligonucleotide complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the modified

oligonucleotide is 8 to 80 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 10 to 30 linked nucleosides in length. In certain embodiments, the compound or composition comprising the modified oligonucleotide can be single-stranded. In certain embodiments, the compound or composition comprising the modified oligonucleotide can be part of a duplex that is double-stranded.

In any of the foregoing methods or uses, the compound or composition can comprise an antisense compound targeted to the Notch signaling pathway. In certain embodiments, the compound comprises a modified oligonucleotide, for example a modified oligonucleotide consisting of 8 to 80 linked nucleosides, 10 to 30 linked nucleosides, 12 to 30 linked nucleosides, or 20 linked nucleosides. In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar and/or at least one modified nucleobase. In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage, the modified sugar is a bicyclic sugar or a 2'-O-methoxyethyl, and the modified nucleobase is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide comprises a gap segment consisting of linked 2'-deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein the nucleoside of each wing segment immediately adjacent to the gap segment comprises a modified sugar. In certain embodiments, the compound can comprise a modified oligonucleotide 12 to 80 linked nucleosides in length and having a nucleobase sequence complementary to a Notch signaling pathway member transcript. In certain embodiments, the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the Notch signaling pathway member transcript is a JAG1 transcript. In certain embodiments, the compound is an antisense compound or oligomeric compound. In certain embodiments, the compound is single-stranded. In certain embodiments, the compound is part of a duplex that is double-stranded. In certain embodiments, the modified oligonucleotide is 12 to 30 linked nucleosides in length. In certain embodiments, the compounds or compositions disclosed herein comprise a pharmaceutically acceptable carrier or diluent.

In any of the foregoing methods or uses, the compound or composition comprises or consists of a modified oligonucleotide 12 to 30 linked nucleosides in length, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked 2'-deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein the nucleoside of each wing segment that is immediately adjacent to the gap segment each comprises a modified sugar.

In any of the foregoing methods or uses, the compound or composition can be administered via inhalation, parenterally, or non-parenterally. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration. In certain embodiments, the administration is via inhalation. In certain embodiments, the compound or composition is co-administered with a second agent that is not a Notch signaling pathway modulator. In certain embodiments, the compound or composition and the second agent are administered concomitantly.

Certain Compounds

In certain embodiments, compounds described herein are antisense compounds. In certain embodiments, the antisense compound comprises or consists of an oligomeric compound. In certain embodiments, the oligomeric compound or antisense compound comprises a modified oligonucleotide. In certain embodiments, the modified oligonucleotide has a nucleobase sequence complementary to that of a target nucleic acid.

In certain embodiments, a compound described herein comprises or consists of a modified oligonucleotide. In certain embodiments, the modified oligonucleotide has a nucleobase sequence complementary to that of a target nucleic acid.

In certain embodiments, a compound or antisense compound is single-stranded. Such a single-stranded compound or antisense compound comprises or consists of an oligomeric compound. In certain embodiments, such an oligomeric compound comprises or consists of a modified oligonucleotide. In certain embodiments, the modified oligonucleotide is an antisense oligonucleotide.

In certain embodiments, antisense compounds are double-stranded. Such double-stranded compounds comprise a first oligomeric compound comprising or consisting of a modified oligonucleotide having a region complementary to a target nucleic acid and a second oligomeric compound comprising or consisting of a modified oligonucleotide having a region complementary to the first modified oligonucleotide. In certain embodiments, the modified oligonucleotide is an RNA oligonucleotide. In certain embodiments, the thymine nucleobase in the modified oligonucleotide is replaced by a uracil nucleobase. In certain embodiments, the compound comprises a conjugate group. In certain embodiments, each modified oligonucleotide is 12-30 linked nucleosides in length. The oligomeric compounds of double-stranded compounds may include non-complementary overhanging nucleosides.

Examples of single-stranded and double-stranded compounds include but are not limited to oligonucleotides, siRNAs, microRNA targeting oligonucleotides, and single-stranded RNAi compounds, such as small hairpin RNAs (shRNAs), single-stranded siRNAs (ssRNAs), and microRNA mimics.

In certain embodiments, a compound described herein has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is complementary.

In certain embodiments, a compounds described herein comprise a modified oligonucleotide 12 to 30 linked nucleosides in length. In certain embodiments, compounds described herein comprise a modified oligonucleotide 12 to 22 linked nucleosides in length. In certain embodiments, compounds described herein comprise a modified oligonucleotide 14 to 30 linked nucleosides in length. In certain embodiments, compounds described herein comprise a modified oligonucleotide 14 to 20 linked nucleosides in length. In certain embodiments, compounds described herein comprise a modified oligonucleotide 15 to 30 linked nucleosides in length. In certain embodiments, compounds described herein comprise a modified oligonucleotide 15 to 20 linked nucleosides in length. In certain embodiments, compounds described herein comprise a modified oligonucleotide 16 to 30 linked nucleosides in length. In certain embodiments, compounds described herein comprise a modified oligonucleotide 16 to 20 linked nucleosides in length. In other words, such modified oligonucleotides are from 12 to 30 linked nucleosides, 12 to 22 linked nucleosides, 14 to 30 linked nucleosides, 14 to 20 nucleosides, 15 to 30 nucleosides, 15 to 20 nucleosides, 16 to 30 nucleosides, or 16 to 20 nucleosides, respectively. In certain embodiments, a compound described herein comprises a modified oligonucleotide 16 linked nucleosides in length. In certain embodiments, a compound described herein comprises a modified oligonucleotide 17 linked nucleosides in length. In certain embodiments, compound described herein comprises a modified oligonucleotide 18 linked nucleosides in length. In certain embodiments, a compound described herein comprises a modified oligonucleotide 19 linked nucleosides in length. In certain embodiments, a compound described herein comprises a modified oligonucleotide 20 linked nucleosides in length. In other embodiments, a compound described herein comprises a modified oligonucleotide 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked nucleosides. In certain such embodiments, the compound described herein comprises a modified oligonucleotide 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked nucleosides in length, or a range defined by any two of the above values.

In certain embodiments, compounds described herein are interfering RNA compounds (RNAi), which include double-stranded RNA duplexes (also referred to as short-interfering RNA or siRNA) and single-stranded RNAi compounds (or ssRNA). Such compounds work at least in part through the RISC pathway to degrade and/or sequester a target nucleic acid (thus, include microRNA/microRNA-mimic compounds). As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as

used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics.

In certain embodiments, a double-stranded compound or duplex comprises a first oligomeric compound comprising the nucleobase sequence complementary to a target region of a Notch signaling pathway nucleic acid and a second oligomeric compound. In certain such embodiments, the double-stranded duplex comprises ribonucleotides in which the first strand has uracil (U) in place of thymine (T) and is complementary to a target region. In certain embodiments, the double-stranded duplex comprises one or more modified nucleosides comprising a 2'-F modified sugar moiety or 2'-O-alkyl modified sugar moiety (such as a methoxy group; 2'-OMe). In certain embodiments, the double-stranded duplex comprises at least one 2'-F sugar modification and at least one 2'-OMe sugar modification. In certain embodiments, the at least one 2'-F sugar modification and at least one 2'-OMe sugar modification are arranged in an alternating pattern for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases along an oligomeric compound of the duplex. In certain embodiments, the double-stranded duplex comprises one or more linkages between adjacent nucleosides other than a phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The double-stranded duplexes may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the duplex contains one or two capped oligomeric compounds, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000. In certain embodiments, the first oligomeric compound of the double-stranded duplex is an siRNA guide strand and the second oligomeric compound of the double-stranded duplex is an siRNA passenger strand. In certain embodiments, the second oligomeric compound of the double-stranded duplex is complementary to the first oligomeric compound. In certain embodiments, each oligomeric comound of the double-stranded duplex consists of 16, 17, 18, 19, 20, 21, 22, or 23 linked nucleosides. In certain embodiments, one oligomeric compound of the duplex comprises a conjugate group. In certain embodimenets, both oligomeric compounds of the duplex each comprise a conjugate group.

Further description of the compounds herein is provided below:

I. Certain Oligonucleotides

In certain embodiments, compounds described herein comprise oligonucleotides consisting of linked nucleosides. Oligonucleotides may be unmodified oligonucleotides (RNA or DNA) or may be modified oligonucleotides. Modified oligonucleotides comprise at least one modification relative to unmodified RNA or DNA (i.e., comprise at least one modified nucleoside (comprising a modified sugar moiety and/or a modified nucleobase) and/or at least one modified internucleoside linkage).

A. Certain Modified Nucleosides

Modified nucleosides comprise a modified sugar moiety or a modified nucleobase or both a modifed sugar moiety and a modified nucleobase.

1. Certain Sugar Moieties

In certain embodiments, modified sugar moieties are non-bicyclic modified sugar moieties. In certain embodiments, modified sugar moieties are bicyclic or tricyclic sugar moieties. In certain embodiments, modified sugar moieties are sugar surrogates. Such sugar surrogates may comprise one or more substitutions corresponding to those of other types of modified sugar moieties.

In certain embodiments, modified sugar moieties are non-bicyclic modified furanosyl sugar moieties comprising one or more acyclic substituent, including but not limited to substituents at the 2', 4', and/or 5' positions. In certain embodiments, the furanosyl sugar moiety is a ribosyl sugar moiety. In certain embodiments one or more acyclic substituent of non-bicyclic modified sugar moieties is branched. Examples of 2'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 2'-F, 2'-OCH_3 ("OMe" or "O-methyl"), and 2'-$O(CH_2)_2OCH_3$ ("MOE"). In certain embodiments, 2'-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF_3, OCF_3, O-C_1-C_{10} alkoxy, O-C_1-C_{10} substituted alkoxy, O-C_1-C_{10} alkyl, O-C_1-C_{10} substituted alkyl, S-alkyl, N(R_m)-alkyl, O-alkenyl, S-alkenyl, N(R_m)-alkenyl, O-alkynyl, S-alkynyl, N(R_m)-alkynyl, O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$ or $OCH_2C(=O)$-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl, and the 2'-substituent groups described in Cook et al., U.S. 6,531,584; Cook et al., U.S. 5,859,221; and Cook et al., U.S. 6,005,087. Certain embodiments of these 2'-substituent groups can be further substituted with one or more substituent groups independently selected from among: hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO_2), thiol, thioalkoxy, thioalkyl, halogen, alkyl, aryl, alkenyl and alkynyl. Examples of 4'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to alkoxy (*e.g.*, methoxy), alkyl, and those described in Manoharan et al., WO 2015/106128. Examples of 5'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 5'-methyl (R or S), 5'-vinyl, and 5'-methoxy. In certain embodiments, non-bicyclic modified sugars comprise more than one non-bridging sugar substituent, for example, 2'-F-5'-methyl sugar moieties and the modified sugar moieties and modified nucleosides described in Migawa et al., WO 2008/101157 and Rajeev et al., US2013/0203836.).

In certain embodiments, a 2'-substituted nucleoside or 2'- non-bicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, NH_2, N_3, OCF_3, OCH_3, $O(CH_2)_3NH_2$, $CH_2CH{=}CH_2$, $OCH_2CH{=}CH_2$, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and N-substituted acetamide ($OCH_2C(=O)$-$N(R_m)(R_n)$), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl.

In certain embodiments, a 2'-substituted nucleoside or 2'- non-bicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, OCF_3, OCH_3, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(CH_3)_2$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and $OCH_2C(=O)$-$N(H)CH_3$ ("NMA").

In certain embodiments, a 2'-substituted nucleoside or 2'- non-bicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, OCH_3, and $OCH_2CH_2OCH_3$.

Nucleosides comprising modified sugar moieties, such as non-bicyclic modified sugar moieties, may be referred to by the position(s) of the substitution(s) on the sugar moiety of the nucleoside. For example, nucleosides comprising 2'-substituted or 2-modified sugar moieties are referred to as 2'-substituted nucleosides or 2-modified nucleosides.

Certain modifed sugar moieties comprise a bridging sugar substituent that forms a second ring resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4' and the 2' furanose ring atoms. In certain such embodiments, the furanose ring is a ribose ring. Examples of such 4' to 2' bridging sugar substituents include but are not limited to: 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2' ("LNA"), 4'-CH_2-S-2', 4'-$(CH_2)_2$-O-2' ("ENA"), 4'-$CH(CH_3)$-O-2' (referred to as "constrained ethyl" or "cEt" when in the *S* configuration), 4'-CH_2-O-CH_2-2', 4'-CH_2-N(R)-2', 4'-$CH(CH_2OCH_3)$-O-2' ("constrained MOE" or "cMOE") and analogs thereof (*see, e.g.,* Seth et al., U.S. 7,399,845, Bhat et al., U.S. 7,569,686, Swayze et al., U.S. 7,741,457, and Swayze et al., U.S. 8,022,193), 4'-$C(CH_3)(CH_3)$-O-2' and analogs thereof (*see, e.g.,*Seth et al., U.S. 8,278,283), 4'-CH_2-N(OCH_3)-2' and analogs thereof (*see, e.g.,* Prakash et al., U.S. 8,278,425), 4'-CH_2-O-N(CH_3)-2' (*see, e.g.,* Allerson et al., U.S. 7,696,345 and Allerson et al., U.S. 8,124,745), 4'-CH_2-C(H)(CH_3)-2' (*see, e.g.,* Zhou, *et al., J. Org. Chem.,*2009, *74*, 118-134), 4'-CH_2-C(=CH_2)-2' and analogs thereof (*see e.g.*, Seth et al., U.S. 8,278,426), 4'-$C(R_aR_b)$-N(R)-O-2', 4'-$C(R_aR_b)$-O-N(R)-2', 4'-CH_2-O-N(R)-2', and 4'-CH_2-N(R)-O-2', wherein each R, R_a, and R_b is, independently, H, a protecting group, or C_1-C_{12} alkyl (*see, e.g.* Imanishi et al., U.S. 7,427,672).

In certain embodiments, such 4' to 2' bridges independently comprise from 1 to 4 linked groups independently selected from: -$[C(R_a)(R_b)]_n$-, -$[C(R_a)(R_b)]_n$-O-, -$C(R_a)$=$C(R_b)$-, -$C(R_a)$=N-, -C(=NR_a)-, -C(=O)-, -C(=S)-, -O-, -$Si(R_a)_2$-, -$S(=O)_x$-, and -$N(R_a)$-;

wherein:

x is 0, 1, or 2;

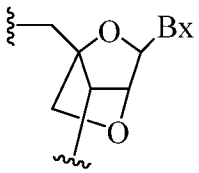
n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl (S(=O)-J_1); and

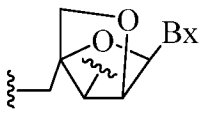
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl, or a protecting group.

Additional bicyclic sugar moieties are known in the art, see, for example: Freier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443, Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740, Singh et al., *Chem. Commun.*, 1998, *4*, 455-456; Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630; Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039; Srivastava et al., *J. Am. Chem. Soc.*, 20017, *129,* 8362-8379; Elayadi *et al.,*; Wengel et a., U.S. 7,053,207; Imanishi et al., U.S. 6,268,490; Imanishi et al. U.S. 6,770,748; Imanishi et al., U.S. RE44,779; Wengel et al., U.S. 6,794,499; Wengel et al., U.S. 6,670,461; Wengel et al., U.S. 7,034,133; Wengel et al., U.S. 8,080,644; Wengel et al., U.S. 8,034,909; Wengel et al., U.S. 8,153,365; Wengel et al., U.S. 7,572,582; and Ramasamy et al., U.S. 6,525,191;; Torsten et al., WO 2004/106356;Wengel et al., WO 1999/014226; Seth et al., WO 2007/134181; Seth et al., U.S. 7,547,684; Seth et al., U.S. 7,666,854; Seth et al., U.S. 8,088,746; Seth et al., U.S. 7,750,131; Seth et al., U.S. 8,030,467; Seth et al., U.S. 8,268,980; Seth et al., U.S. 8,546,556; Seth et al., U.S. 8,530,640; Migawa et al., U.S. 9,012,421; Seth et al., U.S. 8,501,805; and U.S. Patent Publication Nos. Allerson et al., US2008/0039618 and Migawa et al., US2015/0191727..

In certain embodiments, bicyclic sugar moieties and nucleosides incorporating such bicyclic sugar moieties are further defined by isomeric configuration. For example, an LNA nucleoside (described herein) may be in the α-L configuration or in the β-D configuration.



LNA (β-D-configuration)
bridge = 4'-CH_2-O-2'



α-*L*-LNA (α-*L*-configuration)
bridge = 4'-CH_2-O-2'

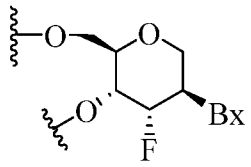
α-L-methyleneoxy (4'-CH_2-O-2') or α-L-LNA bicyclic nucleosides have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden et al., *Nucleic Acids Research,* 2003, *21*, 6365-6372). Herein, general descriptions of bicyclic nucleosides include both isomeric configurations. When the positions of specific bicyclic nucleosides (*e.g.*, LNA or cEt) are identified in exemplified embodiments herein, they are in the β-D configuration, unless otherwise specified.

In certain embodiments, modified sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (e.g., 5'-substituted and 4'-2' bridged sugars).

In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the sugar moiety is replaced, *e.g.,* with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moieties also comprise bridging and/or non-bridging substituents as described herein. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-position (*see*, *e.g.,* Bhat et al., U.S. 7,875,733 and Bhat et al., U.S. 7,939,677) and/or the 5' position.

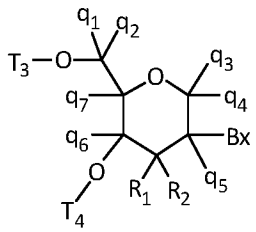
In certain embodiments, sugar surrogates comprise rings having other than 5 atoms. For example, in certain embodiments, a sugar surrogate comprises a six-membered tetrahydropyran ("THP"). Such

tetrahydropyrans may be further modified or substituted. Nucleosides comprising such modified tetrahydropyrans include but are not limited to hexitol nucleic acid ("HNA"), anitol nucleic acid ("ANA"), manitol nucleic acid ("MNA") (*see, e.g.,* Leumann, CJ. *Bioorg. & Med. Chem.* 2002, *10*, 841-854), fluoro HNA:



F-HNA

("F-HNA", *see e.g.* Swayze et al., U.S. 8,088,904; Swayze et al., U.S. 8,440,803; Swayze et al., U.S. 8,796,437; and Swayze et al., U.S. 9,005,906; F-HNA can also be referred to as a F-THP or 3'-fluoro tetrahydropyran), and nucleosides comprising additional modified THP compounds having the formula:



wherein, independently, for each of said modified THP nucleoside:

Bx is a nucleobase moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide or one of T_3 and T_4 is an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

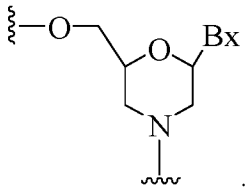
q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl; and

each of R_1 and R_2 is independently selected from among: hydrogen, halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, and CN, wherein X is O, S or NJ_1, and each J_1, J_2, and J_3 is, independently, H or C_1-C_6 alkyl.

In certain embodiments, modified THP nucleosides are provided wherein q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is other than H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is methyl. In certain embodiments, modified THP nucleosides are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is F and R_2 is H, in

certain embodiments, R_1 is methoxy and R_2 is H, and in certain embodiments, R_1 is methoxyethoxy and R_2 is H.

In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example, nucleosides comprising morpholino sugar moieties and their use in oligonucleotides have been reported (*see, e.g.,* Braasch et al., Biochemistry, 2002, *41*, 4503-4510 and Summerton et al., U.S. 5,698,685; Summerton et al., U.S. 5,166,315; Summerton et al., U.S. 5,185,444; and Summerton et al., U.S. 5,034,506). As used here, the term "morpholino" means a sugar surrogate having the following structure:



In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are refered to herein as "modifed morpholinos."

In certain embodiments, sugar surrogates comprise acyclic moieites. Examples of nucleosides and oligonucleotides comprising such acyclic sugar surrogates include but are not limited to: peptide nucleic acid ("PNA"), acyclic butyl nucleic acid (*see, e.g.,* Kumar et al., *Org. Biomol. Chem.*, 2013, *11*, 5853-5865), and nucleosides and oligonucleotides described in Manoharan et al., WO2011/133876.

Many other bicyclic and tricyclic sugar and sugar surrogate ring systems are known in the art that can be used in modified nucleosides).

2. Certain Modified Nucleobases

In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising an unmodified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising a modified nucleobase.

In certain embodiments, modified nucleobases are selected from: 5-substituted pyrimidines, 6-azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from: 2-aminopropyladenine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine, 6-N-methyladenine, 2-propyladenine , 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl (-C≡C-CH_3) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-

benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Further modified nucleobases include tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one and 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in Merigan et al., U.S. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch *et al.*, *Angewandte Chemie*, International Edition, 1991, *30*, 613; Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; and those disclosed in Chapters 6 and 15, *Antisense Drug Technology*, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

Publications that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, Manohara et al., US2003/0158403; Manoharan et al., US2003/0175906;; Dinh et al., U.S. 4,845,205; Spielvogel et al., U.S. 5,130,302; Rogers et al., U.S. 5,134,066; Bischofberger et al., U.S. 5,175,273; Urdea et al., U.S. 5,367,066; Benner et al., U.S. 5,432,272; Matteucci et al., U.S. 5,434,257; Gmeiner et al., U.S. 5,457,187; Cook et al., U.S. 5,459,255; Froehler et al., U.S. 5,484,908; Matteucci et al., U.S. 5,502,177; Hawkins et al., U.S. 5,525,711; Haralambidis et al., U.S. 5,552,540; Cook et al., U.S. 5,587,469; Froehler et al., U.S. 5,594,121; Switzer et al., U.S. 5,596,091; Cook et al., U.S. 5,614,617; Froehler et al., U.S. 5,645,985; Cook et al., U.S. 5,681,941; Cook et al., U.S. 5,811,534; Cook et al., U.S. 5,750,692; Cook et al., U.S. 5,948,903; Cook et al., U.S. 5,587,470; Cook et al., U.S. 5,457,191; Matteucci et al., U.S. 5,763,588; Froehler et al., U.S. 5,830,653; Cook et al., U.S. 5,808,027; Cook et al., 6,166,199; and Matteucci et al., U.S. 6,005,096.

B. Certain Modified Internucleoside Linkages

In certain embodiments, nucleosides of modified oligonucleotides may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus-containing internucleoside linkages include but are not limited to phosphates, which contain a phosphodiester bond ("P=O") (also referred to as unmodified or naturally occurring linkages), phosphotriesters, methylphosphonates, phosphoramidates, and phosphorothioates ("P=S"), and phosphorodithioates ("HS-P=S"). Representative non-phosphorus containing internucleoside linking groups include but are not limited to methylenemethylimino ($-CH_2-N(CH_3)-O-CH_2-$), thiodiester, thionocarbamate (-O-C(=O)(NH)-S-); siloxane ($-O-SiH_2-O-$); and N,N'-dimethylhydrazine ($-CH_2-N(CH_3)-N(CH_3)-$). Modified internucleoside linkages, compared to naturally occurring phosphate linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. In certain embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral internucleoside linkages include but are not limited to alkylphosphonates

and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

Neutral internucleoside linkages include, without limitation, phosphotriesters, methylphosphonates, MMI (3'-CH_2-N(CH_3)-O-5'), amide-3 (3'-CH_2-C(=O)-N(H)-5'), amide-4 (3'-CH_2-N(H)-C(=O)-5'), formacetal (3'-O-CH_2-O-5'), methoxypropyl, and thioformacetal (3'-S-CH_2-O-5'). Further neutral internucleoside linkages include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: *Carbohydrate Modifications in Antisense Research*; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580; Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S and CH_2 component parts.

C. Certain Motifs

In certain embodiments, modified oligonucleotides comprise one or more modified nucleoside comprising a modified sugar. In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more modified internucleoside linkage. In such embodiments, the modified, unmodified, and differently modified sugar moieties, nucleobases, and/or internucleoside linkages of a modified oligonucleotide define a pattern or motif. In certain embodiments, the patterns of sugar moieties, nucleobases, and internucleoside linkages are each independent of one another. Thus, a modified oligonucleotide may be described by its sugar motif, nucleobase motif and/or internucleoside linkage motif (as used herein, nucleobase motif describes the modifications to the nucleobases independent of the sequence of nucleobases).

1. Certain Sugar Motifs

In certain embodiments, oligonucleotides comprise one or more type of modified sugar and/or unmodified sugar moiety arranged along the oligonucleotide or region thereof in a defined pattern or sugar motif. In certain instances, such sugar motifs include but are not limited to any of the sugar modifications discussed herein.

In certain embodiments, modified oligonucleotides comprise or consist of a region having a gapmer motif, which comprises two external regions or "wings" and a central or internal region or "gap." The three regions of a gapmer motif (the 5'-wing, the gap, and the 3'-wing) form a contiguous sequence of nucleosides wherein at least some of the sugar moieties of the nucleosides of each of the wings differ from at least some of the sugar moieties of the nucleosides of the gap. Specifically, at least the sugar moieties of the nucleosides of each wing that are closest to the gap (the 3'-most nucleoside of the 5'-wing and the 5'-most nucleoside of the 3'-wing) differ from the sugar moiety of the neighboring gap nucleosides, thus defining the boundary between the wings and the gap (i.e., the wing/gap junction). In certain embodiments, the sugar moieties within the gap are the same as one another. In certain embodiments, the gap includes one or more nucleoside having a sugar moiety that differs from the sugar moiety of one or more other nucleosides of the gap. In certain embodiments, the sugar motifs of the two wings are the same as one another (symmetric gapmer). In

certain embodiments, the sugar motif of the 5'-wing differs from the sugar motif of the 3'-wing (asymmetric gapmer).

In certain embodiments, the wings of a gapmer comprise 1-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 2-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 3-5 nucleosides. In certain embodiments, the nucleosides of a gapmer are all modified nucleosides.

In certain embodiments, the gap of a gapmer comprises 7-12 nucleosides. In certain embodiments, the gap of a gapmer comprises 7-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 8-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 10 nucleosides. In certain embodiment, each nucleoside of the gap of a gapmer is an unmodified 2'-deoxy nucleoside.

In certain embodiments, the gapmer is a deoxy gapmer. In such embodiments, the nucleosides on the gap side of each wing/gap junction are unmodified 2'-deoxy nucleosides and the nucleosides on the wing sides of each wing/gap junction are modified nucleosides. In certain such embodiments, each nucleoside of the gap is an unmodified 2'-deoxy nucleoside. In certain such embodiments, each nucleoside of each wing is a modified nucleoside.

In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif. In such embodiments, each nucleoside of the fully modified region of the modified oligonucleotide comprises a modified sugar moiety. In certain such embodiments, each nucleoside to the entire modified oligonucleotide comprises a modified sugar moiety. In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif, wherein each nucleoside within the fully modified region comprises the same modified sugar moiety, referred to herein as a uniformly modified sugar motif. In certain embodiments, a fully modified oligonucleotide is a uniformly modified oligonucleotide. In certain embodiments, each nucleoside of a uniformly modified comprises the same 2'-modification.

2. Certain Nucleobase Motifs

In certain embodiments, oligonucleotides comprise modified and/or unmodified nucleobases arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, each nucleobase is modified. In certain embodiments, none of the nucleobases are modified. In certain embodiments, each purine or each pyrimidine is modified. In certain embodiments, each adenine is modified. In certain embodiments, each guanine is modified. In certain embodiments, each thymine is modified. In certain embodiments, each uracil is modified. In certain embodiments, each cytosine is modified. In certain embodiments, some or all of the cytosine nucleobases in a modified oligonucleotide are 5-methylcytosines.

In certain embodiments, modified oligonucleotides comprise a block of modified nucleobases. In certain such embodiments, the block is at the 3'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleosides of the 3'-end of the oligonucleotide. In certain embodiments, the block is at the 5'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleosides of the 5'-end of the oligonucleotide.

In certain embodiments, oligonucleotides having a gapmer motif comprise a nucleoside comprising a modified nucleobase. In certain such embodiments, one nucleoside comprising a modified nucleobase is in the central gap of an oligonucleotide having a gapmer motif. In certain such embodiments, the sugar moiety of said nucleoside is a 2'-deoxyribosyl moiety. In certain embodiments, the modified nucleobase is selected from: a 2-thiopyrimidine and a 5-propynepyrimidine.

3. Certain Internucleoside Linkage Motifs

In certain embodiments, oligonucleotides comprise modified and/or unmodified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, essentially each internucleoside linking group is a phosphate internucleoside linkage (P=O). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is a phosphorothioate (P=S). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is independently selected from a phosphorothioate and phosphate internucleoside linkage. In certain embodiments, the sugar motif of a modified oligonucleotide is a gapmer and the internucleoside linkages within the gap are all modified. In certain such embodiments, some or all of the internucleoside linkages in the wings are unmodified phosphate linkages. In certain embodiments, the terminal internucleoside linkages are modified.

D. Certain Lengths

In certain embodiments, oligonucleotides (including modified oligonucleotides) can have any of a variety of ranges of lengths. In certain embodiments, oligonucleotides consist of X to Y linked nucleosides, where X represents the fewest number of nucleosides in the range and Y represents the largest number nucleosides in the range. In certain such embodiments, X and Y are each independently selected from 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50; provided that $X \leq Y$. For example, in certain embodiments, oligonucleotides consist of 12 to 13, 12 to 14, 12 to 15, 12 to 16, 12 to 17, 12 to 18, 12 to 19, 12 to 20, 12 to 21, 12 to 22, 12 to 23, 12 to 24, 12 to 25, 12 to 26, 12 to 27, 12 to 28, 12 to 29, 12 to 30, 13 to 14, 13 to 15, 13 to 16, 13 to 17, 13 to 18, 13 to 19, 13 to 20, 13 to 21, 13 to 22, 13 to 23, 13 to 24, 13 to 25, 13 to 26, 13 to 27, 13 to 28, 13 to 29, 13 to 30, 14 to 15, 14 to 16, 14 to 17, 14 to 18, 14 to 19, 14 to 20, 14 to 21, 14 to 22, 14 to 23, 14 to 24, 14 to 25, 14 to 26, 14 to 27, 14 to 28, 14 to 29, 14 to 30, 15 to 16, 15 to 17, 15 to 18, 15 to 19, 15 to 20, 15 to 21, 15 to 22, 15 to 23, 15 to 24, 15 to 25, 15 to 26, 15 to 27, 15 to 28, 15 to 29, 15 to 30, 16 to 17, 16 to 18, 16 to 19, 16 to 20, 16 to 21, 16 to 22, 16 to 23, 16 to 24, 16 to 25, 16 to 26, 16 to 27, 16 to 28, 16 to 29, 16 to 30, 17 to 18, 17 to 19, 17 to 20, 17 to 21, 17 to 22, 17 to 23, 17 to 24, 17 to 25, 17 to 26, 17 to 27, 17 to 28, 17 to 29, 17 to 30, 18 to 19, 18 to 20, 18 to 21, 18 to 22, 18 to 23, 18 to 24, 18 to 25, 18 to 26, 18 to 27, 18 to 28, 18 to 29, 18 to 30, 19 to 20, 19 to 21, 19 to 22, 19 to 23, 19 to 24, 19 to 25, 19 to 26, 19 to 29, 19 to 28, 19 to 29, 19 to 30, 20 to 21, 20 to 22, 20 to 23, 20 to 24, 20 to 25, 20 to 26, 20 to 27, 20 to 28, 20 to 29, 20 to 30, 21 to 22, 21 to 23, 21 to 24, 21 to 25, 21 to 26, 21 to 27, 21 to 28, 21 to 29, 21 to 30, 22 to 23, 22 to 24, 22 to 25, 22 to 26, 22 to 27, 22 to 28, 22 to 29, 22 to 30, 23 to 24, 23 to 25, 23 to 26, 23 to

27, 23 to 28, 23 to 29, 23 to 30, 24 to 25, 24 to 26, 24 to 27, 24 to 28, 24 to 29, 24 to 30, 25 to 26, 25 to 27, 25 to 28, 25 to 29, 25 to 30, 26 to 27, 26 to 28, 26 to 29, 26 to 30, 27 to 28, 27 to 29, 27 to 30, 28 to 29, 28 to 30, or 29 to 30 linked nucleosides

E. Certain Modified Oligonucleotides

In certain embodiments, the above modifications (sugar, nucleobase, internucleoside linkage) are incorporated into a modified oligonucleotide. In certain embodiments, modified oligonucleotides are characterized by their modification motifs and overall lengths. In certain embodiments, such parameters are each independent of one another. Thus, unless otherwise indicated, each internucleoside linkage of an oligonucleotide having a gapmer sugar motif may be modified or unmodified and may or may not follow the gapmer modification pattern of the sugar modifications. For example, the internucleoside linkages within the wing regions of a sugar gapmer may be the same or different from one another and may be the same or different from the internucleoside linkages of the gap region of the sugar motif. Likewise, such sugar gapmer oligonucleotides may comprise one or more modified nucleobase independent of the gapmer pattern of the sugar modifications. Furthermore, in certain instances, an oligonucleotide is described by an overall length or range and by lengths or length ranges of two or more regions (e.g., a regions of nucleosides having specified sugar modifications), in such circumstances it may be possible to select numbers for each range that result in an oligonucleotide having an overall length falling outside the specified range. In such circumstances, both elements must be satisfied. For example, in certain embodiments, a modified oligonucleotide consists if of 15-20 linked nucleosides and has a sugar motif consisting of three regions, A, B, and C, wherein region A consists of 2-6 linked nucleosides having a specified sugar motif, region B consists of 6-10 linked nucleosides having a specified sugar motif, and region C consists of 2-6 linked nucleosides having a specified sugar motif. Such embodiments do not include modified oligonucleotides where A and C each consist of 6 linked nucleosides and B consists of 10 linked nucleosides (even though those numbers of nucleosides are permitted within the requirements for A, B, and C) because the overall length of such oligonucleotide is 22, which exceeds the upper limit of the overall length of the modified oligonucleotide (20). Herein, if a description of an oligonucleotide is silent with respect to one or more parameter, such parameter is not limited. Thus, a modified oligonucleotide described only as having a gapmer sugar motif without further description may have any length, internucleoside linkage motif, and nucleobase motif. Unless otherwise indicated, all modifications are independent of nucleobase sequence.

F. Nucleobase Sequence

In certain embodiments, oligonucleotides (unmodified or modified oligonucleotides) are further described by their nucleobase sequence. In certain embodiments oligonucleotides have a nucleobase sequence that is complementary to a second oligonucleotide or an identified reference nucleic acid, such as a target nucleic acid. In certain such embodiments, a region of an oligonucleotide has a nucleobase sequence that is complementary to a second oligonucleotide or an identified reference nucleic acid, such as a target nucleic acid. In certain embodiments, the nucleobase sequence of a region or entire length of an

oligonucleotide is at least 70%, at least 80%, at least 90%, at least 95%, or 100% complementary to the second oligonucleotide or nucleic acid, such as a target nucleic acid.

As an example, a compound in which 18 of 20 nucleobases of the compound are complementary to a target region would represent 90 percent complementarity to the target region. In this example, the remaining non-complementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. Percent complementarity of a compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

In certain embodiments, compounds described herein also include those which are complementary to a portion of a target nucleic acid. In certain embodiments, the compounds are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 15 nucleobase portion of a target segment. In certain embodiments, the compounds are complementary to at least a 16 nucleobase portion of a target segment. Also contemplated are compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

In certain embodiments, compounds herein comprise oligonucleotides that are complementary to the target nucleic acid over the entire length of the oligonucleotide. In certain embodiments, such oligonucleotides are 99% complementary to the target nucleic acid. In certain embodiments, such oligonucleotides are 95% complementary to the target nucleic acid. In certain embodiments, such oligonucleotides are 90% complementary to the target nucleic acid. In certain embodiments, such oligonucleotides are 85% complementary to the target nucleic acid. In certain embodiments, such oligonucleotides are 80% complementary to the target nucleic acid. In certain embodiments, antisense oligonucleotides are at least 80% complementary to the target nucleic acid over the entire length of the oligonucleotide and comprise a region that is 100% or fully complementary to a target nucleic acid. In certain

such embodiments, the region of full complementarity is from 6 to 20 nucleobases in length. In certain such embodiments, the region of full complementarity is from 10 to 18 nucleobases in length. In certain such embodiments, the region of full complementarity is from 18 to 20 nucleobases in length.

In certain embodiments, compounds comprising an oligonucleotide comprise one or more mismatched nucleobases relative to the target nucleic acid. In certain such embodiments, antisense activity against the target is reduced by such mismatch, but activity against a non-target is reduced by a greater amount. Thus, in certain such embodiments selectivity of the antisense compound is improved. In certain embodiments, the mismatch is specifically positioned within an oligonucleotide having a gapmer motif. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, or 8 from the 5'-end of the gap region. In certain such embodiments, the mismatch is at position 9, 8, 7, 6, 5, 4, 3, 2, 1 from the 3'-end of the gap region. In certain such embodiments, the mismatch is at position 1, 2, 3, or 4 from the 5'-end of the wing region. In certain such embodiments, the mismatch is at position 4, 3, 2, or 1 from the 3'-end of the wing region.

II. Certain Oligomeric Compounds

In certain embodiments, the invention provides oligomeric compounds, which consist of an oligonucleotide (modified or unmodified) and optionally one or more conjugate groups and/or terminal groups. Conjugate groups consist of one or more conjugate moiety and a conjugate linker which links the conjugate moiety to the oligonucleotide. Conjugate groups may be attached to either or both ends of an oligonucleotide and/or at any internal position. In certain embodiments, conjugate groups are attached to the 2'-position of a nucleoside of a modified oligonucleotide. In certain embodiments, conjugate groups that are attached to either or both ends of an oligonucleotide are terminal groups. In certain such embodiments, conjugate groups or terminal groups are attached at the 3' and/or 5'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached at the 3'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 3'-end of oligonucleotides. In certain embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 5'-end of oligonucleotides.

Examples of terminal groups include but are not limited to conjugate groups, capping groups, phosphate moieties, protecting groups, abasic nucleosides, modified or unmodified nucleosides, and two or more nucleosides that are independently modified or unmodified.

A. Certain Conjugate Groups

In certain embodiments, oligonucleotides are covalently attached to one or more conjugate groups. In certain embodiments, conjugate groups modify one or more properties of the attached oligonucleotide, including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, tissue distribution, cellular distribution, cellular uptake, charge and clearance. In certain embodiments, conjugate

groups impart a new property on the attached oligonucleotide, *e.g.*, fluorophores or reporter groups that enable detection of the oligonucleotide. Certain conjugate groups and conjugate moieties have been described previously, for example: cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, *4*, 1053-1060), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, *660*, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, *3*, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, *20*, 533-538), an aliphatic chain, *e.g.*, do-decan-diol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, *10*, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, *259*, 327-330; Svinarchuk et al., *Biochimie*, 1993, *75*, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, *36*, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, *18*, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, *14*, 969-973), or adamantane acetic acid a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, *1264,* 229-237), an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, *277*, 923-937), a tocopherol group (Nishina et al., *Molecular Therapy Nucleic Acids*, 2015, *4*, e220; and Nishina et al., *Molecular Therapy,* 2008, *16*, 734-740), or a GalNAc cluster (*e.g.*, WO2014/179620).

1. Conjugate Moieties

Conjugate moieties include, without limitation, intercalators, reporter molecules, polyamines, polyamides, peptides, carbohydrates (e.g., GalNAc), vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins, fluorophores, and dyes.

In certain embodiments, a conjugate moiety comprises an active drug substance, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fen-bufen, ketoprofen, (*S*)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, fingolimod, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

2. Conjugate linkers

Conjugate moieties are attached to oligonucleotides through conjugate linkers. In certain oligomeric compounds, the conjugate linker is a single chemical bond (i.e., the conjugate moiety is attached directly to an oligonucleotide through a single bond). In certain oligomeric compounds, a conjugate moiety is attached to an oligonucleotide via a more complex conjugate linker comprising one or more conjugate linker moieities, which are sub-units making up a conjugate linker. In certain embodiments, the conjugate linker comprises a chain structure, such as a hydrocarbyl chain, or an oligomer of repeating units such as ethylene glycol, nucleosides, or amino acid units.

In certain embodiments, a conjugate linker comprises one or more groups selected from alkyl, amino,

oxo, amide, disulfide, polyethylene glycol, ether, thioether, and hydroxylamino. In certain such embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and amide groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and ether groups. In certain embodiments, the conjugate linker comprises at least one phosphorus moiety. In certain embodiments, the conjugate linker comprises at least one phosphate group. In certain embodiments, the conjugate linker includes at least one neutral linking group.

In certain embodiments, conjugate linkers, including the conjugate linkers described above, are bifunctional linking moieties, *e.g.,* those known in the art to be useful for attaching conjugate groups to parent compounds, such as the oligonucleotides provided herein. In general, a bifunctional linking moiety comprises at least two functional groups. One of the functional groups is selected to bind to a particular site on a parent compound and the other is selected to bind to a conjugate group. Examples of functional groups used in a bifunctional linking moiety include but are not limited to electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In certain embodiments, bifunctional linking moieties comprise one or more groups selected from amino, hydroxyl, carboxylic acid, thiol, alkyl, alkenyl, and alkynyl.

Examples of conjugate linkers include but are not limited to pyrrolidine, 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEX or AHA). Other conjugate linkers include but are not limited to substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl or substituted or unsubstituted C_2-C_{10} alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

In certain embodiments, conjugate linkers comprise 1-10 linker-nucleosidesIn certain embodiments, such linker-nucleosides are modified nucleosides. In certain embodiments such linker-nucleosides comprise a modified sugar moiety. In certain embodiments, linker-nucleosides are unmodified. In certain embodiments, linker-nucleosides comprise an optionally protected heterocyclic base selected from a purine, substituted purine, pyrimidine or substituted pyrimidine. In certain embodiments, a cleavable moiety is a nucleoside selected from uracil, thymine, cytosine, 4-N-benzoylcytosine, 5-methylcytosine, 4-N-benzoyl-5-methylcytosine, adenine, 6-N-benzoyladenine, guanine and 2-N-isobutyrylguanine. It is typically desirable for linker-nucleosides to be cleaved from the oligomeric compound after it reaches a target tissue. Accordingly, linker-nucleosides are typically linked to one another and to the remainder of the oligomeric compound through cleavable bonds. In certain embodimements, such cleavable bonds are phosphodiester bonds.

Herein, linker-nucleosides are not considered to be part of the oligonucleotide. Accordingly, in embodiments in which an oligomeric compound comprises an oligonucleotide consisting of a specified number or range of linked nucleosides and/or a specified percent complementarity to a reference nucleic acid

and the oligomeric compound also comprises a conjugate group comprising a conjugate linker comprising linker-nucleosides, those linker-nucleosides are not counted toward the length of the oligonucleotide and are not used in determining the percent complementarity of the oligonucleotide for the reference nucleic acid. For example, an oligomeric compound may comprise (1) a modified oligonucleotide consisting of 8-30 nucleosides and (2) a conjugate group comprising 1-10 linker-nucleosides that are contiguous with the nucleosides of the modified oligonucleotide. The total number of contiguous linked nucleosides in such an oligomeric compound is more than 30. Alternatively, an oligomeric compound may comprise a modified oligonucleotide consisting of 8-30 nucleosides and no conjugate group. The total number of contiguous linked nucleosides in such an oligomeric compound is no more than 30. Unless otherwise indicated conjugate linkers comprise no more than 10 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 5 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 3 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 2 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 1 linker-nucleoside.

In certain embodiments, it is desirable for a conjugate group to be cleaved from the oligonucleotide. For example, in certain circumstances oligomeric compounds comprising a particular conjugate moiety are better taken up by a particular cell type, but once the oligomeric compound has been taken up, it is desirable that the conjugate group be cleaved to release the unconjugated or parent oligonucleotide. Thus, certain conjugate linkers may comprise one or more cleavable moieties. In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety is a group of atoms comprising at least one cleavable bond. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds. In certain embodiments, a cleavable moiety is selectively cleaved inside a cell or subcellular compartment, such as a lysosome. In certain embodiments, a cleavable moiety is selectively cleaved by endogenous enzymes, such as nucleases.

In certain embodiments, a cleavable bond is selected from among: an amide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, or a disulfide. In certain embodiments, a cleavable bond is one or both of the esters of a phosphodiester. In certain embodiments, a cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is a phosphate linkage between an oligonucleotide and a conjugate moiety or conjugate group.

In certain embodiments, a cleavable moiety comprises or consists of one or more linker-nucleosides. In certain such embodiments, the one or more linker-nucleosides are linked to one another and/or to the remainder of the oligomeric compound through cleavable bonds. In certain embodiments, such cleavable bonds are unmodified phosphodiester bonds. In certain embodiments, a cleavable moiety is 2'-deoxy nucleoside that is attached to either the 3' or 5'-terminal nucleoside of an oligonucleotide by a phosphate internucleoside linkage and covalently attached to the remainder of the conjugate linker or conjugate moiety by a phosphate or phosphorothioate linkage. In certain such embodiments, the cleavable moiety is 2'-deoxyadenosine.

III. Certain Antisense Compounds

In certain embodiments, the present invention provides antisense compounds, which comprise or consist of an oligomeric compound comprising an antisense oliognucleotide, having a nucleobase sequences complementary to that of a target nucleic acid. In certain embodiments, antisense compounds are single-stranded. Such single-stranded antisense compounds typically comprise or consist of an oligomeric compound that comprises or consists of a modified oligonucleotide and optionally a conjugate group. In certain embodiments, antisense compounds are double-stranded. Such double-stranded antisense compounds comprise a first oligomeric compound having a region complementary to a target nucleic acid and a second oligomeric compound having a region complementary to the first oligomeric compound. The first oligomeric compound of such double stranded antisense compounds typically comprises or consists of a modified oligonucleotide and optionally a conjugate group. The oligonucleotide of the second oligomeric compound of such double-stranded antisense compound may be modified or unmodified. Either or both oligomeric compounds of a double-stranded antisense compound may comprise a conjugate group. The oligomeric compounds of double-stranded antisense compounds may include non-complementary overhanging nucleosides.

In certain embodiments, oligomeric compounds of antisense compounds are capable of hybridizing to a target nucleic acid, resulting in at least one antisense activity. In certain embodiments, antisense compounds selectively affect one or more target nucleic acid. Such selective antisense compounds comprises a nucleobase sequence that hybridizes to one or more target nucleic acid, resulting in one or more desired antisense activity and does not hybridize to one or more non-target nucleic acid or does not hybridize to one or more non-target nucleic acid in such a way that results in significant undesired antisense activity.

In certain antisense activities, hybridization of an antisense compound to a target nucleic acid results in recruitment of a protein that cleaves the target nucleic acid. For example, certain antisense compounds result in RNase H mediated cleavage of the target nucleic acid. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. The DNA in such an RNA:DNA duplex need not be unmodified DNA. In certain embodiments, the invention provides antisense compounds that are sufficiently "DNA-like" to elicit RNase H activity. Further, in certain embodiments, one or more non-DNA-like nucleoside in the gap of a gapmer is tolerated.

In certain antisense activities, an antisense compound or a portion of an antisense compound is loaded into an RNA-induced silencing complex (RISC), ultimately resulting in cleavage of the target nucleic acid. For example, certain antisense compounds result in cleavage of the target nucleic acid by Argonaute. Antisense compounds that are loaded into RISC are RNAi compounds. RNAi compounds may be double-stranded (siRNA) or single-stranded (ssRNA).

In certain embodiments, hybridization of an antisense compound to a target nucleic acid does not result in recruitment of a protein that cleaves that target nucleic acid. In certain such embodiments,

hybridization of the antisense compound to the target nucleic acid results in alteration of splicing of the target nucleic acid. In certain embodiments, hybridization of an antisense compound to a target nucleic acid results in inhibition of a binding interaction between the target nucleic acid and a protein or other nucleic acid. In certain such embodiments, hybridization of an antisense compound to a target nucleic acid results in alteration of translation of the target nucleic acid.

Antisense activities may be observed directly or indirectly. In certain embodiments, observation or detection of an antisense activity involves observation or detection of a change in an amount of a target nucleic acid or protein encoded by such target nucleic acid, a change in the ratio of splice variants of a nucleic acid or protein, and/or a phenotypic change in a cell or animal.

IV. Target Nucleic Acids

In certain embodiments, compounds described herein comprise or consist of an oligonucleotide that is complementary to a target nucleic acid. In certain embodiments, the target nucleic acid is an endogenous RNA transcript. In certain such embodiments, the target transcript is selected from: an mRNA and a pre-mRNA, including intronic, exonic and untranslated regions. In certain embodiments, the target transcript is a mRNA. In certain embodiments, the target transcript is a pre-mRNA. In certain such embodiments, the target region of the target transcript is entirely within an intron. In certain embodiments, the target region spans an intron/exon junction. In certain embodiments, the target region is entirely within an exon. In certain embodiments, the target transcript is a Notch signaling pathway member transcript. In certain embodiments, the target transcript is a Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, DLL4, or Hes-1 transcript. In certain embodiments, the target transcript is a JAG1 transcript.

In certain embodiments, a compound comprising a Notch signaling pathway inhibitor inhibits the expression or activity of multiple members of the Notch signaling pathway but targets only one member of the Notch signaling pathway. In certain such embodiments, the Notch signaling pathway inhibitor is a modified oligonucleotide complementary to a Notch signaling pathway member. In such embodiments, the target nucleic acid or target transcript of the Notch signaling pathway inhibitor is the nucleic acid or transcript of the Notch signaling pathway to which the modified oligonucleotide has the greatest complementarity. In certain embodiments, the target Notch signaling pathway transcript is inhibited and at least one Notch signaling pathway member that is not the target transcript is also inhibited.

Nucleobase sequences of Notch signaling pathway member transcripts include, without limitation, SEQ ID Numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

Compositions and Methods for Formulating Pharmaceutical Compositions

Compounds described herein may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods

for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

In certain embodiments, the present invention provides pharmaceutical compositions comprising one or more compounds or a salt thereof. In certain embodiments, the compounds are antisense compounds or oligomeric compounds. In certain embodiments, the compounds comprise or consist of a modified oligonucleotide. In certain such embodiments, the pharmaceutical composition comprises a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises a sterile saline solution and one or more compound. In certain embodiments, such pharmaceutical composition consists of a sterile saline solution and one or more compound. In certain embodiments, the sterile saline is pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises one or more compound and sterile water. In certain embodiments, a pharmaceutical composition consists of one compound and sterile water. In certain embodiments, the sterile water is pharmaceutical grade water. In certain embodiments, a pharmaceutical composition comprises one or more compound and phosphate-buffered saline (PBS). In certain embodiments, a pharmaceutical composition consists of one or more compound and sterile PBS. In certain embodiments, the sterile PBS is pharmaceutical grade PBS. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

A compound described herein comprising a Notch signaling pathway member inhibitor can be utilized in pharmaceutical compositions by combining the compound with a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutically acceptable diluent is water, such as sterile water suitable for injection. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising a Notch signaling pathway member inhibitor and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is water. In certain embodiments, the compound comprises or consists of a modified oligonucleotide provided herein.

Certain embodiments provide pharmaceutical compositions suitable for aerosolization and/or dispersal by a nebulizer or inhaler. Such devices are well known in the art. In certain such embodiments, the pharmaceutical composition is a solid comprising particles of compounds that are of respirable size. A solid particulate composition can optionally contain a dispersant which serves to facilitate the formation of an aerosol, *e.g.*, lactose. Solid pharmaceutical compositions comprising a modified oligonucleotide can also be aerosolized using any solid particulate medicament aerosol generator known in the art, *e.g.*, a dry powder inhaler. In certain embodiments, the powder employed in the inhaler consists of the compound comprising the active compound or of a powder blend comprising the active compound, a suitable powder diluent, and an optional surfactant.

In certain embodiments, the pharmaceutical composition is a liquid. In certain such embodiments, the liquid is administered as an aerosol that is produced by any suitable means, such as with a nebulizer or

inhaler. See, *e.g.*, U.S. Pat. No. 4,501,729. Nebulizers are devices that transform solutions or suspensions into an aerosol mist and are well known in the art. Suitable nebulizers include jet nebulizers, ultrasonic nebulizers, electronic mesh nebulizers, and vibrating mesh nebulizers. Companies such as PARI and Vectura sell some types of such suitable nebulziers. In certain embodiments, the aerosol is produced by a metered dose inhaler, which typically contains a suspension or solution formulation of the active compound in a liquefied propellant. Inhalers suitable for dispensing liquid aerosol also include certain inhalers sold by Respimat (See, *e.g.*, Anderson, *Int J Chron Obstruct Pulmon Dis.* 1, 251 (2006).) Pharmaceutical compositions suitable for aerosolization can comprise propellants, surfactants, co-solvents, dispersants, preservatives, and/or other additives or excipients.

A compound described herein complementary to a Notch signaling pathway member nucleic acid can be utilized in pharmaceutical compositions by combining the compound with a suitable pharmaceutically acceptable diluent or carrier and/or additional components such that the pharmaceutical composition is suitable for aerosolization by a nebulizer. In certain embodiments, a pharmaceutically acceptable diluent is phosphate buffered saline. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising a compound complementary to a Notch signaling pathway member nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is phosphate buffered saline. In certain embodiments, the compound comprises or consists of a modified oligonucleotide provided herein.

Pharmaceutical compositions comprising compounds provided herein encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an individual, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. In certain embodiments, the compounds are antisense compounds or oligomeric compounds. In certain embodiments, the compound comprises or consists of a modified oligonucleotide. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of a compound which are cleaved by endogenous nucleases within the body, to form the active compound.

In certain embodiments, the compounds or compositions further comprise a pharmaceutically acceptable carrier or diluent.

Certain Combinations and Combination Therapies

In certain embodiments, a first agent comprising the compound described herein is co-administered with one or more additional agents. In certain embodiments, such second agents are designed to treat the

same disease, disorder, or condition as the first agent described herein. In certain embodiments, such second agents are designed to treat a different disease, disorder, or condition as the first agent described herein. In certain embodiments, a first agent is designed to treat an undesired side effect of a second agent. In certain embodiments, second agents are co-administered with the first agent to treat an undesired effect of the first agent. In certain embodiments, such second agents are designed to treat an undesired side effect of one or more pharmaceutical compositions as described herein. In certain embodiments, second agents are co-administered with the first agent to produce a combinational effect. In certain embodiments, second agents are co-administered with the first agent to produce a synergistic effect. In certain embodiments, the co-administration of the first and second agents permits use of lower dosages than would be required to achieve a therapeutic or prophylactic effect if the agents were administered as independent therapy.

In certain embodiments, one or more compounds or compositions provided herein are co-administered with one or more secondary agents. In certain embodiments, a method of treating an individual suffering from a respiratory disorder associated with excessive mucus production comprises administering a compound or composition provided herein and and one or more secondary agents. In certain embodiments, one or more compounds or compositions provided herein and one or more secondary agents are administered at different times. In certain embodiments, one or more compounds or compositions provided herein and one or more secondary agents are prepared together in a single formulation. In certain embodiments, one or more compounds or compositions provided herein and one or more secondary agents are prepared separately.

Certain embodiments are directed to the use of a compound comprising a Notch signaling pathway inhibitor as described herein in combination with a secondary agent. Certain embodiments are directed to use of a compound comprising a Notch signaling pathway inhibitor as described herein and a secondary agent in the preparation or manufacture of a medicament for treating a respiratory disorder associated with excessive mucus production. In certain embodiments the respiratory disorder associated with excessive mucus production is selected from: asthma, COPD, IPF, and CF.

Certain embodiments are drawn to a combination comprising a compound comprising a Notch signaling pathway inhibitor as described herein and a secondary agent. In such embodiments, the secondary agent is not a Notch signaling pathway inhibitor. In certain embodiments, such a combination is useful for increasing trans-differentiation from club cells or goblet to ciliated cells, decreasing mucus in the lungs, or increasing lung function, or a combination thereof and/or treating a respiratory disorder associated with excessive mucus production. In certain embodiments the respiratory disorder associated with excessive mucus production is selected from: asthma, COPD, IPF, and CF.

In certain embodiments, the compound comprising a Notch signaling pathway inhibitor, as described herein and the secondary agent are used in combination treatment by administering the two agents simultaneously, separately or sequentially. In certain embodiments, the two agents are formulated as a fixed dose combination product. In other embodiments, the two agents are provided to the patient as separate units which can then either be taken simultaneously or serially (sequentially).

Nonlimiting disclosure and incorporation by reference

Each of the literature and patent publications listed herein is incorporated by reference in its entirety.

While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references, GenBank accession numbers, and the like recited in the present application is incorporated herein by reference in its entirety.

Although the sequence listing accompanying this filing identifies each sequence as either "RNA" or "DNA" as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH in place of one 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) in place of a uracil of RNA). Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligomeric compound having the nucleobase sequence "ATCGATCG" encompasses any oligomeric compounds having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG" and oligomeric compounds having other modified nucleobases, such as "AT^{m}CGAUCG," wherein ^{m}C indicates a cytosine base comprising a methyl group at the 5-position.

Certain compounds described herein (e.g., modified oligonucleotides) have one or more asymmetric center and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), as α or β, such as for sugar anomers, or as (D) or (L), such as for amino acids, etc. Compounds provided herein that are drawn or described as having certain stereoisomeric configurations include only the indicated compounds. Compounds provided herein that are drawn or described with undefined stereochemistry include all such possible isomers, including their racemic and optically pure forms. All tautomeric forms of the compounds provided herein are included unless otherwise indicated.

The compounds described herein include variations in which one or more atoms are replaced with a non-radioactive isotope or radioactive isotope of the indicated element. For example, compounds herein that comprise hydrogen atoms encompass all possible deuterium substitutions for each of the ^{1}H hydrogen atoms. Isotopic substitutions encompassed by the compounds herein include but are not limited to: ^{2}H or ^{3}H in place

of ^{1}H, ^{13}C or ^{14}C in place of ^{12}C, ^{15}N in place of ^{14}N, ^{17}O or ^{18}O in place of ^{16}O, and ^{33}S, ^{34}S, ^{35}S, or ^{36}S in place of ^{32}S. In certain embodiments, non-radioactive isotopic substitutions may impart new properties on the oligomeric compound that are beneficial for use as a therapeutic or research tool. In certain embodiments, radioactive isotopic substitutions may make the compound suitable for research or diagnostic purposes such as imaging.

EXAMPLES

Example 1: Inhibition of the Notch signaling pathway by modified oligonucleotides complementary to JAG1

Modified oligonucleotides 100% complementary to mouse JAG1 were tested at various doses in HEPA1-6 (mouse hepatoma) cells. The cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.22 μM, 0.66 μM, 2 μM, or 6 μM modified oligonucleotide, as specified in the tables below. After a treatment period of approximately 24 hours, total RNA was isolated from the cells and JAG1 mRNA levels were measured by RT-qPCR. Mouse JAG1 primer probe set RTS35952 (Forward sequence: ACCGTAATCGCATCGTACTG (SEQ ID No: 13) Reverse sequence: TGCTATCAGGTTGAATAGTGTCA (SEQ ID No: 14) Probe sequence: CCTGGCCGAGGTCCTACACTTTG (SEQ ID No: 15) was used to measure mRNA levels. JAG1 mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN. Results are presented as the percent level of JAG1 mRNA, relative to that of the untreated control cells. As illustrated in the tables below, JAG1 mRNA transcript levels were reduced in a dose-dependent manner in cells treated with modified oligonucleotides complementary to JAG1.

The modified oligonucleotides in the tables below are gapmers, wherein the central gap segment consists of ten 2'-deoxynucleosides linked via phosphorothioate internucleoside linkages, and each wing segment consists of three cEt nucleosides linked via phosphorothioate internucleoside linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. In the tables below, "start Site" indicates the 5'-most nucleoside to which the gapmer is complementary in the mouse nucleic acid target sequence. "Stop Site" indicates the 3'-most nucleoside to which the gapmer is complementary in the mouse nucleic acid target sequence. The modified oligonucleotides are 100% complementary to the pre-mRNA sequence of mouse JAG1 (the complement of GENBANK No. NC_000068.7 truncated from 137078001 to 13712000, herein referred to as SEQ ID No. 1) and/or the mRNA sequence of mouse JAG1 (NM_013822.5, hereinreferred to as SEQ ID No. 2). An entry of "N/A" in the table below indicates that the modified oligonucleotide is not 100% complementary to the corresponding nucleic acid.

Table 1: Modified oligonucleotides complementary to JAG1

Compound Number	Sequence	SEQ ID 1 start site	SEQ ID 1 stop site	SEQ ID 2 start site	SEQ ID 2 stop site	SEQ ID NO.
897215	AAGTATCACTCTCCCC	34671	34686	2861	2876	37
897272	GGCACATTCACTAGTT	37184	37199	4127	4142	38
897316	GTAATGAGATTCAACC	38094	38109	5037	5052	39
897317	GTAAGATTGGGATGCT	38139	38154	5082	5097	40
897319	CAGCATTACATAACGA	38183	38198	5126	5141	41
897353	GCAATATAGGGCTCGG	4635	4650	N/A	N/A	42
897363	ATGTACTTGGCCCAGC	6521	6536	N/A	N/A	43
897367	GCGAATGAAGCTGTGC	6883	6898	N/A	N/A	44
897368	GCTTATGTGGCTATGA	7133	7148	N/A	N/A	45
897372	GCGATACTGAGATGGC	7390	7405	N/A	N/A	46
897375	GTGTGACACGGGTTCA	7919	7934	N/A	N/A	47
897376	CAGCATAATCATACCC	8019	8034	N/A	N/A	48
897382	GGATTACCAAGCTGGC	8854	8869	N/A	N/A	49
897386	AGAATACCAGGGAGCC	9368	9383	N/A	N/A	50
897393	TGCATTGGAGTTCCAG	11088	11103	N/A	N/A	51
897417	CACAATGAGACAGCGC	14223	14238	N/A	N/A	52
897426	AGTTTTTGCAAATAGA	15634	15649	N/A	N/A	53
897427	GAGTTTTTGCAAATAG	15635	15650	N/A	N/A	54
897439	TGTGATCCGTATCCTT	17410	17425	N/A	N/A	55
897454	CAGTATTGTCCCTGGA	20564	20579	N/A	N/A	56
897498	CTGTTCAAGCAATGAC	28083	28098	N/A	N/A	57
897505	TGTCATGTGTCAAGCA	28105	28120	N/A	N/A	58
897506	CCAGACTAGCGGTTCC	28243	28258	N/A	N/A	59
897530	TGGACAATGGCTTGGC	33218	33233	N/A	N/A	60
897533	ACCACAACAGTTCTGA	33811	33826	N/A	N/A	61

Table 2: Dose Response

Compound Number	JAG1 mRNA (% control)			
	222 nM	666 nM	2,000 nM	6,000 nM
897272	92	66	37	19
897316	77	52	25	12

897353	72	52	20	4
897363	77	63	32	6
897375	69	41	15	4
897376	81	79	30	7
897382	79	50	16	5
897393	84	50	27	6
897417	92	74	41	11
897454	103	66	38	9
897533	88	74	36	11

Table 3: Dose Response

Compound Number	JAG1 mRNA (% control)			
	222 nM	666 nM	2,000 nM	6,000 nM
897215	83	60	40	12
897317	66	45	22	17
897319	79	69	28	15
897367	81	45	18	5
897368	64	54	19	5
897372	45	16	4	3
897386	67	49	24	12
897426	98	81	52	19
897427	83	48	19	7
897439	79	57	26	8
897498	90	72	40	17
897505	64	45	18	8
897506	79	55	27	17
897530	82	69	27	9

Example 2: Inhibition of the Notch signaling pathway by modified oligonucleotides complementary to Notch1

Modified oligonucleotides 100% complementary to mouse Notch1 were tested at various doses in b.END cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.37 μM, 1.1 μM, 3.3 μM, or 10 μM modified oligonucleotide, as specified in the tables below. After a treatment period of approximately 24 hours, total RNA was isolated from the cells and Notch1 mRNA levels were measured by RT-qPCR. Mouse Notch1 primer probe set RTS1458 (Forward sequence: CGTGGTCTTCAAGCGTGATG (SEQ ID No: 16) Reverse sequence: GGTGCTTGCGCAGCTCTT (SEQ ID No: 17) Probe sequence: CCAGCAGATGATCTTCCCGTACTATG (SEQ ID No: 18) was used to measure Notch1 mRNA levels. The resulting Notch1 mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN. Results are presented as the percent level of Notch1 mRNA

transcript, relative to that of the untreated control cells. As illustrated in the tables below, Notch1 mRNA levels were reduced in a dose-dependent manner in cells treated with modified oligonucleotides complementary to Notch1.

The modified oligonucleotides in the tables below are cEt gapmers (as described in Example 1). The nucleobase sequences of the modified oligonucleotides, shown in the tables below, are 100% complementary to mouse Notch1 pre-mRNA (the complement of GENBANK No. NW_000174.1_truncated from 3935000 to 3983000, hereinreferred to as SEQ ID No. 3) , and/or to mouse Notch1 mRNA (Genbank No. NM_008714.3, herein referred to as SEQ ID: 4). An entry of "N/A" in the table below indicates that the modified oligonucleotide is not 100% complementary to the corresponding nucleic acid.

Table 4: Modified oligonucleotides complementary to Notch1

Compound Number	Sequence	SEQ ID 3 start site	SEQ ID 3 stop site	SEQ ID 4 start site	SEQ ID 4 stop site	SEQ ID NO
784187	ACTCAAAGGGCAGGCA	20358	20373	727	742	62
784217	ATACACCTTCATAACC	25344	25359	1705	1720	63
784227	GTAGGAGTTGTCACGG	26792	26807	2124	2139	64
784255	CTCGCAGTGGATGCCA	32080	32095	3192	3207	65
784303	CTCAATCTGCGGTGGG	36410	36425	4587	4602	66
784421	CGATTTTGGAAAGAAG	45740	45755	8225	8240	67
784432	AAGTTGTCAGGAAGGG	46117	46132	8602	8617	68
784446	ACACTTGTTCCTTTAG	46549	46564	9034	9049	69
784448	CAAGGTCTGGGTCACA	46612	46627	9097	9112	70
784455	AACATCTTAGGATGCG	46817	46832	9302	9317	71
784496	CAAGACTGACAGTCCA	10006	10021	N/A	N/A	72
784511	GCAAGAAAGATCTCTC	15515	15530	N/A	N/A	73
784527	ATGTCAAGTCAACAAA	19786	19801	N/A	N/A	74
784563	CTTCATGTTTCCACAA	30213	30228	N/A	N/A	75
784585	GATCAATTCTCTCTCT	38985	39000	N/A	N/A	76
784596	GACAAAGGATTTAGGG	39041	39056	N/A	N/A	77
784600	CTGCGCTCGCATTGAG	39064	39079	N/A	N/A	78

Table 5: Dose response

Compound Number	Notch1 mRNA (% control)				IC_{50} (μM)
	370 nM	1111 nM	3333 nM	10,000 nM	
784511	62	44	23	14	0.7
784600	92	76	51	29	3.6
784187	84	66	49	40	4.0
784455	68	46	38	34	1.3
784421	75	50	40	20	1.5
784227	79	58	46	25	2.2
784563	67	55	34	22	1.3
784527	78	61	49	17	2.1
784217	76	62	42	24	2.1
784432	63	56	38	27	1.4
784446	61	48	30	27	0.9

Table 6: Dose response

Compound Number	Notch 1 mRNA (% control)				IC_{50} (μM)
	370 nM	1111 nM	3333 nM	10,000 nM	
784255	114	92	82	46	>10
784585	103	87	71	36	6.5
784303	100	83	64	45	7.5
784448	88	55	44	27	2.3
784496	65	50	38	16	1.1

Example 3: Inhibition of the Notch signaling pathway by modified oligonucleotides complementary to Notch2

Modified oligonucleotides 100% complementary to mouse Notch2 were tested at various doses in HEPA1-6 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.33 μM, 1.0 μM, 3.0 μM, or 9.0 μM modified oligonucleotide, as specified in the tables below. After a treatment period of approximately 24 hours, total RNA was isolated from the cells and Notch2 mRNA levels were measured by RT-qPCR. Mouse Notch2 primer probe set RTS36985 (Forward sequence: CGACTTCACTTTCGAATGCAAC (SEQ ID No: 19) Reverse sequence: CACCATCCACACAAACTCCT (SEQ ID No: 20) Probe sequence: AATATCGACGACTGCCCCAACCAC (SEQ ID No: 21) was used to

measure Notch2 mRNA levels. The resulting Notch2 mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN. Results are presented as the percent level of Notch2 mRNA transcript, relative to that of the untreated control cells. As illustrated in the tables below, Notch2 mRNA levels were reduced in a dose-dependent manner in cells treated with modified oligonucleotides complementary to Notch2.

The modified oligonucleotides in the tables below are cEt gapmers (as described in Example 1). The nucleobase sequences of the modified oligonucleotides, shown in the tables below, are 100% complementary to mouse Notch2 pre-mRNA (GENBANK No. NC_000069.6 truncated from 98011001 to 98153000, SEQ ID No. 5) and/or to Notch2 mRNA (GENBANK No. NM_010928.2, SEQ ID No 6). An entry of "N/A" in the table below indicates that the modified oligonucleotide is not 100% complementary to the corresponding nucleic acid.

Table 7: Modified oligonucleotides complementary to Notch2

Compound Number	Sequence	SEQ ID 5 start site	SEQ ID 5 stop site	SEQ ID 6 start site	SEQ ID 6 stop site	SEQ ID No.
977277	GGACGCAGAGCGGGCA	2700	2715	163	178	79
977296	GTCTGAATGACACTCG	89309	89324	1535	1550	80
977302	GTCGATCCCATCCTGG	93419	93434	1913	1928	81
977306	GCGATTGATGCCGTCC	96111	96126	2141	2156	82
977313	CATACACGGCTTGGAG	106227	106242	2795	2810	83
977338	TGTATTCCCAGCAGCG	124731	124746	4572	4587	84
977359	GGTTACACGGTTGCGG	133370	133385	5954	5969	85
977375	GGGCAACTGGACTGCG	135999	136014	7138	7153	86
977376	TGGTACATAGAGGGCA	136035	136050	7174	7189	87
977380	AGGTATGGGTGCTCGC	136257	136272	7396	7411	88
977406	CAGGAAGCAGGTTCGG	137854	137869	8993	9008	89
977408	GACTGATGGCATGGCC	137980	137995	9119	9134	90
977413	GGTTACTGTTCGCAGG	138422	138437	9561	9576	91
977428	ACAAGACATAGCCCCA	3623	3638	N/A	N/A	92
		3664	3679	N/A	N/A	
		3705	3720	N/A	N/A	
		3746	3761	N/A	N/A	
		3787	3802	N/A	N/A	
977429	TACAAGACATAGCCCC	3624	3639	N/A	N/A	93
		3665	3680	N/A	N/A	
		3706	3721	N/A	N/A	

		3747	3762	N/A	N/A	
		3788	3803	N/A	N/A	
977430	GTACAAGACATAGCCC	3625	3640	N/A	N/A	94
		3666	3681	N/A	N/A	
		3707	3722	N/A	N/A	
		3748	3763	N/A	N/A	
		3789	3804	N/A	N/A	
977431	AGTACAAGACATAGCC	3626	3641	N/A	N/A	95
		3667	3682	N/A	N/A	
		3708	3723	N/A	N/A	
		3749	3764	N/A	N/A	
		3790	3805	N/A	N/A	
977454	TGAGTCTAGTCATGCA	22842	22857	N/A	N/A	96
977472	GTTATATAATCTTCCA	37896	37911	N/A	N/A	97
977474	TGCAAGATTGCACAGG	40230	40245	N/A	N/A	98
977499	TAATATAGGTGACAGC	63604	63619	N/A	N/A	99
977500	GATAATATAGGTGACA	63606	63621	N/A	N/A	100
977515	TCAGTATGCCTCTTGC	70718	70733	N/A	N/A	101
977525	GTGTCTCACCCCAGGG	86267	86282	N/A	N/A	102
977526	AGTGTCTCACCCCAGG	86268	86283	N/A	N/A	103
977539	ATAGTTGTCACACAGT	98757	98772	N/A	N/A	104
977545	AGCGATATTAAATGGC	114166	114181	N/A	N/A	105
977557	GGTGTGCTGAATGCTA	121156	121171	N/A	N/A	106
977568	GCTACTGCGGTCACTG	121110	121125	N/A	N/A	107
977569	TGCTACTGCGGTCACT	121111	121126	N/A	N/A	108
977571	AATGCTACTGCGGTCA	121113	121128	N/A	N/A	109
977572	GAATGCTACTGCGGTC	121114	121129	N/A	N/A	110
977574	CTGAATGCTACTGCGG	121116	121131	N/A	N/A	111
977575	GCTGAATGCTACTGCG	121117	121132	N/A	N/A	112
977581	GCACATAAATTACTGG	130943	130958	N/A	N/A	113

Table 8: Dose response

Compound Number	Notch2 mRNA (% control)				IC_{50} (μM)
	333 nM	1000 nM	3000 nM	9000 nM	
977499	40	16	6	4	0.1

977431	83	52	16	5	1.1
977515	92	58	13	8	1.3
977375	71	34	15	8	0.7
977571	112	49	20	15	1.7
977539	105	63	28	6	1.8
977575	100	76	29	17	2.1
977359	55	33	20	15	0.4
977472	51	27	5	4	0.3
977428	77	41	15	2	0.8
977500	84	43	17	3	1.0
977572	74	48	18	10	0.9
977296	99	69	38	9	2.0
977380	87	62	32	23	1.8
977408	89	59	34	28	2.0
977568	117	82	39	18	2.7
977376	112	75	44	17	2.6

Table 9: Notch2 Expression

Compound Number	Notch2 mRNA (% control)				IC50 (μM)
	333 nM	1000 nM	3000 nM	9000 nM	
977557	42	24	18	11	0.1
977525	63	36	23	20	0.6
977545	71	41	23	10	0.8
977413	76	51	24	17	1.1
977313	100	63	26	21	1.9
977569	123	80	44	18	2.9
977581	128	90	55	20	3.5
977429	101	59	21	7	1.6
977277	68	49	26	11	0.9
977574	46	24	14	8	0.2
977474	72	36	11	2	0.7
977526	79	44	14	n.d.	0.9
977454	85	52	13	3	1.1
977306	94	51	34	9	1.5
977406	80	59	28	18	1.4

977338	99	73	33	19	2.2
977430	118	81	20	12	2.1
977302	102	70	31	15	2.0

Example 4: Inhibition of the Notch signaling pathway by modified oligonucleotides complementary to Notch3

Modified oligonucleotides 100% complementary to mouse Notch3 were tested at various doses in C2C12 (mouse myoblast) cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.259 μM, 0.778 μM, 2.33 μM, and 7.0 μM modified oligonucleotide, as specified in the tables below. After a treatment period of approximately 24 hours, RNA was isolated from the cells and Notch3 mRNA levels were measured by RT-qPCR. Mouse Notch3 primer probe set RTS36974 (Forward sequence: CTTTGGAGTTTGCCGTGATG (SEQ ID No: 22) Reverse sequence: TCATTGATCTCCACGTTGCAG (SEQ ID No: 23) Probe sequence: ACCGTTATGACTGTGTCTGTCAGCC (SEQ ID No: 24)) was used to measure Notch3 mRNA levels. The resulting Notch3 mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN. Results are presented as the percent level of Notch3 mRNA transcript, relative to that of the untreated control cells. As illustrated in the tables below, Notch3 mRNA levels were reduced in a dose-dependent manner in cells treated with modified oligonucleotides complementary to Notch3.

The modified oligonucleotides in the tables below are cEt gapmers (as described in Example 1). The nucleobase sequences of the modified oligonucleotides, shown in the tables below, are 100% complementary to mouse Notch3 pre-mRNA (the complement of GENBANK No. NC_000083.6 truncated from 32118001 to 32170000, SEQ ID No. 7), and/or to mouse Notch3 mRNA NM_008716.2, SEQ ID 8). An entry of "N/A" in the table below indicates that the modified oligonucleotide is not 100% complementary to the corresponding nucleic acid.

Table 10: Modified oligonucleotides complementary to Notch3

Compound Number	Sequence	Start Site SEQ ID 7	Stop Site SEQ ID 7	Start Site SEQ ID 8	Stop Site SEQ ID 8	SEQ ID No.
976941	ATCTATGTCACTTTGG	11368	11383	527	542	114
976942	CCAGATCGGCACTCAT	11382	11397	541	556	115
976960	CTGCACAGCGACACTC	15669	15684	1650	1665	116
976963	TGCCATCGACACAGCG	16080	16095	1734	1749	117
976964	GGCACAAGCACACGAG	16106	16121	1760	1775	118
976977	CCGCAGGGTGAGGCAC	19907	19922	2509	2524	119
976989	GCACAGGCGGCCACTC	22962	22977	3146	3161	120

976990	TGTATGTCGCACAGGC	22970	22985	3154	3169	121
976992	TGTCTATGCACTTTCC	23442	23457	3237	3252	122
977008	CGCAGCGGAAATGCCC	25598	25613	3744	3759	123
977032	GTGTTCTCGCTTTCGC	30754	30769	5060	5075	124
977034	TCAAGTCTGTGACCAC	32135	32150	5211	5226	125
977057	CAGGATTGAGCAGACC	47705	47720	6540	6555	126
977081	GTCTTATCTGGAATGC	48817	48832	7652	7667	127
977103	AGCAAGATGATGCGGG	6033	6048	N/A	N/A	128
977107	TCACTCTGTGAGAGCC	6576	6591	N/A	N/A	129
977113	TCGAAGCTCAACCCTG	7861	7876	N/A	N/A	130
		7877	7892	N/A	N/A	
977114	GTCGAAGCTCAACCCT	7862	7877	N/A	N/A	131
		7878	7893	N/A	N/A	
977115	TGTCGAAGCTCAACCC	7863	7878	N/A	N/A	132
		7879	7894	N/A	N/A	
977116	TGCAACTATGCAATGA	8075	8090	N/A	N/A	133
977117	GTAGTCAAACAATCCT	8096	8111	N/A	N/A	134
977119	TCCTCTCATGGATCGG	8437	8452	N/A	N/A	135
977129	TCAGTATTATCTGTTA	12995	13010	N/A	N/A	136
977130	GAATATTGGTTCAGTA	13005	13020	N/A	N/A	137
977131	GGAATATTGGTTCAGT	13006	13021	N/A	N/A	138
977154	GTGATCTCACTGCCAG	20525	20540	N/A	N/A	139
977156	TGTAGTGCCACTGCCT	20616	20631	N/A	N/A	140
977170	ACAATTCTATGGTCTC	24812	24827	N/A	N/A	141
977191	CTACCTGTGTACCACA	32564	32579	N/A	N/A	142
		32967	32982	N/A	N/A	
977192	ACTACCTGTGTACCAC	32565	32580	N/A	N/A	143
		32968	32983	N/A	N/A	
977213	ACTTAGATGCTACCAG	38941	38956	N/A	N/A	144
977234	GCAACTCATGTCCACA	46126	46141	N/A	N/A	145

Table 11: Dose response

Compound Number	Notch3 mRNA (% control)				IC_{50} (μM)
	259 nM	778 nM	2333 nM	7000 nM	
977131	40	18	9	2	0.07
977119	41	13	19	4	0.05
977107	69	38	16	4	0.54
977103	72	40	9	5	0.57
976963	115	61	18	6	1.35
977115	79	50	16	8	0.80
977191	45	27	9	4	0.14
977156	45	15	3	1	0.10
976992	57	28	7	7	0.27
977032	36	12	13	4	0.03
977116	81	41	13	2	0.70
977192	65	33	12	4	0.42
977008	78	38	14	6	0.65
976964	63	34	9	11	0.39
976960	89	46	44	13	1.21

Table 12: Dose response

Compound Number	Notch3 mRNA (% control)				IC50(μM)
	259 nM	778 nM	2333 nM	7000 nM	
977117	35	14	6	5	0.02
977057	55	27	19	10	0.24
977129	30	14	4	1	0.02
976941	62	28	17	6	0.36
977113	61	31	12	7	0.35
976989	100	55	38	15	1.44
977213	98	54	36	10	1.32
977081	64	22	16	7	0.32
976977	58	53	26	16	0.57
977114	36	14	2	7	0.03
977170	46	22	6	3	0.13

977130	68	37	16	5	0.52
976990	41	18	7	4	0.07
976942	80	34	14	5	0.64
977034	71	46	15	8	0.65
977154	125	56	15	5	1.36
977234	79	59	18	10	0.92

Example 5: Inhibition of the Notch signaling pathway by modified oligonucleotides complementary to JAG2

Modified oligonucleotides 100% complementary to mouse JAG2 were tested at various doses in primary mouse embryonic cortical neuron. Cells were plated at a density of 60,000 cells per well and treated via free uptake with 0.313 μM, 1.25 μM, 5.0 μM, or 20.0 μM modified oligonucleotide, as specified in the tables below. After a treatment period of approximately 24 hours, total RNA was isolated from the cells and JAG2 mRNA levels were measured by RT-qPCR using primer probe set RTS35955 (Forward sequence: CTGACTGCCGTATCAACATTG (SEQ ID No: 25) Reverse sequence: GCCTCGTGAATATGACCACTT (SEQ ID No: 26) Probe sequence: CAGTCCTCGCCCTGTGCCTAC (SEQ ID No: 27)) was used to measure JAG2 mRNA levels. The resulting JAG2 mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN. Results are presented as the percent level of JAG2 mRNA transcript, relative to that of the untreated control cells. As illustrated in the tables below, JAG2 mRNA levels were reduced in a dose-dependent manner in cells treated with modified oligonucleotides complementary to JAG2.

The modified oligonucleotides in the tables below are cEt gapmers (as described in Example 1). The nucleobase sequences of the modified oligonucleotides, shown in the tables below, are 100% complementary to mouse JAG2 pre-mRNA (the complement of GENBANK No. NC_000078.6 truncated from 112905001 to 112933000, SEQ ID No. 9), and/or to mouse Jagged2 mRNA (GENBANK No. NM_010588.2, SEQ ID 10). An entry of "N/A" in the table below indicates that the modified oligonucleotide is not 100% complementary to the corresponding nucleic acid.

Table 13: Modified oligonucleotides complementary to JAG2

Compound Number	Sequence	Start Site SEQ ID 9	Stop Site SEQ ID 9	Start Site SEQ ID 10	Stop Site SEQ ID 10	SEQ ID No.
897605	TACCACCCGCTGCACA	17034	17049	1191	1206	146
897613	CTCTAGTTCGCAATGG	17680	17695	1490	1505	147
897614	CGTACTCTAGTTCGCA	17684	17699	1494	1509	148
897619	GTAGTAGTCACCCTCA	18553	18568	1673	1688	149

897621	TCTACATGCCCCGCCA	18628	18643	1748	1763	150
897622	TCGAACCCGCAGCCAT	18729	18744	1771	1786	151
897664	GTCCACCATACGCAGA	23676	23691	3319	3334	152
897695	CAGTACGCCAGCCCAG	24743	24758	N/A	N/A	153
897718	AGTAGTTCAGGTCTGG	16274	16289	N/A	N/A	154
897720	TGTTAGTGTCTCTTCC	4665	4680	N/A	N/A	155
897721	ACAATAAAACATCCGC	4718	4733	N/A	N/A	156
897724	CACCATAAGACTTCCT	4888	4903	N/A	N/A	157
897727	GCTTGATACCCCCCCT	5063	5078	N/A	N/A	158
897728	CTAACCAAAAGTCTCT	5171	5186	N/A	N/A	159
897742	AGAACTTAAGCAGGAG	6819	6834	N/A	N/A	160
897751	GTTACTCACAGCCTAG	7979	7994	N/A	N/A	161
897756	CGCTTCGGATGATCCA	8721	8736	N/A	N/A	162
897758	TTTATACTCGCTCAGC	8889	8904	N/A	N/A	163
897762	TGCCATCTAAATCCCC	9601	9616	N/A	N/A	164
897763	TATAAGTACTCTCTCT	9758	9773	N/A	N/A	165
897764	TCCTATCTGTTGGCAG	9957	9972	N/A	N/A	166
897765	AACTTATCCCACTGCC	10017	10032	N/A	N/A	167
897771	GATAATTATCCCTGGC	10701	10716	N/A	N/A	168
897775	GTATGAGCAGCTCTGC	11187	11202	N/A	N/A	169
897776	CACTTGAGGGTATCTC	11268	11283	N/A	N/A	170
897777	TACTAGCTTGGATCCT	11463	11478	N/A	N/A	171
897780	GAGAATAGCCAGAACT	11707	11722	N/A	N/A	172
897794	TCCTACTGTGTTCACC	13371	13386	N/A	N/A	173
897795	TGCAGAATCATGTCAG	13415	13430	N/A	N/A	174
897798	GACAATCATCCCTACC	13670	13685	N/A	N/A	175
897803	ACACATCACTAATGCC	14219	14234	N/A	N/A	176
897805	GTGGATGGACGATTTC	14434	14449	N/A	N/A	177
897813	GTAAGTAGGTGGCCAG	15425	15440	N/A	N/A	178
897833	AAGTTAAGCAGAACCC	19872	19887	N/A	N/A	179
897835	GTTGGAATGGGACCTA	20076	20091	N/A	N/A	180
897836	AGAAGTACGAGGAAGG	20133	20148	N/A	N/A	181
897862	GTTATAGCCACTGCCC	23214	23229	N/A	N/A	182

Table 14: Dose response

Compound Number	JAG2 mRNA (% control)				IC_{50} (μM)
	312.5 nM	1250.0 nM	5000.0 nM	20000.0 nM	
897836	28	10	8	6	< 0.3
897780	22	14	10	6	< 0.3
897728	65	40	21	7	0.74
897756	47	33	20	16	< 0.3
897720	85	50	20	6	1.50
897724	83	52	27	15	1.78
897764	97	77	43	21	4.17
897776	71	56	39	27	2.10
897664	72	47	32	15	1.33
897775	42	25	13	7	< 0.3
897803	52	33	12	5	< 0.3
897763	68	44	20	6	0.90
897751	72	47	20	12	1.08
897619	45	29	17	12	< 0.3
897835	75	44	19	8	1.10
897795	63	47	23	9	0.84
897727	84	57	36	18	2.33
897695	67	52	28	20	1.24
897771	92	68	41	20	3.38

Table 15: Dose response

Compound Number	JAG2 mRNA (% control)				IC50 (μM)
	312.5 nM	1250.0 nM	5000.0 nM	20000.0 nM	
897836	31	17	8	6	< 0.3
897721	53	41	22	5	0.46
897805	40	25	13	15	< 0.3
897605	51	38	23	15	0.32
897813	46	40	20	16	< 0.3
897777	61	48	29	14	0.91
897621	67	44	28	31	1.07

897765	85	75	37	16	2.97
897833	74	53	21	7	1.28
897613	77	40	19	11	1.06
897758	63	44	14	5	0.70
897798	63	41	15	9	0.67
897742	48	30	13	6	< 0.3
897862	67	50	19	9	1.00
897762	87	53	25	9	1.77
897794	94	36	23	11	1.55
897614	72	50	22	11	1.21
897622	73	58	26	18	1.63

Example 6: Inhibition of the Notch signaling pathway by modified oligonucleotides complementary to DLL4

Modified oligonucleotides 100% complementary to mouse DLL4 were tested at various doses in b.END1 cells. Compound 380876 was included as a comparison in all experiments. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.74 μM,2.2 μM, 6.7 μM, and 20 μM modified oligonucleotide, as specified in the tables below. After a treatment period of approximately 24 hours, total RNA was isolated from the cells and DLL4 mRNA levels were measured by RT-qPCR. Mouse DLL4 primer probe set RTS2518 (Forward sequence: GCCTTCCTTCTGCATTGTTTACA (SEQ ID No: 28) Reverse sequence: CTCCGCAGAGCAGCACTGT (SEQ ID No: 29) Probe sequence: TGCATCCTGTATGGGACATCTTT (SEQ ID No: 30)) was used to measure DLL4 mRNA levels. The resulting DLL4 mRNA levels were adjusted according to total RNA content as measured by RIBOGREEN. Results are presented as the percent level of DLL4 mRNA transcript, relative to that of the untreated control cells. As illustrated in the tables below, DLL4 mRNA levels were reduced in a dose-dependent manner in cells treated with modified oligonucleotides complementary to DLL4.

The modified oligonucleotides in the tables below are cEt gapmers (as described in Example 1). The nucleobase sequences of the modified oligonucleotides, shown in the tables below, are 100% complementary to mouse DLL4 pre-mRNA (GENBANK No. NC_000068.7 truncated from 119322001 to 119338000, SEQ ID No. 11), and/or to mouse DLL4 mRNA (GENBANK No. NM_019454.3, SEQ ID: 12). An entry of "N/A" in the table below indicates that the modified oligonucleotide is not 100% complementary to the corresponding nucleic acid.

Table 16: Modified oligonucleotides complementary to DLL4

Compound Number	Sequence	SEQ ID 11 start site	SEQ ID 11 stop site	SEQ ID 12 start site	SEQ ID 12 stop site	SEQ ID No.
380876	GCTCACAGTGCTCACCAGTG	8713	8732	1308	1327	183
797555	GCAAATCCTAGGGTCT	3908	3923	125	140	184
797562	GCTCGATGCCTCGGTA	3978	3993	195	210	185
797569	AGGGATGTCGCTCTCC	4080	4095	297	312	186
797580	CGCTGCTGCGGCCACA	N/A	N/A	413	428	187
797619	GGCAACTGCAGAGGGT	4553	4568	662	677	188
797656	GTCCAGCCCGGCAGGC	6157	6172	983	998	189
797676	GGATACATTCATTGCA	6710	6725	1108	1123	190
797700	TCACAGTGCTCACCAG	8715	8730	1310	1325	191
797710	GGTACTATGCTCACAG	9015	9030	1434	1449	192
797730	CCATTGGCACACGGGT	9167	9182	1586	1601	193
797731	CTCCATTGGCACACGG	N/A	N/A	1588	1603	194
797738	CGCTGATGTGCAGTTC	10214	10229	1672	1687	195
797750	GTCCGGAGGCACAGGC	10349	10364	1807	1822	196
797793	GCATGCCGCCCCGTCC	10769	10784	2227	2242	197
797801	GGCTGATATTCGACAC	12060	12075	2316	2331	198
797811	GGCAATCACACACTCG	12135	12150	2391	2406	199
797813	TCTGAGTAGGCTCCTG	12636	12651	2421	2436	200
797822	GTTCATGCCATTTCCT	12754	12769	2539	2554	201
797835	TCGAGAGGCACCTTAG	12901	12916	2686	2701	202
797836	TCCAAGTTCGAGAGGC	12908	12923	2693	2708	203
797843	GCCAAGACCCACTAGG	12986	13001	2771	2786	204
797844	CTCATTTGGGCCCAGC	13066	13081	2851	2866	205
797847	CTTAATGCCAAACTCC	13135	13150	2920	2935	206
797860	TAGCATGAAGGCCCTG	13356	13371	3141	3156	207
797868	GAAGATCGGCTTCAAG	13493	13508	3278	3293	208
797871	GATTTTTGAAGATCGG	13500	13515	3285	3300	209
797941	GGTGTTCGCGCAGCGC	4910	4925	N/A	N/A	210
797964	TGGCAAGTGTCACTGG	7420	7435	N/A	N/A	211
797966	GCACAGTACTTGACCC	7582	7597	N/A	N/A	212
797978	ACCATTGGCACACGGG	9168	9183	N/A	N/A	213
797983	AGCACTGGGTATTCCA	9599	9614	N/A	N/A	214

797987	GGCTTGATCTCTCTGG	9874	9889	N/A	N/A	215
797992	TGTGACTGCACCGTCT	11395	11410	N/A	N/A	216

Table 17: Dose response

Compound Number	DLL4 mRNA (% control)				IC50 (μM)
	740.5 nM	2222 nM	6667 nM	20,000 nM	
380876	112	88	92	69	>20
797656	74	106	43	40	5.9
797844	78	52	46	25	3.9
797759	96	94	33	28	6.6
797868	85	55	28	29	3.7
797843	87	125	49	53	15.8
797700	76	65	54	19	4.8
797801	78	128	56	49	13.1
797730	65	65	41	23	3.4
797987	124	157	84	42	18.3
797676	58	36	31	23	1.1
797580	77	76	62	40	13.1
797813	170	141	69	60	16.0
797941	86	64	37	35	5.3
797731	85	70	52	57	>20
797964	62	114	31	25	2.5
797983	67	71	36	39	5.2
797555	115	138	21	15	4.6
797562	97	71	46	30	6.6

Table 18: Dose response

Compound Number	DLL4 mRNA (% control)				IC50 (μM)
	740.5 nM	2222 nM	6667 nM	20,000 nM	
380876	152	140	127	66	>20
797978	18	16	11	6	<0.74
797966	67	43	76	30	6.7

797793	61	40	38	28	1.6
797569	63	39	43	17	2.3
797847	81	50	47	41	5.8
797750	88	55	47	30	5.1
797835	79	46	41	30	3.6
797822	57	53	51	42	4.7
797710	74	46	27	23	2.4
797860	102	66	62	60	>20
797738	59	62	54	39	6.8
797836	99	80	73	55	>20
797871	117	85	78	34	13.2
797992	87	63	57	41	9.4
797811	115	66	62	62	>20
797619	82	81	72	53	>20

Example 7: Inhibition of the Notch signaling pathway *in vivo* by modified oligonucleotides complementary to Notch1

Groups of 6 week old female BALB/c mice were administered 50 mg/kg of a modified oligonucleotide on study days 1, 6, and 12, via intraperitoneal (IP) delivery. Compound 549144 is control oligonucleotide. It is a cEt gapmer, as described in Example 1, with a nucleobase sequence that is not 100% complementary to any known mouse transcript. Each group contained 4 mice. One group of male mice was administered a saline control via IP delivery. Mice were sacrificed 48 hours after the last dose, and liver tissue was harvested. Total RNA was isolated from the liver tissue, and mRNA levels of Notch1 were measured RT-qPCR using primer probe set RTS1458 described above and normalized to Ribogreen.

Table 19: mRNA levels

Compound Number	Notch1 (% control)
Saline	100
549144	94
784192	54
784421	64
784432	83
784446	51
784496	57
784511	48
784563	28
784586	35

Example 8: Inhibition of the Notch signaling pathway *in vivo* by modified oligonucleotides complementary to Notch1

Groups of 6 week old female BALB/c mice were administered 6.25, 12.5, 25, or 50 mg/kg of a modified oligonucleotide described above once per week for 6 weeks via subcutaneous delivery. Each group contained 4 mice. One group of male BALB/c mice was administered a saline control via subcutaneous delivery. Mice were sacrificed 48 hours after the last dose, and liver tissue was harvested. Total RNA was isolated from the liver tissue and, mRNA levels of Notch1 were measured by RT-qPCR using primer probe set RTS1458 described above, normalized to Ribogreen. Results are presented as the average percent level of Notch1 mRNA transcript for each treatment group, relative to that of the saline treated group. As illustrated in the tables below, Notch1 mRNA levels were reduced in animals treated with a modified oligonucleotide complementary to Notch1.

Table 20: Dose response

Compound No./Dose (mg/kg)		Notch 1 mRNA (% control)
Saline		100
549144	50	107
784563	6.25	45
	12.5	27
	25	27
	50	29

Example 9: Inhibition of the Notch signaling pathway *in vivo* by modified oligonucleotides complementary to Notch2 or Notch3

Groups of 6 week old male BALB/c mice were administered 50 mg/kg of a modified oligonucleotide described above once per week for 4 weeks via subcutaneous delivery. Each group contained 4 mice. One group of male mice was administered a saline (PBS) control via subcutaneous delivery. Mice were sacrificed 24 hours after the last dose, and liver and lung tissues were harvested. Total RNA was isolated from the tissues, and mRNA levels of Notch2 or Notch3 were measured with RT-qPCR using primer probe set RTS36982 (forward sequence CAACCAGTGTGATGAGCAGT, designated herein as SEQ ID NO: 31; reverse sequence GTTGTCTTTGAAGTGGTCTGC, designated herin as SEQ ID NO: 32; probe sequence TTGTCATACTTGCACGTCTTGCTATTCCT, designated herein as SEQ ID: 33) for Notch2, or primer probe set RTS36974 for Notch3, described above. The resulting mRNA levels were normalized to cyclophilin A levels. Results are presented as the average percent level of Notch2 mRNA transcript or Notch3 mRNA transcript for each treatment group, relative to that of the saline treated group. An entry of "n.d." means that the corresponding data was not analyzed.

Table 21: mRNA levels (% PBS control)

Compound No.	Target transcript	Notch2, liver	Notch2, lung	Notch3, lung
PBS	None	106	100	101
549144	None	89	89	88
977277	Notch2	54	93	n.d.
977359	Notch2	13	71	n.d.
977375	Notch2	21	84	n.d.
977406	Notch2	16	79	n.d.
977413	Notch2	11	51	n.d.
977431	Notch2	6	60	n.d.
977472	Notch2	3	41	n.d.
977474	Notch2	23	68	n.d.
977499	Notch2	6	54	n.d.
977500	Notch2	12	70	n.d.
977545	Notch2	16	71	n.d.
977572	Notch2	2	43	n.d.
977574	Notch2	2	52	n.d.
976941	Notch3	n.d.	n.d.	27
976944	Notch3	n.d.	n.d.	64
976990	Notch3	n.d.	n.d.	18
977057	Notch3	n.d.	n.d.	32
977081	Notch3	n.d.	n.d.	23
977103	Notch3	n.d.	n.d.	74
977113	Notch3	n.d.	n.d.	49
977114	Notch3	n.d.	n.d.	25
977117	Notch3	n.d.	n.d.	33
977119	Notch3	n.d.	n.d.	35
977129	Notch3	n.d.	n.d.	27
977130	Notch3	n.d.	n.d.	28
977131	Notch3	n.d.	n.d.	17
977170	Notch3	n.d.	n.d.	16

Example 10: Inhibition of the Notch signaling pathway *in vivo* by a modified oligonucleotide complementary to Notch1

Modified oligonucleotides described above were tested in C57B/6 mice for their effect on mRNA level of Notch1 in mouse lung. Groups of 6 week old male C57B/6 mice were administered 200μg modified oligonucleotide in 50 μL saline on study day 1, 4, 7, 11, 14 and 19 via oropharyngeal delivery while under anesthesia. Each treatment group that received compound 784563 contained 6 mice, while each control group contained 4 mice (saline, 549144). Mice were sacrificed 48 hours after the last dose, and lung tissue was harvested. Total RNA was isolated from the lung tissue and mRNA levels of Notch1 were measured by RT-qPCR as described above, using primer probe set Mm00627185_m1 (ABI catalog 4351370). Results were normalized to cyclophilin A and are presented as the average percent level of Notch1 mRNA transcript for each treatment group, relative to that of the saline treated group.

Table 22: mRNA levels (% saline)

Compound Number	Notch1 mRNA
Saline	100
549144	105
784563	64

Example 11: Effects on Notch signaling pathway inhibition and trans-differentiation of lung cells by modified oligonucleotides complementary to a member of the Notch signaling pathway

Modified oligonucleotides described above were tested in C57B/6 mice for their effects on mRNA levels of cell differentiation markers and members of the Notch signaling pathway in mouse lung. Groups of 8 week old male C57B/6 mice were administered 200μg modified oligonucleotide in 50 μL saline every other day for 5 days via oropharyngeal delivery while under anesthesia. Each group contained 4 mice. One group of male C57B/6 mice was administered a saline control. Mice were sacrificed 72 hours after the last dose, and lung tissue was harvested. Total RNA was isolated from the lung tissue, and mRNA levels of JAG1, JAG2, and cell differentiation markers were measured by RT-qPCR. JAG1 was detected by Taqman probe Mm00496902_m1 (Thermo Fisher), and JAG2 was detected by Taqman probe Mm01325629_m1(Thermo Fisher). Notch1 was detected with Mm00627185_m1, and DLL4 was detected with Mm0044619_m1. Other Taqman primer probe sets (ThermoFisher) were used for gene detection as follows: Muc5ac: Mm01276718_m1; Muc5b: Mm00466391_m1; Scgb1a1: Mm01230908_m1; FoxJ1: Mm01267279_m1; and Tubb4a: mM00726185. Results were normalized to cyclophilin A, as detected by primer probe set RTS9317 (forward sequence TCGCCGCTTGCTGCA, designated herein as SEQ ID NO: 34; reverse sequence ATCGGCCGTGATGTCGA, designated herein as SEQ ID NO: 35; probe sequence CCATGGTCAACCCCACCGTGTTC, designated herein as SEQ ID: 36). The normalized results are shown in the tables below as the average percent for each treatment group, relative to that of the saline treated group.

The results show that each modified oligonucleotide that is 100% complementary to only one member of the Notch signaling pathway decreased mRNA transcript levels of multiple members of the Notch signaling pathway and decreased mRNA transcript levels of goblet or club cell markers, and/or increased mRNA transcript levels of ciliated cell markers. An entry of "n.d." means that the corresponding data was not analyzed.

Table 23: mRNA levels (% saline) in mouse lung

Compound No.	Target transcript	Notch1	JAG1	JAG2	DLL4	Notch2	Notch3
Saline	None	100	100	100	100	100	100
549144	None	99	97	88	79	90	90
784563	Notch1	54	77	71	41	58	49
897368	JAG1	39	25	46	33	35	27
897427	JAG1	49	25	76	43	49	38
897758	JAG2	57	66	46	55	62	43
897763	JAG2	52	71	51	62	59	51
797555	DLL4	42	52	53	35	45	31
797868	DLL4	57	74	66	55	65	47

Table 24: mRNA levels (% saline) in mouse lung

Compound No.	Target transcript	Goblet Cells	Club Cells	Ciliated cells
		Muc5ac mRNA	Scgb1a1 mRNA	FOXJ1 mRNA
Saline	none	100	100	100
549144	none	130	88	117
784563	Notch1	n.d.	54	90
897368	JAG1	61	17	84
897427	JAG1	51	30	206
897758	JAG2	192	60	101
897763	JAG2	111	76	105
797555	DLL4	249	43	75
797868	DLL4	199	94	106

Example 12: Effects on Notch signaling pathway inhibition and trans-differentiation of lung cells by modified oligonucleotides complementary to a member of the Notch signaling pathway

Modified oligonucleotides described above were tested in A/J mice (Jackson Labs). Groups of 8

week old male A/J mice were administered 200μg of modified oligonucleotide every other day for 5 days via oropharyngeal delivery while under anesthesia. Each group contained 4 mice. One group of control male A/J mice was administered saline. Mice were sacrificed 72 hours after the last dose, and lung tissue was harvested. Total RNA was isolated from the lung tissue, and mRNA levels of JAG1, JAG2, and lung cell differentiation markers were measured by RT-qPCR using Taqman probe set Mm00496902_m1 (Thermo Fisher) for JAG1, primer probe set RTS35955 (see Example 5) for JAG2, Taqman probe set Mm01230908_m1 for Scgb1a1, and Taqman probe set Mm01267279_m1 for FoxJ1. Results were normalized to cyclophilin A levels and are presented in the tables below as the average mRNA level for each treatment group relative to the saline treated group. The results show that each modified oligonucleotide that is 100% complementary to only one member of the Notch signaling pathway decreased mRNA transcript levels of multiple members of the Notch signaling pathway and decreased mRNA transcript levels of a club cell marker and/or increased mRNA transcript levels of a ciliated cell marker.

Table 25: mRNA levels in mouse lung relative to saline treated animals

Compound No.	Notch signaling pathway		Ciliated Cells	Club cells
	JAG1 mRNA	JAG2 mRNA	FoxJ1 mRNA	Scgb1a1 mRNA
Saline	1.0	1.0	1.0	1.0
549144	0.9	1.0	0.8	0.7
897368	0.2	0.4	0.9	0.3
897427	0.2	0.8	2.4	0.3
897316	0.5	0.8	1.5	0.6
897372	0.5	0.6	1.9	0.5
897439	0.5	0.8	2.5	0.8

Example 13: House dust mite model and methacholine challenge with pre-treatment of modified oligonucleotide

House dust mite (HDM) is a common allergen that has been previously demonstrated to induce asthma-like disease in mice (Johnson, *Am J Respir Crit Care Med* Vol 169. pp 378–385, 2004), with increases in airway inflammation, goblet cell hyperplasia, and airway hyperreactivity to methacholine. Modified oligonucleotides described above were tested in A/J mice in combination with administration of HDM and methacholine to induce asthma-like symptoms. Each treatment group contained 4 mice. Modified oligonucleotides and HDM were administered to anesthetized mice via oropharyngeal delivery.

Mice were administered 200 μg of a modified oligonucleotide twice per week for 2 weeks (5 total treatments) before the first HDM treatment (100μg/mouse/treatment) on day 16. Treatment with modified oligonucleotide twice per week continued until study day 30. HDM treatment was repeated once per week

for 3 weeks. One group of mice was administered saline in place of modified oligonucleotide and HDM. 48 hours after the final HDM treatment and 24 hours after the final oligonucleotide treatment, mice were challenged with methacholine, which causes bronchoconstriction. Lung function was measured using the Penh score obtained through unrestrained plethysmography. A higher Penh score indicates more constriction than a lower Penh number. The results in the table below show that mice treated with a modified oligonucleotide complementary to a member of the Notch signaling pathway had improved lung function compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

72 hours after the final HDM treatment and 48 hours after the final oligonucleotide treatment, mice were sacrificed and lung tissue was harvested for histological analysis and RNA isolation. Sections prepared for histology were stained with Schiff stain in order to detect mucus. The resulting images showed that mucus staining was reduced in both groups of mice treated with a modified oligonucleotide 100% complementary to JAG1 compared to the group of mice treated with a modified oligonucleotide that is not 100% complementary to any member of the Notch signaling pathway. Furthermore, compound 897427, which reduced JAG1 mRNA levels to a greater extent than compound 897372, also reduced mucus staining to a greater extent than compound 897372.

Total RNA was isolated from lung tissue, and mRNA levels were measured by RT-qPCR using primer probe sets described above and normalized to cyclophilin levels. Results are presented in the tables below as the average mRNA level for each treatment group relative to saline treated animals. The results in the tables below show that in an asthma disease model, mice treated with a modified oligonucleotide complementary to a member of the Notch signaling pathway had improved lung function and trans-differentiation to ciliated cells compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

Table 26: Penh scores

Treatment group	Methacholine (mg/mL)					
	0	3	6	12	25	50
	Penh score					
Naïve (saline + saline)	0.7	0.9	1.8	3.8	4.8	7.3
549144 + HDM	1.0	1.4	3.4	7.1	11.5	12.4
897427 + HDM	1.0	2.2	2.4	2.9	3.4	3.8
897372 + HDM	1.2	1.7	2.8	4.3	8.3	10.8

Table 27: mRNA levels in lung relative to saline treated animals

Treatment group	Notch signaling pathway	Goblet cell markers				Club cell marker	Ciliated cell marker
	JAG1	Muc5b	Gob5 (Clca1)	Foxa3	SPDEF	Scgb1a1	FOXJ1
Naïve (saline + saline)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
549144 + HDM	0.8	9.1	81.9	4.4	5.8	0.3	1.1
897427 + HDM	0.5	4.5	18.9	2.2	2.7	0.2	3.4
897372 + HDM	0.7	7.7	38.9	2.7	4.0	0.3	2.3

Example 14: House dust mite model and methacholine challenge, followed by treatment with modified oligonucleotide

Modified oligonucleotides described above were administered to A/J mice after treatment with HDM and methacholine to induce asthma-like symptoms. Modified oligonucleotides and HDM were administered to anesthetized mice via oropharyngeal delivery, as outlined in the table below. Each group contained 4-6 mice. The three groups that received a modified oligonucleotide and the one group that received house dust mites only ("HDM-only") received HDM treatment (100μg/mouse/treatment) weekly for four weeks. The group of naïve mice received no HDM or oligonucleotide treatment. On day 11, 72 hours after the second HDM treatment, mouse lung function was tested following various doses of methacholine ("methacholine challenge"). After the methacholine challenge, mice in the appropriate groups were administered 200 μg of modified oligonucleotide, as indicated in the table below. Mice were sacrificed on day 12 or day 27, and lung tissue was harvested.

Table 28: Study design for each treatment group

Study Day	Naïve	HDM-only	549144	897427	897372
1	none	HDM	HDM	HDM	HDM
8	none	HDM	HDM	HDM	HDM
11	Methacholine challenge				
11	none	none	549144	897427	897372
12	Sac/RNA analysis		none	none	none
13	N/A	N/A	549144	897427	897372
14	N/A	N/A	HDM	HDM	HDM
15	N/A	N/A	549144	897427	897372
17	N/A	N/A	549144	897427	897372

20	N/A	N/A	549144	897427	897372
21	N/A	N/A	HDM	HDM	HDM
22	N/A	N/A	549144	897427	897372
25	N/A	N/A	549144	897427	897372
26	N/A	N/A	Methacholine challenge		
27	N/A	N/A	Sac/RNA analysis		

The methacholine challenge doses and results are shown in the table below. Day 11 scores were obtained prior to that day's administration of modified oligonucleotides. The results in the table below show that mice treated with a modified oligonucleotide complementary to a member of the Notch signaling pathway after inducement of asthma-like symptoms generally had improved lung function compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

Total RNA was isolated from the lung tissue of sacrificed mice, and mRNA levels were measured by RT-qPCR, as described in Example 13. Results are presented in the table below as normalized mRNA levels relative to saline treated animals. The results show that mice treated with a modified oligonucleotide complementary to a member of the Notch signaling pathway after inducement of asthma-like symptoms exhibited increased trans-differentiation to ciliated cells compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

Sections of lung tissue were prepared for histology and stained with Schiff stain in order to detect mucus. The resulting images showed that mucus staining was reduced in both groups of mice treated with a modified oligonucleotide 100% complementary to JAG1 compared to the group of mice treated with a modified oligonucleotide that is not 100% complementary to any member of the Notch signaling pathway. Furthermore, compound 897427, which reduced JAG1 mRNA levels to a greater extent than compound 897372, also reduced mucus staining to a greater extent than compound 897372. These results show that asthma-like symptoms were reversed following administration of modified oligonucleotides 100% complementary to a member of the Notch signaling pathway.

Table 29: Penh scores

Treatment group, study day	Methacholine (mg/mL)				
	0	3	6	12	25
	Penh score				
Naïve, day 11	0.7	0.8	1.6	2.9	6.0
HDM-only, day 11	0.8	2.6	4.1	7.1	9.3
549144, day 11	0.8	2.4	5.9	7.6	9.4

897427, day 11	0.7	2.9	3.8	5.8	10.3
897372, day 11	0.8	2.7	4.5	7.2	10.7
549144, day 26	1.1	3.5	5.0	10.9	13.4
897427, day 26	1.2	2.0	3.4	6.6	8.6
897372, day 26	1.3	1.9	5.6	9.2	12.5

Table 30: mRNA levels in lung relative to saline treated animals

Treatment group, study day	Notch signaling pathway	Goblet cells	Ciliated cells
	JAG1	Muc5b	FoxJ1
Naïve, day 12	1.0	1	1
HDM-only, day 12	1.1	6.3	1.4
549144, day 27	0.7	5.9	1.1
897427, day 27	0.3	1.6	1.7
897372, day 27	0.6	3.4	2.8

Example 15: Inhibition of the Notch signaling pathway *in vivo* by modified oligonucleotides complementary to Notch1, Notch2, or Notch3

Modified oligonucleotides described in Examples 2, 3, and 4 were tested in BALB/c mice. Groups of 8 week old male mice were administered 50mg/kg modified oligonucleotide once per week for four weeks via subcutaneous delivery. Each group contained 4 mice. One group received PBS via subcutaneous delivery. Mice were sacrificed 48 hours after the last dose, and lung tissue and other tissues were harvested. Total RNA was isolated from the lung tissue and other tissues, and mRNA levels were measured by RT-qPCR.

Table 31: Notch1 mRNA levels (% PBS control)

Compound No.	Target transcript	Liver	Lung	Quadricep	Kidney
549144	None	102	94	79	92
784563	Notch1	34	57	55	64
784586	Notch1	50	66	66	69
977472	Notch2	105	89	79	98
977499	Notch2	90	88	91	89
977129	Notch3	96	89	88	96
977130	Notch3	75	78	65	86

Table 32: Notch2 mRNA levels (% PBS control)

Compound No.	Target transcript	Liver	Lung	Quadricep	Kidney
549144	None	97	102	87	95
784563	Notch1	96	87	112	104
784586	Notch1	102	107	101	107
977472	Notch2	6	41	20	50
977499	Notch2	6	54	45	59
977129	Notch3	97	100	116	105
977130	Notch3	90	91	99	102

Table 33: Notch3 mRNA levels (% PBS control)

Compound No.	Target transcript	Liver	Lung	Quadricep	Kidney
549144	None	84	104	104	95
784563	Notch1	87	85	84	97
784586	Notch1	35	66	79	67
977472	Notch2	92	79	98	96
977499	Notch2	106	83	120	92
977129	Notch3	31	29	27	70
977130	Notch3	33	18	20	59

Plasma levels of liver transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the table below.

Table 34: Plasma Transaminases

Compound No.	ALT (U/L)	AST (U/L)
549144	25.0	58.5
784563	36.3	57.3
784586	234.5	157.8
977472	89.8	109.8
977499	32.8	47.3
977129	33.8	84.3
977130	38.0	68.8

Example 16: House dust mite model and methacholine challenge, followed by treatment with modified oligonucleotide

Modified oligonucleotides described above were administered to A/J mice after treatment with HDM and methacholine to induce asthma-like symptoms. Modified oligonucleotides and HDM were administered to anesthetized mice as described in Example 14, via oropharyngeal delivery. Each group contained 10-14 mice. A group of naïve mice received no HDM or oligonucleotide treatment, and a group of HDM-only mice received HDM treatment but no modified oligonucleotide. For HDM-only and HDM+oligonucleotide-treated groups, mice were administered HDM (100μg/mouse/treatment) weekly for 5 weeks. For HDM+oligonucleotide-treated groups, mice were administered 200 μg/dose of compound no. 549144 (control) or compound no. 897427 (Jag1) three times a week for 3.5 weeks.

A methacholine challenge was performed, as described above, on day 11 (baseline) and day 38. Day 11 scores were obtained prior to that day's administration of modified oligonucleotides, and animals were randomized to normalize the baseline Penh score. The results in the table below show that mice treated with a modified oligonucleotide complementary to a member of the Notch signaling pathway after inducement of asthma-like symptoms generally had improved lung function compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

Table 35: Penh scores on day 38

Treatment group	Methacholine (mg/mL)			
	0	3	6	12
	Penh score			
Naïve	0.65	0.84	1.18	2.53
HDM-only	0.73	3.72	12.6	15.3
HDM + 549144	0.94	4.10	10.5	16.4
HDM+897427	1.04	1.99	4.12	7.3

Mice were sacrificed, total RNA was isolated from the lung tissue, and mRNA levels were measured by RT-qPCR, as described in Example 13. Results are presented in the table below as normalized mRNA levels relative to naïve animals. The results show that mice treated with a modified oligonucleotide 100% complementary to a member of the Notch signaling pathway after inducement of asthma-like symptoms exhibited increased trans-differentiation to ciliated cells compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

Table 36: mRNA levels in lungs of treated mice relative to naïve mice on day 40 (% control)

Treatment group, study day	Notch signaling pathway	Goblet cell marker	Ciliated cell marker
	JAG1	Muc5b	FoxJ1
Naïve	100	100	100
HDM-only	58	476	64
HDM + 549144	39	363	41
HDM+897427	13	136	66

Example 17: Inhibition of the Notch signaling pathway by modified oligonucleotides complementary to Hes-1

Modified oligonucleotides 100% complementary to mouse Hes-1 were tested at various doses in HEPA1-6 (mouse hepatoma) cells. The cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.11 μM, 0.33 μM, 10 μM, or 30 μM modified oligonucleotide, as specified in the tables below. After a treatment period of approximately 24 hours, total RNA was isolated from the cells and Hes-1 mRNA levels were measured by RT-qPCR. Mouse Hes-1 primer probe set RTS38987 was used to measure Hes-1 (Forward sequence GCACAGAAAGTCATCAAAGCC, SEQ ID NO: 219; Reverse sequence ATGTCTGCCTTCTCTAGCTTG, SEQ ID NO: 220; Probe sequence ATTCTTGCCCTTCGCCTCTTCTCC, SEQ ID NO: 221). Hes-1 mRNA levels were normalized according to total RNA content as measured by RIBOGREEN. Results are presented as the percent level of normalized Hes-1 mRNA, relative to that of the untreated control cells. As illustrated in the tables below, Hes-1 mRNA transcript levels were reduced in a dose-dependent manner in cells treated with modified oligonucleotides complementary to Hes-1.

The modified oligonucleotides in the tables below are cEt gapmers (as described in Example 1). The nucleobase sequences of the modified oligonucleotides, shown in the tables below, are 100% complementary to mouse Hes-1 pre-mRNA (GENBANK No. NC_000082.6_TRUNC_30063857_30069296, SEQ ID No. 217), and/or to mouse Hes-1 mRNA (GENBANK No. NM_008235.2, SEQ ID: 218).

Table 37: Modified oligonucleotides complementary to Hes-1

Compound Number	Sequence	SEQ ID 217 start site	SEQ ID 217 stop site	SEQ ID 218 start site	SEQ ID 218 stop site	SEQ ID NO.
1057776	CACTATTCCAGGACCA	1545	1560	45	60	222
1057778	AGCACTATTCCAGGAC	1547	1562	47	62	223
1057781	ATCGGTAGCACTATTC	1553	1568	53	68	224
1057782	GATCGGTAGCACTATT	1554	1569	54	69	225

1057784	GTGATCGGTAGCACTA	1556	1571	56	71	226
1057788	CTACTTAGTGATCGGT	1563	1578	63	78	227
1057789	GCTACTTAGTGATCGG	1564	1579	64	79	228
1057796	TTATTATGTCTTAGGG	1579	1594	79	94	229
1057797	TTTATTATGTCTTAGG	1580	1595	80	95	230
1057799	GGTTTATTATGTCTTA	1582	1597	82	97	231
1057800	AGGTTTATTATGTCTT	1583	1598	83	98	232
1057804	GCAGTTGAAGGTTTAT	1591	1606	91	106	233
1057805	AGCAGTTGAAGGTTTA	1592	1607	92	107	234
1057813	TTTTTGGAATCCTTCA	1674	1689	174	189	235
1057906	GGACTTTACGGGTAGC	3588	3603	1099	1114	236
1057910	CGTTTTTAGTGTCCGT	3625	3640	1136	1151	237
1057975	AGAGCTTAGTTCTTTG	2130	2145	45	60	238
1057979	GTAAGATCCACATGCA	2154	2169	47	62	239
1057980	GGTAAGATCCACATGC	2155	2170	53	68	240
1057987	CAGTCCTCCTTGTCAG	2263	2278	54	69	241
1057994	GGAATGCCGGGAGCTC	2306	2321	56	71	242
1058018	GGCAGTAAAATGTAGC	2490	2505	63	78	243
1058024	GGCTATAAATAAGACC	2534	2549	64	79	244
1058030	GTAACAACTTGGGAGC	2553	2568	79	94	245
1058031	AGTAACAACTTGGGAG	2554	2569	80	95	246
1058043	CTTCTCGGCTACAGCC	2590	2605	82	97	247
1058045	ACCGGCTTCTACCACA	2624	2639	83	98	248
1058055	GTGCTAAACCACTGAC	2693	2708	91	106	249
1058071	TTCTCCCTAGGTTGGG	2855	2870	92	107	250

Table 38: Dose response

Compound Number	Hes-1 mRNA (% control)				IC50 (μM)
	1,111 nM	3,333 nM	10,000 nM	30,000 nM	
1057994	33	35	27	14	<1.1
1057906	102	88	52	35	>30
1058071	102	97	111	94	>30
1057782	86	94	79	52	>30
1057799	76	60	32	17	4.7

1057910	71	65	39	18	5.3
1058031	82	80	77	55	>30
1058018	80	84	83	71	>30
1057987	85	96	90	108	>30
1058055	71	68	72	52	>30
1057975	94	92	78	58	>30
1057778	65	56	37	16	3.7
1058043	66	91	105	88	>30
1058030	81	64	27	8	4.7
1057979	62	56	30	15	3.1

Table 39: Dose response

Compound Number	Hes-1 mRNA (% control)				IC50 (μM)
	1,111 nM	3,333 nM	10,000 nM	30,000 nM	
1057784	100	77	42	19	8.5
1058024	105	105	90	70	>30
1057797	90	72	41	27	8.4
1057788	104	86	51	25	11.1
1058045	89	91	77	62	>30
1057980	85	64	46	24	7.5
1057813	82	73	56	31	11.3
1057776	98	89	59	39	17.8
1057789	83	78	53	37	13.7
1057796	79	73	47	30	9.2
1057805	80	58	48	39	9.6
1057800	72	54	31	12	3.8
1057804	84	50	33	22	5.0
1057781	86	74	47	26	8.9

Example 18: Inhibition of the Notch signaling pathway *in vivo* by modified oligonucleotides complementary to Hes-1

Groups of 6 week old male BALB/c mice were administered 50 mg/kg of a modified oligonucleotide described above once per week for 4 weeks via subcutaneous delivery. Each group contained 4 mice. One

group was administered only saline as a control. Mice were sacrificed 48 hours after the last dose, and tissues were harvested. Total RNA was isolated from the liver and lung tissue, and mRNA levels of Hes-1 were measured via RT-qPCR as described above, and normalized to Cyclophilin A levels.

Table 40: mRNA levels

Compound Number	Hes-1, lung (% Control)	Hes-1, liver (% control)
Saline	100	100
549144	111	95
1057778	112	53
1057781	122	58
1057797	95	21
1057799	86	27
1057800	117	66
1057804	103	67
1057910	110	65
1057979	63	18
1057994	4	2.5
1058030	41	15

Example 19: Effects on Notch signaling pathway inhibition and trans-differentiation of lung cells by modified oligonucleotides complementary to members of the Notch signaling pathway

Modified oligonucleotides were tested in A/J mice (Jackson Labs). Groups of 8 week old male A/J mice were administered 10 mg/kg of modified oligonucleotide three times in one week via oropharyngeal delivery while under anesthesia. Each group contained 4 mice. One group of control male A/J mice was administered saline, and another group was administered the control oligonucleotide 549144, described herein (see Example 7). Mice were sacrificed 72 hours after the last dose, and lung tissue was harvested. Total RNA was isolated from the lung tissue, and mRNA levels of Hes1, Notch1, Notch2, Notch3, FOXJ1, and Jag1 were measured by RT-qPCR using RTS38987 for Hes1 (See Example 17), RTS1458 for Notch 1 (see Example 2), RTS36982 for Notch 2 (see Example 9), RTS36974 for Notch 3 (see Example 4), Mm01267279 for FoxJ1 (see Example 11), Mm01230908 for Scgb1a1 (see Example 11), and RTS35953 for Jag 1 (Forward sequence GCACAGAAAGTCATCAAAGCC, SEQ ID NO: 219; Reverse sequence ATGTCTGCCTTCTCTAGCTTG, SEQ ID NO: 220; Probe sequence ATTCTTGCCCTTCGCCTCTTCTCC, SEQ ID NO: 221). RNA levels were normalized to cyclophilin A levels and are presented in the tables below as the average, normalized mRNA level for each treatment group relative to the saline treated group. The results show that each modified oligonucleotide that is 100%

complementary to only one member of the Notch signaling pathway decreased mRNA transcript levels of multiple members of the Notch signaling pathway and decreased mRNA transcript levels of a club cell marker and/or increased mRNA transcript levels of a ciliated cell marker.

Table 41: mRNA levels in lung of oligonucleotide treated mice relative to saline treated mice

Compound No.	Notch signaling pathway components					Ciliated Cell marker	Club cell marker
	Hes-1 mRNA	Notch1 mRNA	Notch2 mRNA	Notch3 mRNA	Jag1 mRNA	FoxJ1 mRNA	Scgb1a1 mRNA
549144	75	72	71	67	82	71	58
977472	48	48	19	57	75	149	16
977499	62	70	25	72	102	226	28
1057797	30	63	73	64	70	92	36
1057979	59	77	87	82	88	95	38
1058030	44	66	75	69	68	67	45

Example 20: House dust mite model and methacholine challenge with pre-treatment of modified oligonucleotide

Modified oligonucleotides described above were tested in A/J mice in combination with administration of HDM and methacholine to induce asthma-like symptoms as described in Example 13 above. Each treatment group contained 6 mice for mRNA analysis and 8 mice for the methacholine challenge. Modified oligonucleotides and HDM were administered to anesthetized mice via oropharyngeal delivery.

Mice were administered 200 μg of a modified oligonucleotide twice per week for 2 weeks (5 total treatments) before the first HDM treatment (100μg/mouse/treatment) on day 16. Treatment with modified oligonucleotide twice per week continued until study day 30. HDM treatment was repeated once per week for 3 weeks. One group of mice was administered saline in place of modified oligonucleotide and HDM, and served as the control group to which other groups were compared. 48 hours after the final HDM treatment and 24 hours after the final oligonucleotide treatment, mice were challenged with methacholine, which causes bronchoconstriction. Lung function was measured using the Penh score obtained through unrestrained plethysmography. A higher Penh score indicates more constriction than a lower Penh number.

Total RNA was isolated from lung tissue, and mRNA levels were measured by RT-qPCR using primer probe sets described above and normalized to cyclophilin levels. Results are presented in the tables below as the average mRNA level for each treatment group relative to saline treated animals. The results in the tables below show that in an asthma disease model, mice treated with a modified oligonucleotide complementary to a member of the Notch signaling pathway had improved trans-differentiation to ciliated

cells compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

Table 42: Penh scores

Treatment group	Methacholine (mg/mL)			
	0	3	6	12
	Penh score			
Naïve (saline + saline)	0.8	1.0	1.8	4.1
549144 + HDM	0.9	3.8	4.8	6.1
977472 + HDM	1.0	1.4	3.0	7.4
977499 + HDM	1.1	1.6	3.6	7.1

Table 43: mRNA levels in lung of oligonucleotide treated mice relative to saline treated mice

Treatment group	Notch signaling pathway			Goblet cell markers		Club cell marker	Ciliated cell marker
	Notch2	Jag1	Jag2	Muc5b	Muc5ac	Scgb1a1	FOXJ1
549144 + HDM	46	78	74	472	3707	18	56
977472 + HDM	21	92	96	184	1322	21	120
977499 + HDM	21	96	88	176	1110	17	171

Example 21: House dust mite model and methacholine challenge, followed by treatment with modified oligonucleotide

Modified oligonucleotides described above were administered to A/J mice after treatment with HDM and methacholine to induce asthma-like symptoms. Modified oligonucleotides and HDM were administered to anesthetized mice as described in the table below, via oropharyngeal delivery. Each group contained 8 mice. A group of naïve mice received no HDM or oligonucleotide treatment and one group received 5 doses of HDM and no oligonucleotide treatment ("HDM-only"). For HDM-only and HDM+oligonucleotide-treated groups, mice were administered HDM (100µg/mouse/treatment) weekly for 5 weeks. For HDM+oligonucleotide-treated groups, mice were administered 200 µg/dose of compound no. 549144 (control), compound no. 897427 (Jag1) or compound no. 977472 or 977499 (Notch2) three times a week for 3.5 weeks.

Table 44: Study design for each treatment group

Study Day	Naïve	HDM-only	549144	897427	977472	977499
1	N/A	HDM	HDM	HDM	HDM	HDM

8	N/A	HDM	HDM	HDM	HDM	HDM
11	Methacholine challenge					
15	N/A	N/A	549144	897427	977472	977499
16	N/A	HDM	HDM	HDM	HDM	HDM
17	N/A	N/A	549144	897427	977472	977499
19	N/A	N/A	549144	897427	977472	977499
22	N/A	N/A	549144	897427	977472	977499
23	N/A	HDM	HDM	HDM	HDM	HDM
24	N/A	N/A	549144	897427	977472	977499
26	N/A	N/A	549144	897427	977472	977499
29	N/A	N/A	549144	897427	977472	977499
31	N/A	N/A	549144	897427	977472	977499
32	N/A	HDM	HDM	HDM	HDM	HDM
33	N/A	N/A	549144	897427	977472	977499
37	N/A	N/A	549144	897427	977472	977499
39	N/A	N/A	549144	897427	977472	977499
40	Methacholine challenge					
41	Sac/RNA analysis					

A methacholine challenge was performed, as described above, on day 11 (baseline) and day 40. The results are shown in the table below.

Table 45: Penh scores

Treatment group	Test day	Methacholine (mg/mL)				
		0	3	6	12	25
		Penh score				
Naïve	11	0.67	0.77	1.26	2.34	3.83
HDM-only	11	0.76	1.12	2.75	3.42	5.38
HDM + 549144	11	0.80	1.26	2.73	5.19	6.82
HDM + 897427	11	0.73	1.38	2.18	3.05	5.00
HDM + 977472	11	0.73	1.02	2.34	2.91	4.36
HDM + 977499	11	0.77	1.30	3.41	4.75	9.65
Naïve	40	0.82	0.82	1.00	2.54	5.32
HDM-only	40	0.85	3.94	7.33	11.6	13.3

HDM + 549144	40	0.94	2.45	5.64	8.59	9.70
HDM + 897427	40	1.24	1.67	2.56	4.13	5.59
HDM + 977472	40	1.86	4.20	8.43	10.1	11.4
HDM + 977499	40	1.31	3.03	10.86	13.5	14.4

Mice were sacrificed, total RNA was isolated from the lung tissue, and mRNA levels were measured by RT-qPCR, as described in Example 13. Results are presented in the table below as normalized mRNA levels relative to naïve animals. The results show that mice treated with a modified oligonucleotide 100% complementary to a member of the Notch signaling pathway after inducement of asthma-like symptoms exhibited increased trans-differentiation to ciliated cells compared to mice treated with a modified oligonucleotide that is not 100% complementary to a member of the Notch signaling pathway.

Table 46: mRNA levels in lung of oligonucleotide treated mice relative to saline treated mice

Treatment group	Notch signaling pathway		Goblet cell markers				Ciliated cell marker
	Notch2	Jag1	Muc5b	Muc5ac	Gob5 (Clca1)	SPDEF	FOXJ1
HDM-only	73	89	510	1062	20419	191	114
HDM + 549144	45	76	682	2294	31529	285	66
HDM + 897427	33	25	214	681	8325	132	115
HDM + 977472	18	62	155	199	1820	66	92
HDM + 977499	20	61	185	337	5534	97	101

Example 22: Inhibition of the Notch signaling pathway *in vivo* by modified oligonucleotides complementary to Jag1 or Notch2

Modified oligonucleotides described in the examples above were tested in BALB/c mice. Groups of 7 week old male mice were administered 50mg/kg modified oligonucleotide once per week for four weeks via subcutaneous delivery. Each group contained 8 mice. One group received PBS via subcutaneous delivery. Mice were sacrificed 48 hours after the last dose, and tracheal tissue was harvested. Total RNA was isolated from the trachea, and mRNA levels were measured by RT-qPCR.

Table 47: mRNA levels in lung of oligonucleotide treated mice relative to saline treated mice

Compound ID	Notch signaling pathway		Club Cell marker	Ciliated cell marker
	Notch2	Jag1	Scgb1a1	FOXJ1
549144	82	69	111	158
897427	78	33	47	300
977472	39	70	29	502
977499	63	78	60	291

What is claimed is:

1. A method of treating, preventing, delaying the onset, slowing the progression, or ameliorating a respiratory disorder associated with excessive mucus production in an individual having, or at risk of having, a respiratory disorder associated with excessive mucus production comprising administering a compound comprising a Notch signaling pathway inhibitor to the individual, thereby treating, preventing, delaying the onset, slowing the progression, or ameliorating the respiratory disorder associated with excessive mucus production in the individual.

2. The method of claim 1, wherein the respiratory disorder associated with excessive mucus production is asthma, chronic obstructive pulmonary disorder (COPD), idiopathic pulmonary fibrosis (IPF), or cystic fibrosis (CF).

3. The method of claim 2, wherein the respiratory disorder associated with excessive mucus production is asthma.

4. The method of claim 2, wherein the respiratory disorder associated with excessive mucus production is COPD.

5. The method of claim 2, wherein the respiratory disorder associated with excessive mucus production is IPF.

6. The method of claim 2, wherein the respiratory disorder associated with excessive mucus production is CF.

7. The method of any of claims 1-6, wherein the compound increases trans-differentiation from club cells or goblet cells to ciliated cells, decreases mucus in the lungs, and/or increases lung function.

8. The method of claim 7, wherein the compound decreases mucus in the lungs.

9. The method of claim 7, wherein the compound increases lung function.

10. A method of inhibiting expression or activity of the Notch signaling pathway in a cell comprising contacting the cell with a compound comprising a Notch signaling pathway inhibitor, thereby inhibiting expression or activity of at least one member of the Notch signaling pathway in the cell.

11. The method of claim 10, wherein the cell is a lung cell.

12. The method of claim 11, wherein the cell is in an individual.

13. The method of claim 12, wherein the individual has, or is at risk of having asthma, COPD, IPF, or CF.

14. The method of any of claims 1-9 or 12-13, wherein the individual is human.

15. The method of any of claims 1-14, comprising administering to the individual or contacting the cell with no more than one compound comprising a Notch signaling pathway inhibitor.

16. The method of any of claims 1-15, wherein the compound inhibits the expression of at least one Notch signaling pathway member transcript.

17. The method of any of claims 1-16, wherein the compound inhibits the expression of at least two Notch signaling pathway members.

18. The method of any of claims 1-17, wherein the Notch signaling pathway inhibitor is a modified oligonucleotide complementary to a Notch signaling pathway member transcript.

19. The method of any of claims 1-17, wherein the compound comprises a modified oligonucleotide complementary to a member of the Notch signaling pathway.

20. The method of claim 18 or 19, wherein the modified oligonucleotide is single-stranded.

21. The method of claim 18 or 19, wherein the modified oligonucleotide is part of a double-stranded duplex.

22. The method of any of claims 18-21, wherein the modified oligonucleotide is 12 to 30 linked nucleosides in length.

23. The method of any of claims 18-22, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

24. The method of claim 23, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.

25. The method of any of claims 18-24, wherein the modified oligonucleotide comprises at least one modified sugar moiety.

26. The method of claim 25, wherein the at least one modified sugar moiety is a bicyclic sugar or 2'-O-methyoxyethyl modified sugar moiety.

27. The method of claim 26, wherein the at least one modified sugar is a cEt, LNA, or ENA.

28. The method of any of claims 18-27, wherein the modified oligonucleotide comprises at least one 5-methylcytosine modified nucleobase.

29. The method of any of claims 24-28, wherein each modified internucleoside linkage is a phosphorothioate linkage.

30. The method of any of claims 18-29, wherein each cytosine nucleobase is a 5-methylcytosine.

31. The method of any one of claims 18-30, wherein the modified oligonucleotide comprises:
a gap segment consisting of 7-11 linked 2'-deoxynucleosides;
a 5' wing segment consisting of 1-7 linked nucleosides;
a 3' wing segment consisting of 1-7 linked nucleosides;
wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein the nucleoside of each wing segment that is immediately adjacent to the gap segment each comprises a modified sugar.

32. The method of any of claims 18-31, wherein the modified oligonucleotide is at least 90% complementary to a Notch signaling pathway member nucleic acid.

33. The method of any of claims 18-31, wherein the modified oligonucleotide is 100% complementary to a Notch signaling pathway member nucleic acid.

34. The method of claims 32 or 33, wherein the Notch signaling pathway member nucleic acid is a Notch signaling pathway member transcript.

35. The method of claim 34, wherein the Notch signaling pathway member transcript is a Notch signaling pathway member pre-mRNA.

36. The method of claim 34, wherein the Notch signaling pathway member transcript is a Notch signaling pathway member mRNA.

37. The method of any of claims 32-36, wherein the Notch signaling pathway member is a Notch receptor, ligand of a Notch receptor, or intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

38. The method of claim 37, wherein the Notch signaling pathway member is a Notch receptor or a ligand of a Notch receptor.

39. The method of claim 38, wherein the Notch signaling pathway member is a Notch receptor.

40. The method of claim 39, wherein the Notch receptor is Notch1, Notch2, Notch 3, or Notch4.

41. The method of claim 40, wherein the Notch receptor is Notch1, Notch2, or Notch3.

42. The method of claim 41, wherein the Notch receptor is Notch1.

43. The method of claim 41, wherein the Notch receptor is Notch2.

44. The method of claim 41, wherein the Notch receptor is Notch3.

45. The method of claim 38, wherein the Notch signaling pathway member is a ligand of a Notch receptor.

46. The method of claim 45, wherein the ligand is DLL1, DLL3, DLL4, JAG1, or JAG2.

47. The method of claim 46, wherein the ligand is DLL4, JAG1, or JAG2.

48. The method of claim 47, wherein the ligand is DLL4.

49. The method of claim 47, wherein the ligand is JAG1.

50. The method of claim 47, wherein the ligand is JAG2.

51. The method of claim 37, wherin the Notch signaling pathway member is an intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

52. The method of claim 51, wherein the intracellular protein that transmits the Notch signal to or within in the nucleus of a cell is Hes-1.

53. The method of any of claims 16-52, wherein the at least one Notch signaling pathway member that is inhibited is the target transcript.

54. The method of any of claims 17-53, wherein the expression or activity of at least one Notch signaling pathway member that is not the target transcript is inhibited.

55. The method of any of claims 1-9 or 12-54, wherein the compound is administered parenterally.

56. The method of claim 55, wherein the compound is administered parenterally by subcutaneous administration.

57. The method of any of claims 1-9 or 12-54, wherein the compound is administered via inhalation.

58. The method of any of claims 55 or 56, wherein a pharmaceutical composition comprising the compound and at least one pharmaceutically acceptable carrier or diluent is administered.

59. The method of claim 57, wherein a pharmaceutical composition comprising the compound and at least one pharmaceutically acceptable carrier or diluent is administered.

60. The method of claim 59, wherein the pharmaceutical composition is a solution suitable for administration to an individual using a nebulizer or inhaler.

61. The method of claim 59, wherein th pharmaceutical composition is a powder suitable for administration to an individual using an inhaler.

62. The method of any of the preceding claims, comprising co-administering the compound and at least one additional therapy, wherein the additional therapy is not a Notch signaling pathway inhibitor.

63. The method of claim 62, wherein the compound and the additional therapy are administered concomitantly.

64. The method of claim 62, wherein the compound and the additional therapy are administered consecutively.

65. The method of any of claims 7-9 or 14-64, wherein the compound increases trans-differentiation from club cells or goblet cells to ciliated cells.

66. The method of claim 65, wherein the cells are in the respiratory epithelium of the individual.

67. The method of claim 65 or 66, wherein the increased trans-differentiation comprises decreased expression of at least one club cell or goblet cell marker.

68. The method of any of claims 65-67, wherein the increased trans-differentation comprises increased expression of at least one ciliated cell marker.

69. The method of any of claims 65-68, wherein the increased trans-differentiation comprises a decrease in the ratio of the expression of at least one club cell or goblet cell marker to the expression of at least one ciliated cell marker.

70. The method of claim 67 or 69, wherein the at least one goblet cell or club cell marker is MUC5AC, MUC5B, GOB5, FOXA3, SPDEF, or SCGB1A1.

71. The method of claim 68 or 69, wherein the at least one ciliated cell marker is FOXJ1.

72. Use of a compound comprising a modified oligonucleotide complementary to a Notch signaling pathway member transcript for the manufacture or preparation of a medicament for treating a respiratory disorder associated with excessive mucus production.

73. Use of a compound comprising a modified oligonucleotide complementary to a Notch signaling pathway member transcript for the treatment of a respiratory disorder associated with excessive mucus production.

74. The use of claim 72 or 73, wherein the respiratory disorder associated with excessive mucus production is asthma, COPD, IPF, or CF.

75. The use of any one of claims 72-74, wherein the compound is capable of increasing trans-differentiation from club cells or goblet cells to ciliated cells, decreasing mucus in the lungs, and/or increasing lung function.

76. The use of any one of claims 73-75, wherein the modified oligonucleotide is at least 90% complementary to the Notch signaling pathway member transcript.

77. The use of claim 76, wherein the modified oligonucleotide is at least 100% complementary to the Notch signaling pathway member transcript.

78. The use of any one of claims 72-77, wherein the Notch signaling pathway member transcript is a Notch receptor transcript, a transcript of a ligand of a Notch receptor, or a transcript of an intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

79. The use of claim 78, wherein the Notch signaling pathway member transcript is a Notch receptor transcript or a transcript of a ligand of a Notch receptor.

80. The use of claim 79, wherein the Notch signaling pathway member transcript is a Notch1, Notch2, Notch3, or Notch4 trasncript.

81. The use of claim 80, wherein the Notch signaling pathway member transcript is a Notch1, Notch2, or Notch3 transcript.

82. The use of claim 81, wherein the Notch signaling pathway member transcript is a Notch1 transcript.

83. The use of claim 81, wherein the Notch signaling pathway member transcript is a Notch2 transcript.

84. The use of claim 81, wherein the Notch signaling pathway member transcript is a Notch3 transcript.

85. The use of claim 79, wherein the Notch signaling pathway member transcript is a DLL1, DLL3, DLL4, JAG1, or JAG2 transcript.

86. The use of claim 85, wherein the Notch signaling pathway member transcript is a DLL4, JAG1, or JAG2 transcript.

87. The use of claim 86, wherein the Notch signaling pathway member transcript is a DLL4 transcript.

88. The use of claim 86, wherein the Notch signaling pathway member transcript is a JAG1 transcript.

89. The use of claim 86, wherein the Notch signaling pathway member transcript is a JAG2 transcript.

90. The use of claim 78, wherin the Notch signaling pathway member transcript is a transcript of an intracellular protein that transmits the Notch signal to or within the nucleus of a cell.

91. The use of claim 90, wherein the transcript of an intracellular protein that transmits the Notch signal to or within in the nucleus of a cell is a Hes-1 transcript.

92. The use of any one of claims 72-91, wherein the modified oligonucleotide is single-stranded.

93. The use of any one of claims 72-91, wherein the modified oligonucleotide is part of a double-stranded duplex.

94. The use of any one of claims 72-93, wherein the modified oligonucleotide is 12 to 30 linked nucleosides in length.

95. The use of any one of claims 72-94, wherein the modified oligonucleotide comprises at least one phosphorothioate internucleoside linkage, at least one bicyclic sugar moiety or 2'-O-methyoxyethyl modified sugar moiety, and at least one 5-methylcytosine modified nucleobase.

96. The use of claim 95, wherein at least one modified sugar is a cEt, LNA, or ENA.

97. The use of any of claims 72-96, wherein each modified internucleoside linkage of the modified oligonucleotide is a phosphorothioate linkage.

98. The use of any one of claims 72-97, wherein each cytosine nucleobase of the modified oligonucleotide is a 5-methylcytosine.

99. The use of any one of claims 72-98, wherein the modified oligonucleotide comprises:

a gap segment consisting of 7-11 linked 2'-deoxynucleosides;

a 5' wing segment consisting of 1-7 linked nucleosides;

a 3' wing segment consisting of 1-7 linked nucleosides;

wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein the nucleoside of each wing segment that is immediately adjacent to the gap segment comprises a modified sugar moiety.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/46905

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/113; A61K 31/7088, 31/7115 (2018.01)
CPC - C12N 15/113; G01N 2800/12, 2800/12; A61K 31/7088, 31/7115

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/046151 A1 (GLAXOSMITHKLINE INTELLECTUAL PROPERTY DEVELOPMENT, LTD.) 31 March 2016 (31.03.2016). Especially pg 2 para 4, pg 3 para 2, pg 4 para 7, pg 5 para 5-6, pg 6 para 2-3, pg 9 para 2 pg 18 para 2.	1-14, 73, 74/73
A	TSAO et al. Notch signaling prevents mucous metaplasia in mouse conducting airways during postnatal development. Development, August 2011, Vol 138, No 16, Pages 3533-3543. Especially abstract.	1-14, 73, 74/73
A	US 2012/0053112 A1 (WHITSETT) 1 March 2012 (01.03.2012). Especially para [0006], claims 56, 57.	1-14, 73, 74/73

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2018	18 DEC 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/46905

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/46905

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 14-71, 75-99
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-----Go to Extra Sheet for continuation--------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-13, 73, 74 (in part)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/46905

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-13, 73, 74 (in part), drawn to a method involving administering a compound comprising a Notch signaling pathway inhibitor to an individual to treat a respiratory disorder.

Group II: 72, 74 (in part), drawn to use of modified oligonucleotide complementary to a Notch signaling pathway member for the manufacture or preparation of a medicament.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of administering a Notch signaling pathway inhibitor, not required by Group II.

Group II has the special technical feature of use of modified oligonucleotide complementary to a Notch signaling pathway member for the manufacture or preparation of a medicament, not required by Group I.

Common Technical Feature:

1. Groups I and II share the common technical feature of a Notch signaling pathway inhibitor.

2. Groups I and II share the common technical feature of a modified oligonucleotide complementary to a Notch signaling pathway member transcript.

3. Groups I and II share the common technical feature of treating a respiratory disorder associated with excessive mucus production.

However, said common technical features do not represent a contribution over the prior art and is anticipated by WO 2016/046151 A1 to GlaxoSmithKline Intellectual Property Development, Ltd. (hereinafter "GSK")

As to common technical features #1 and #2, GSK teaches (pg 6 para 3; "the inhibitor may be an antisense nucleic acid capable of inhibiting expression of Notch 3 and/or Notch 4. The antisense nucleic acid can comprise all or part of the sequence of the Notch 3 and/or Notch 4 receptor, or of a sequence that is complementary thereto. The antisense sequence can be a DNA, an RNA (e.g. siRNA), a ribozyme, etc."; pg 2 para 4; "In another aspect, there is provided a method of treating or preventing COPD which comprises inhibiting Notch 3 and/or Notch 4 signaling in a mammal"; Pg 4 para 7; "In another aspect, there is provided a method of treating or preventing COPD which comprises inhibiting Notch 3 and/or Notch 4 signaling in a mammal").

As to common technical feature #3, GSK teaches (pg 4 para 7; "According to the present invention it has been found that inhibition of Notch 3 and/or Notch 4 signalling may result in the simultaneous reduction of neutrophilic inflammation and mucus production").

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning item 4: Claims 14-71, 75-99 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).